Invest New Drugs
DOI 10.1007/s10637-011-9693-6

PHASE I STUDIES

Phase I results from a two-part Phase I/II study of cediranib in combination with mFOLFOX6 in Japanese patients with metastatic colorectal cancer

Taroh Satoh · Kensei Yamaguchi · Narikazu Boku · Wataru Okamoto · Tomotaka Shimamura · Kentaro Yamazaki · Xiaojin Shi · Hideyuki Mishima

Received: 12 March 2011 / Accepted: 17 May 2011

Summary *Background* Colorectal cancer (CRC) is the second most common malignancy in Japan. Inhibition of vascular endothelial growth factor (VEGF) signaling is a clinically validated therapeutic strategy in patients with metastatic CRC. Cediranib is an oral, highly potent VEGF signaling inhibitor of all three VEGF receptors. *Methods* This Phase I study investigated the safety, tolerability and pharmacokinetics of cediranib (20 or 30 mg) in combination with mFOLFOX6 in Japanese patients with previously untreated metastatic CRC. If the safety of the 20 mg dose was confirmed, a second cohort of patients was to be recruited to receive cediranib 30 mg + mFOLFOX6. *Results* Six patients received cediranib 20 mg + mFOLFOX6 and seven received cediranib 30 mg + mFOLFOX6. One patient in the initial cediranib 20 mg cohort experienced a dose-limiting toxicity (DLT; grade 3 bilirubin increase); no DLTs were observed in the 30 mg cohort. The most commonly reported adverse events were diarrhea, decreased appetite, peripheral neuropathy, hypertension and fatigue. Two patients in the 20 mg cohort and three in the 30 mg cohort experienced serious adverse events during all treatment courses. Cediranib was generally well tolerated in this patient population with no evidence to suggest any significant pharmacokinetic interactions between cediranib and fluorouracil or oxaliplatin. A preliminary evaluation showed that five of nine evaluable patients achieved a best response of partial response. *Conclusion* Cediranib (20 or 30 mg) in combination with mFOLFOX6 was considered tolerable according to the protocol-defined criteria, providing justification for the Phase II part of this study.

Keywords Cediranib · Colorectal cancer · mFOLFOX6 · Tolerability

T. Satoh · W. Okamoto
Kinki University School of Medicine,
Osaka, Japan

K. Yamaguchi · T. Shimamura
Saitama Cancer Centre,
Saitama, Japan

N. Boku
St. Marianna University School of Medicine,
Kanagawa, Japan

K. Yamazaki
Shizuoka Cancer Centre,
Shizuoka, Japan

X. Shi
AstraZeneca KK,
Osaka, Japan

H. Mishima
National Hospital Organization, Osaka National Hospital,
Osaka, Japan

T. Satoh (✉)
Department of Medical Oncology, Kinki University School of Medicine,
377-2 Onohigashi,
Osakasayama-city, Osaka 589–8511, Japan
e-mail: tarohsatoh@hotmail.com

Present Address:
T. Satoh
Osaka University Graduate School of Medicine,
Osaka, Japan

Published online: 25 May 2011

Introduction

The incidence of colorectal cancer (CRC) in Japan has been increasing rapidly in recent years due to lifestyle changes and it is now the second most common malignancy in this country. In 2008, it was estimated that there were 101,656 new cases of CRC and 43,349 deaths because of this disease in Japan [1]. For many years, fluorouracil (5-FU) was the standard chemotherapy used for the treatment of CRC. Clinical outcomes were improved by the addition of leucovorin, oxaliplatin and irinotecan, and if all these agents are used at some point in patient care, a median overall survival (OS) of up to 20 months can be achieved [2]. The combination of 5-FU, leucovorin and oxaliplatin resulted in the development of the FOLFOX regimen [3]. Japanese guidelines recommend the use of FOLFOX as a standard treatment for metastatic CRC [4]. A number of different FOLFOX regimens have been evaluated and the modified FOLFOX6 regimen (mFOLFOX6) is the current standard in Japan. mFOLFOX6 has a reduced incidence of hematological toxicity and chronic neurotoxicity associated with bolus 5-FU and oxaliplatin, respectively.

Inhibition of vascular endothelial growth factor (VEGF) signaling is a clinically validated therapeutic strategy in patients with metastatic CRC [5]. Cediranib (AZD2171) is an oral, highly potent VEGF signaling inhibitor that inhibits all three VEGF receptors [6, 7]. Early-phase clinical evaluation in patients with advanced cancer confirmed that cediranib is suitable for once-daily oral dosing, with biological activity at doses ≥20 mg/day [8]. In a Phase I study of Japanese patients with advanced solid tumors, cediranib was well tolerated as a monotherapy at doses of ≤30 mg/day [9]. Subsequent Phase I combination studies demonstrated that cediranib was generally well tolerated at doses up to 30 mg/day in combination with various anticancer agents [10–14].

Here, we report the results of the Phase I part of a two-part Phase I/II study of cediranib in combination with mFOLFOX6 in Japanese patients with previously untreated metastatic CRC. Two doses of cediranib (20 mg and 30 mg) were investigated in this Phase I study. This decision was consistent with the previous selection of both doses for the HORIZON Phase II/III program of evaluation in Western patients with CRC [15, 16], which was itself based in part on Phase I data showing cediranib 20 mg or 30 mg in combination with mFOLFOX6 to be tolerable, with preliminary evidence of antitumor activity [11, 13]. Since the present study was the first to investigate the addition of cediranib to mFOLFOX6 in Japanese patients, determination of the tolerability of cediranib 20 mg or 30 mg in combination with mFOLFOX6 was required to provide justification for continuation to the Phase II part of the study.

Study objectives

The primary objective of this open-label Phase I study was the assessment of the safety and tolerability of cediranib in combination with mFOLFOX6. Adverse events were recorded and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Secondary objectives included determination of the steady-state pharmacokinetics of cediranib when given in combination with mFOLFOX6 and determination of the pharmacokinetics of oxaliplatin and 5-FU when administered in combination with repeated doses of cediranib. Exploratory endpoints included preliminary assessment of the efficacy of cediranib in combination with mFOLFOX6, assessed by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 and change in tumor size.

Methods

Eligibility

Eligible patients were aged ≥18 years, with histological or cytological confirmation of carcinoma of the colon or rectum. Patients required chemotherapy for stage IV (metastatic) disease and had a World Health Organization (WHO) performance score of 0 or 1, and adequate hematology and organ function. RECIST-measurable lesions were not mandatory for this part of the study. Any adjuvant oxaliplatin or 5-FU therapy must have been completed (or terminated) >12 and >6 months, respectively, prior to study entry. Patients could be hospitalized if required. Patients with brain or meningeal metastases were eligible if they were clinically stable and had not required corticosteroid treatment for ≥10 days. Exclusion criteria included: history of poorly controlled hypertension, significant proteinuria, hemorrhage, hemoptysis or thrombotic event; prior systemic therapy for metastatic disease; and prior therapy with monoclonal antibodies or small-molecule inhibitors against VEGF or VEGF receptors. Each patient provided written informed consent.

Study design

Patients (minimum $n=3$; maximum $n=9$) received cediranib 20 or 30 mg (orally, once daily, starting on day 1) plus standard 14-day cycles of mFOLFOX6 (oxaliplatin 85 mg/m^2 plus leucovorin 200 mg/m^2, both iv over 2 h on day 1 plus 5-FU 400 mg/m^2 iv bolus immediately after the completion of oxaliplatin/leucovorin, followed immediately by 2,400 mg/m^2 of 5-FU by continuous iv infusion over 46 h).

Patients were considered evaluable if they completed at least 28 days of continuous daily cediranib treatment or

they experienced a dose-limiting toxicity (DLT) prior to completing 28 days of continuous therapy. A safety review of the data was initially performed after at least three evaluable patients had received 28 days of cediranib treatment and was also performed subsequently (based on the information below) to permit decisions on cohort expansion for a maximum of nine patients or stopping enrollment in each cohort (Fig. 1). If <33% patients experienced a DLT, the dose was defined as tolerable. If a DLT was observed in >50% of patients, the dose was considered to be not tolerated. If a DLT was observed in ≥33% but ≤50% of patients in a three- or six-patient cohort, the cohort was expanded for further assessment (three patients for each expansion) and a final decision on tolerability was made by the safety review committee (SRC). A DLT was defined as any of the following: grade 3 or higher toxicity considered to be related to cediranib; a single increase from baseline in the QT interval corrected for heart rate (QTc) of 60 ms resulting in a QTc of at least 460 ms, or a QTc interval of >490 ms on two electrocardiograms taken at least 24 h apart; hypertension necessitating treatment pause of cediranib as detailed in the cediranib hypertension management protocol. The study was approved by each center's institutional review board and was performed in accordance with the Declaration of Helsinki, the International Conference on Harmonization/Good Clinical Practice, applicable regulatory requirements and the AstraZeneca policy on Bioethics.

Pharmacokinetic assessment

Blood samples were collected at multiple time points on day 15 for pharmacokinetic assessment of cediranib (predose, 1, 2, 3, 5, 7 and 24 h post dosing), oxaliplatin (predose, at the end of the 120-min infusion, at 15 and 45 min post infusion and at 1, 3, 5, 22, 24 and 46 h post infusion) and 5-FU (1 h into the infusion and at the end of the 46-h infusion).

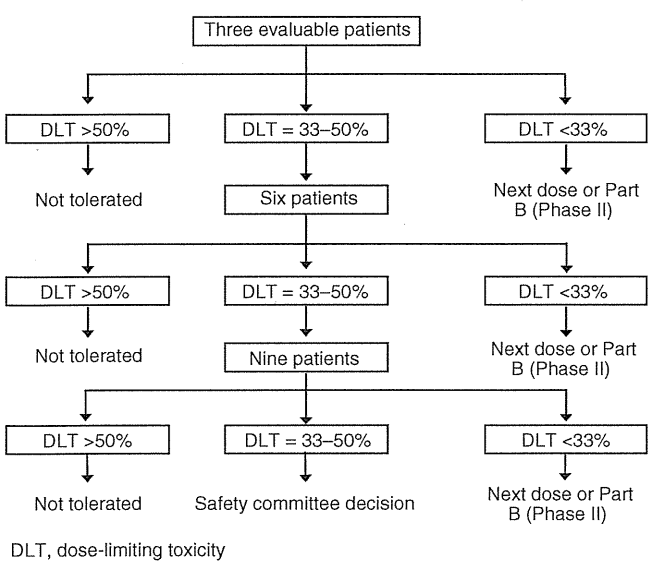

Fig. 1 Study design

Statistical analysis

The number of patients required was based on a desire to gain adequate safety information while exposing as few patients as possible to study medication and procedures. Only descriptive statistics for each dose level were produced.

Results

Patients

Six patients were enrolled into the cediranib 20 mg cohort and seven into the cediranib 30 mg cohort. Patient demographics and characteristics were representative of a Phase I study of patients with metastatic colorectal cancer in Japan (Table 1). All 13 patients enrolled were evaluable for both safety and pharmacokinetics. Nine patients were evaluable for efficacy. At data cut-off (13 October 2009) all 13 patients had discontinued cediranib; five due to an adverse event and eight due to disease progression.

Safety and tolerability

One of the three patients initially enrolled in the cediranib 20 mg cohort experienced a DLT (grade 3 bilirubin increase); therefore three further patients were recruited to this cohort. The patient who developed the DLT was a 72-year-old male with liver metastasis who also experienced grade 2 alanine aminotransferase and aspartate aminotransferase increases on day 36. Study treatment was terminated on the same day and these values returned to approximate normal ranges without medication after 5 days. No further DLTs were observed, and recruitment to the 30 mg cohort was initiated. One of three patients initially enrolled into the cediranib 30 mg cohort developed wound disruption on day 25 at the site of a port placement. This patient was considered to be non-evaluable for DLT because it could not be judged whether the wound disruption was related to cediranib treatment or the port placement procedure. After the discussion at the SRC, a further four patients were enrolled and no DLTs occurred in any of the six evaluable patients in the 30 mg cohort.

Three patients in the cediranib 20 mg cohort and two patients in the cediranib 30 mg cohort had adverse events that led to permanent discontinuation of cediranib: cardiac

Table 1 Patient demographics and baseline characteristics

Characteristic	Cediranib 20 mg + mFOLFOX6 (n=6)	Cediranib 30 mg + mFOLFOX6 (n=7)
Median age, years (range)	55.5 (50–72)	60.0 (48–65)
Sex, n (%)		
Male	6 (100.0)	4 (57.1)
Female	0	3 (42.9)
WHO performance score, n (%)		
0	4 (66.7)	4 (57.1)
1	2 (33.3)	3 (42.9)
Type of cancer, n (%)		
Colon	3 (50.0)	4 (57.1)
Rectal	3 (50.0)	3 (42.9)
Histology type, n (%)		
Adenocarcinoma	6 (100.0)	7 (100.0)
Tumor grading, n (%)		
Well differentiated (G1)	1 (16.7)	2 (28.6)
Moderately differentiated (G2)	3 (50.0)	5 (71.4)
Poorly differentiated (G3)	2 (33.3)	0
Metastatic sites, n (%)		
1	3 (50.0)	4 (57.1)
>1	3 (50.0)	3 (42.9)
Prior adjuvant therapy, n (%)		
Yes	1 (16.7)	1 (14.3)
No	5 (83.3)	6 (85.7)

failure, hepatitis, renal vein occlusion (each n=1, cediranib 20 mg cohort); cerebral hemorrhage, postoperative wound infection (both n=1, in the cediranib 30 mg cohort). Five patients in each cohort (83.3% of patients in the 20 mg cohort; 71.4% of patients in the 30 mg cohort) required dose reductions or pauses of cediranib. During the first 3 months, the relative dose intensity of cediranib was higher in the cediranib 20 mg cohort compared with the 30 mg cohort (89.2% versus 72.2%, respectively) and the mean relative dose intensity of 5-FU, leucovorin and oxaliplatin was slightly higher in the cediranib 20 mg cohort compared with the cediranib 30 mg cohort (5-FU, 72.0% versus 67.0%; leucovorin, 73.1% vs 67.1%; oxaliplatin, 70.3% versus 66.7%).

The adverse event profile was generally similar in both arms. Overall, the most commonly reported adverse events were diarrhea, decreased appetite, peripheral neuropathy and hypertension (Table 2). Five patients in each cohort experienced grade ≥3 adverse events (Table 3). One patient in the cediranib 30 mg cohort experienced grade 4 leukopenia. Hypertension was reported in 11 patients (n=5, 20 mg cohort; n=6, 30 mg cohort). All 11 patients received new antihypertensive medication during the study (the two other patients were receiving antihypertensive medication at baseline). Adverse events of bleeding were observed in ten patients (n=4, 20 mg cohort; n=6, 30 mg cohort); all experienced epistaxis as a bleeding event and no clinically significant bleeding episode was observed. No clinically relevant biochemical toxicities were noted. Increases in median blood thyroid stimulating hormone above normal range (>5 mU/L) were observed but there were no apparent changes in median T3 (free) or T4 (free). Hypothyroidism was reported in one patient of the cediranib 30 mg cohort; this patient did not require hormone-replacement therapy.

Five patients experienced serious adverse events during the study; two in the 20 mg cohort (cardiac failure, renal vein occlusion; both n=1) and three in the 30 mg cohort (cerebral hemorrhage, febrile neutropenia/postoperative ileus, postoperative wound infection; each n=1). No fatal adverse events occurred; the three deaths that occurred on study were due to disease progression.

Pharmacokinetics

On Day 15, the steady-state geometric mean (min–max) plasma concentrations of cediranib ($C_{ss,max}$) in the presence of mFOLFOX6 appeared to be dose related: 52.9 (35.1–69.8) ng/mL and 105 (61.6–217) ng/mL in the 20 mg (n=6) and 30 mg (n=7) cohorts, respectively (Fig. 2). The corresponding geometric mean (min–max) AUC_{ss} values

Table 2 Adverse events reported in ≥3 patients in either cohort

Preferred term, n (%)	Cediranib 20 mg + mFOLFOX6 (n=6)	Cediranib 30 mg + mFOLFOX6 (n=7)
Diarrhea	6 (100.0)	6 (85.7)
Decreased appetite	6 (100.0)	6 (85.7)
Peripheral neuropathy	5 (83.3)	6 (85.7)
Hypertension	5 (83.3)	6 (85.7)
Fatigue	5 (83.3)	5 (71.4)
Nausea	4 (66.7)	6 (85.7)
Epistaxis	4 (66.7)	6 (85.7)
Stomatitis	4 (66.7)	5 (71.4)
PPES	4 (66.7)	4 (57.1)
Dysgeusia	5 (83.3)	2 (28.6)
Constipation	5 (83.3)	5 (71.4)
Headache	2 (33.3)	4 (57.1)
Vomiting	1 (16.6)	5 (71.4)
Alopecia	4 (66.7)	1 (14.3)
Dysphonia	2 (33.3)	3 (42.9)
Weight decreased	2 (33.3)	3 (42.9)
Edema peripheral	3 (50.0)	1 (14.3)
Pyrexia	2 (33.3)	2 (28.6)
Abdominal pain	1 (16.7)	3 (42.9)
Pruritus	1 (16.7)	3 (42.9)
Insomnia	1 (16.7)	3 (42.9)
Drug hypersensitivity	2 (33.3)	1 (14.3)
Abdominal pain upper	1 (16.7)	2 (28.6)

PPES palmar–plantar erythrodysaesthesia syndrome (hand–foot syndrome)

Table 3 Adverse events of CTCAE grade 3 or higher

Preferred term, n (%)	Cediranib 20 mg + mFOLFOX6 (n=6)	Cediranib 30 mg + mFOLFOX6 (n=7)
Patients with CTC grade 3 or higher	5 (83.3)	5 (71.4)
CTCAE grade 4		
Leukopenia	0	1 (14.3)
CTCAE grade 3		
Abdominal pain	0	1 (14.3)
Cardiac failure	1 (16.7)	0
Cerebral hemorrhage	0	1 (14.3)
Decreased appetite	1 (16.7)	0
Diarrhea	0	1 (14.3)
Drug hypersensitivity	0	1 (14.3)
Electrocardiogram QT prolonged	1 (16.7)	0
Fatigue	1 (16.7)	0
Hypertension	0	1 (14.3)
Febrile neutropenia	0	1 (14.3)
Neuropathy peripheral	1 (16.7)	0
Postoperative ileus	0	1 (14.3)
Postoperative wound infection	0	1 (14.3)
Renal vein occlusion	1 (16.7)	0
Thrombocytopenia	1 (16.7)	0

Laboratory abnormalities, including grade 3 bilirubin increase (defined as a DLT), are not included

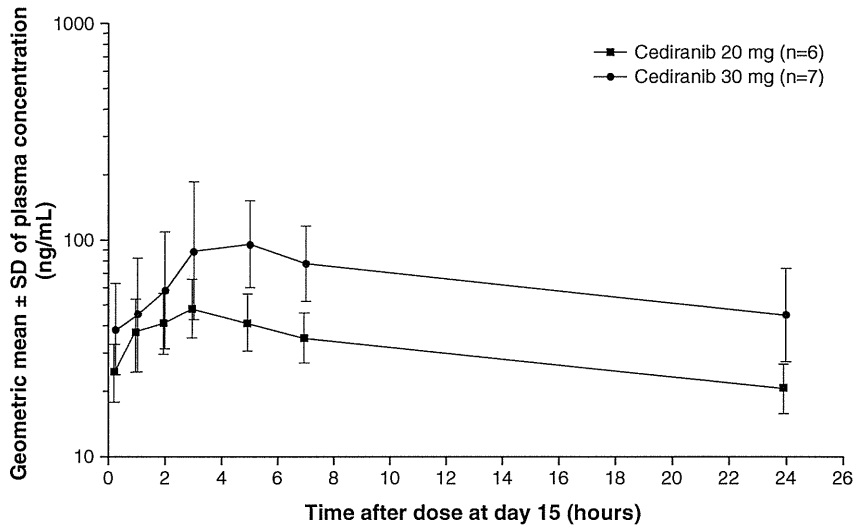

Fig. 2 Geometric mean plasma concentration of cediranib in combination with mFOLFOX6, log scale

were 762 (601–1120) and 1590 (946–3110) ng.h/mL, respectively. Although there was interpatient variability and a small number of patients enrolled at each cohort, the pharmacokinetic parameters of oxaliplatin seemed to be similar when administered with either dose of cediranib ($AUC_{(0-\infty)}$: 20 mg; min 123,000 ng.h/mL, max 231,000 ng.h/mL; 30 mg, min 175,000 ng.h/mL, max 265,000 ng.h/mL). For 5-FU, only two samples were collected post dosing (1 and 46 h). The 5-FU concentrations were similar when administered with either dose of cediranib (20 mg; min 499 ng/mL, max 2,260 ng/mL; 30 mg, min 478 ng/mL, max 1,560 ng/mL).

Efficacy

Four patients in the cediranib 20 mg cohort and five in the 30 mg cohort had measurable disease. A preliminary evaluation showed that five out of nine evaluable patients (55.6%) achieved a best response of partial response (n=2, 20 mg cohort; n=3, 30 mg cohort). One patient in each group had stable disease ≥6 weeks, and one patient in each group had progressive disease. Two patients were treated for more than 2 years (one had a best response of partial response and one had non-measurable disease).

Discussion

Patients were representative of the target population of Japanese patients with previously untreated metastatic CRC. One patient in the cediranib 20 mg cohort experienced a DLT (grade 3 bilirubin increase considered to be related to cediranib) and recovered without medication 5 days after treatment discontinuation. None of the six evaluable patients who received cediranib 30 mg in combination with mFOLFOX6 experienced a DLT. These findings justified continued investigation of both doses in combination with mFOLFOX6 in the placebo-controlled, double-blind, randomized Phase II part of the study,

Consistent with previous studies, the most commonly reported adverse events were diarrhea and hypertension. No new toxicities associated with cediranib were identified in this study. The incidence of palmar–plantar erythrodysaesthesia (hand–foot syndrome) in patients who received cediranib in this study was higher than that reported in Western patients [8, 11], however, it is consistent with values reported in another Phase I study of cediranib in Japanese patients [9] and in studies of other VEGFR-targeted agents in Japanese patient populations [17, 18]. Cediranib was generally well tolerated in combination with mFOLFOX6. However, there were more adverse events leading to discontinuation of cediranib in patients who received cediranib 30 mg compared with cediranib 20 mg. The dose intensity of chemotherapy during the first 3 months was also reduced in the cediranib 30 mg cohort compared with the cediranib 20 mg cohort. The dose intensity should be further investigated with the large number of patients in the Phase II part of this study.

In Japanese patients with advanced solid tumors, Phase I evaluation has shown cediranib monotherapy to be well tolerated at doses ≤30 mg/day [8]. A Phase I study in Western patients with advanced CRC assessed two doses of cediranib (30 and 45 mg) in combination with mFOLFOX6 [11]. Based on the results of the Western study, the recommended Phase II dose of cediranib was 30 mg in combination with mFOLFOX6 (no DLTs were observed at the 45 mg dose level but the overall dose intensities of cediranib and mFOLFOX6 were reduced compared with the 30 mg cohort). In addition, a large randomized Phase II study in Western patients with previously treated metastatic CRC has shown that cediranib 20 mg was better tolerated than cediranib 30 mg when given with mFOLFOX6 [16].

In the current study, both dose levels of cediranib (20 and 30 mg) given in combination with mFOLFOX6 were considered to be tolerable for Japanese patients with previously untreated metastatic CRC.

Comparison of the pharmacokinetic results of this study with previous studies of cediranib monotherapy in a Japanese population [9] and of cediranib in combination with mFOLFOX6 in a Western population [11] showed less than two-fold differences in any parameters, including AUC_{ss} or C_{max}. The Western study reported no pharmacokinetic interactions between cediranib and oxaliplatin or 5-FU. In the present study, relatively large between-patient variability was observed with cediranib treatment. Given this variability, we can only conclude that there is no strong evidence to suggest a clinically significant change in the pharmacokinetics of cediranib (20 or 30 mg) when administered with mFOLFOX6. A preliminary assessment of efficacy showed that five of nine evaluable patients across both doses (n=2, 20 mg cohort; n=3, 30 mg cohort) achieved a best response of partial response.

In conclusion, cediranib (20 or 30 mg) in combination with mFOLFOX6 was active and generally well tolerated in this patient population and the combination was considered suitable for investigation in the Phase II part of this study.

Acknowledgements Funding for this study was provided by AstraZeneca. We thank Dr Helen Jones, from Mudskipper Bioscience, who provided medical writing assistance funded by AstraZeneca.

Conflicts of interest T Satoh, W Okamoto, T Shimamura, K Yamazaki and H Mishima have no conflicts of interest to disclose. K Yamaguchi has received speaker fees (Merck Serono and Chugai Pharmaceutical). N Boku has received honoraria (Takeda, Ono, Daiichi Sankyo and Taiho Co. Ltd). X Shi is an employee of AstraZeneca and owns stock.

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Published OnlineFirst July 20, 2011; DOI:10.1158/0008-5472.CAN-11-0385

Priority Report

Cancer Research

HERC2 Interacts with Claspin and Regulates DNA Origin Firing and Replication Fork Progression

Naoki Izawa[1,2,3], Wenwen Wu[4], Ko Sato[1], Hiroyuki Nishikawa[5], Akihiro Kato[6], Narikazu Boku[3], Fumio Itoh[2], and Tomohiko Ohta[1]

Abstract

DNA replication, recombination, and repair are highly interconnected processes the disruption of which must be coordinated in cancer. HERC2, a large HECT protein required for homologous recombination repair, is an E3 ubiquitin ligase that targets breast cancer suppressor BRCA1 for degradation. Here, we show that HERC2 is a component of the DNA replication fork complex that plays a critical role in DNA elongation and origin firing. In the presence of BRCA1, endogenous HERC2 interacts with Claspin, a protein essential for G_2–M checkpoint activation and replication fork stability. Claspin depletion slowed S-phase progression and additional HERC2 depletion reduced the effect of Claspin depletion. In addition, HERC2 interacts with replication fork complex proteins. Depletion of HERC2 alleviated the slow replication fork progression in Claspin-deficient cells, suppressed enhanced origin firing, and led to a decrease in MCM2 phosphorylation. In a HERC2-dependent manner, treatment of cells with replication inhibitor aphidicolin enhanced MCM2 phosphorylation. Taken together, our results suggest that HERC2 regulates DNA replication progression and origin firing by facilitating MCM2 phosphorylation. These findings establish HERC2 as a critical function in DNA repair, checkpoint activation, and DNA replication. *Cancer Res; 71(17); 5621–5. ©2011 AACR.*

Introduction

DNA replication, recombination, and repair coordinately maintain genome stability, and their defect is a hallmark of cancer cells. The DNA replication and damage response share many critical proteins. Among them are the ATR-Chk1 pathway, which is activated in response to stalled replication forks and prevents inappropriate entry into mitosis, while it also regulates normal DNA replication by stabilizing replication forks and inhibiting excess origin firing (1, 2). Claspin is a checkpoint mediator that facilitates the phosphorylation and activation of Chk1 by ATR (3). In addition, Claspin in combination with TIPIN-TIM1-AND1 complex physically links the DNA polymerase and helicase activities, preventing fork collapse, and is required for a normal rate of fork progression (2, 4, 5).

HERC2 is a large HECT and RCC-like domain-containing protein comprising 4,834 amino acids, and has recently been implicated in homologous recombination repair of DNA double-strand breaks (DSB; ref. 6). HERC2 is recruited to sites of DSBs and facilitates assembly of the RNF8-Ubc13 complex, and is thereby essential for ubiquitin-dependent retention of repair factors (6). HERC2 is also implicated in nucleotide excision repair by ubiquitinating and degrading XPA (7, 8). In addition, we showed that HERC2 is an E3 ubiquitin ligase that targets BARD1-uncoupled BRCA1 for degradation (9). Depletion of HERC2 does not produce G_2–M checkpoint failure (9) in spite of the fact that HERC2 is essential for recruiting the repair factors including BRCA1 that mediate G_2–M checkpoint activation, to sites of DNA damage (6). Because depletion of HERC2 compensated for BRCA1 instability in BARD1-deficient cells and restored G_2–M checkpoint function, we propose that HERC2 inhibits G_2–M checkpoint function by destabilizing BRCA1. HERC2 interacts with BRCA1 and may inhibit its G_2–M checkpoint function during normal S-phase or during recovery from the checkpoint. However, role of HERC2 in normal S-phase is unknown.

Because BRCA1 interacts with Claspin and acts as a second regulator of Chk1 activation (10), HERC2 may interact with Claspin and regulate DNA replication. Here, we show that HERC2 is a component of replication fork complex and regulates the fork progression and origin firing in conjunction with Claspin.

Materials and Methods

Cell culture and transfection

HCT116, Hela, HEK293T, U2OS, and either BRCA1-negative or -positive UWB1.289 cells were purchased from American

Authors' Affiliations: Departments of [1]Translational Oncology, [2]Gastroenterology and Hepatology, [3]Clinical Oncology, and [4]Breast and Endocrine Surgery, [5]Institute of Advanced Medical Science, St. Marianna University Graduate School of Medicine, Kawasaki; and [6]Department of Genome Repair Dynamics, Radiation Biology Center, Kyoto University, Kyoto, Japan

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Corresponding Author: Tomohiko Ohta, Department of Translational Oncology, St. Marianna University Graduate School of Medicine, Kawasaki 216-8511, Japan. Phone: 81-44-977-8111; Fax: 81-44-975-1400; E-mail: to@marianna-u.ac.jp

doi: 10.1158/0008-5472.CAN-11-0385

Published OnlineFirst July 20, 2011; DOI:10.1158/0008-5472.CAN-11-0385

Type Culture Collection and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 1% antibiotic-antimycotic agent. Cell lines were not further tested or authenticated. siRNA oligonucleotides targeting HERC2 (#1: 5′-GGAAAGCACUGGAUUCGUU-3′ and #2: 5′-GAAGGUGGCUGUUCACUCA-3′, sense strand), Claspin (#1: 5′-GGACGUAAUUGAUGAAGUA-3′ and #2: 5′-GGAAAUACCUGGAGGAUGA-3′, sense strand), and nontargeting control (D-001206-14) were purchased from Dharmacon. Myc-tagged *N*-terminus (1-2329, NT) and *C*-terminus (2292-4834, CT) of HERC2 fragments were subcloned in pcDNA3 by fusing smaller fragments of HERC2 as described previously (9). Transfection was carried out as previously described (11). Aphidicolin was purchased from Sigma-Aldrich.

Cell-cycle analysis

Seventy-two hours after siRNA transfection, HCT116 cells were treated with 100 ng/mL of nocodazole for 12 hours to trap cells in mitosis and to analyze a single cycle of S-phase. Bromodeoxyuridine (BrdUrd) was added for the last hour. Cells were then harvested and DNA was stained with anti-BrdUrd monocolonal antibody (mAb; Santa Cruz) and propidium iodide, and analyzed by flow cytometry as described elsewhere (12).

Immunoprecipitation, immunoblotting, and immunocytochemistry

Rabbit polyclonal antibodies to HERC2 (Bethyl Laboratories), Claspin (Bethyl Laboratories), ATRIP (Bethyl Laboratories), phospho-MCM2-S108 (Bethyl Laboratories), goat polyclonal antibodies to MCM2 (Santa Cruz) and mouse mAbs to HERC2 (BD Bioscience), MCM7 (Santa Cruz), PCNA (Neomarkers), TopBP1 (BD Bioscience), α- and β-tubulin (DMIA+BMIB, Neomarkers), Actin (Sigma), and Myc (9E10, BabCo) were purchased commercially. Immunoprecipitation and immunoblotting were done as described (11) with 0.5% NP-40–based lysis buffer in the presence or absence of benzonase nuclease (Novagen) or with RIPA buffer for whole-cell lysates (11, 13). For indirect immunocytochemistry, cells were fixed with cold methanol for 60 minutes and permeablized with cold acetone for 5 seconds. Cells were then stained as previously described (13) with the modification that blocking buffer contained 0.1% Triton X-100.

DNA fiber experiments

Forty-eight hours after siRNA transfection, cells were pulse labeled with 25 μmol/L BrdUrd for indicated time length, followed by 250 μmol/L iododeoxyuridine (IdUrd) for 20 minutes. DNA combing was carried out as described elsewhere (14, 15) with modifications. Briefly, 3,000 labeled cells spread on a glass slide were overlaid with 10 μL of buffer containing 0.5% sodium dodecyl sulfate, 200 mmol/L Tris-HCl (pH 7.4), and 50 mmol/L EDTA. After 10 minutes, the slide was tilted at 30 degrees and the resulting DNA spreads were air-dried, and fixed in 3:1 methanol/acetic acid for 5 minutes. The slides were treated with 2.5 mol/L HCl for 60 minutes, washed in PBS, and blocked in 2% bovine serum albumin in PBS for 30 minutes. The DNA fibers were then immunostained with rat anti-BrdUrd mAb and mouse anti-BrdUrd/IdUrd mAb (BD Biosciences) followed by AlexaFluor 488-conjugated chicken anti-rat IgG (Invitrogen) and AlexaFluor 555-conjugated goat anti-mouse IgG (Millipore). Fluorescent-labeled fibers were then examined with an LSM 510 confocal microscope (Carl Zeiss). The lengths of BrdUrd- (green) and IdUrd (red)-labeled patches were measured using LSM software (Carl Zeiss).

Results and Discussion

HERC2 interacts with Claspin and affects S-phase progression

To examine whether HERC2 cooperates with Claspin, we first analyzed the interaction between endogenous HERC2 and Claspin by coupled immunoprecipitation and Western blotting. HERC2 was readily detected in Claspin immunocomplexes precipitated from HCT116 or HeLa cell lysate (Fig. 1A). The interaction was diminished in BRCA1-defective UWB 1.289 cells (Fig. 1B), suggesting that BRCA1 facilitates the interaction. We next examined whether HERC2 has some role in the effect of Claspin on S-phase progression. HCT116 cells

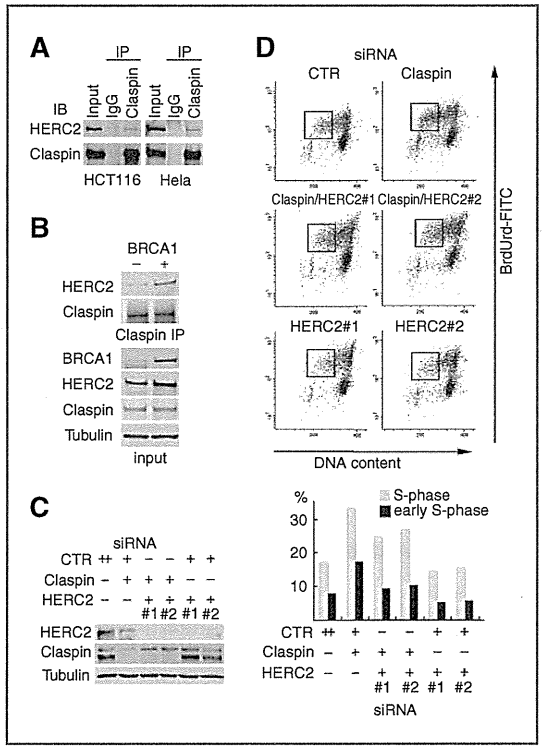

Figure 1. HERC2 interacts with Claspin and affects S-phase progression. A and B, lysates prepared from HeLa, HCT116 (A), and BRCA1-negative and positive UWB 1.289 (B) cells were immunoprecipitated (IP) and immunoblotted (IB) with indicated antibodies. Inputs (1.5%) were also loaded. C, HCT116 cells transfected with indicated siRNA were IB with indicated antibodies. Tubulin was the loading control. D, cells from (C) were analyzed for cell cycle. The percentages of early S- (indicated with square boxes) and S-phase cells are shown in bottom. CTR, control.

Published OnlineFirst July 20, 2011; DOI:10.1158/0008-5472.CAN-11-0385

were transfected with siRNA for Claspin and/or HERC2, and the S-phase progression was analyzed with BrdUrd. The siRNA treatment successfully inhibited expression of Claspin and HERC2 (Fig. 1C; Supplementary Fig. S1). Depletion of Claspin expression increased cells in S-phase, including that in early S-phase (Fig. 1D), indicating slowed S-phase progression as previously reported (4). Importantly, additional HERC2 depletion with 2 different siRNAs both reduced the effect of Claspin depletion and decreased cells in S-phase to the level of control cells. This suggests that HERC2 suppresses S-phase progression in the absence of Claspin. Single HERC2 knockdown also slightly decreased cells in S-phase when compared with the control cells.

HERC2 localizes at DNA replication fork

We previously showed that HERC2 and BRCA1 colocalize at S-phase nuclear foci (9). To analyze whether HERC2 localizes at DNA replication foci, exponentially proliferating HeLa cells were immunostained with anti-HERC2 antibody in combination with antibodies to proteins in the replication fork complex. Interphase cells exhibited nuclear HERC2 foci as we showed previously (9). Importantly, clear colocalization of HERC2 with nuclear PCNA foci was visualized (Fig. 2A). The colocalization was especially remarkable in cells with larger PCNA foci (light upper cell in top panels), an indication of cells in late S-phase (16). To further show the interaction between HERC2 and PCNA, HEK-293T cells were transfected with HERC2 fragments. As shown in Fig. 2B, PCNA was detected in the Myc-HERC2-CT immunocomplexes. Reciprocally, Myc-HERC2-CT was detected in PCNA immunocomplexes. The detection of the interaction was dependent on endonuclease treatment of cell lysates, suggesting that HERC2 physically interacts with PCNA complex on chromatin. HERC2 also colocalized at nuclear foci with TopBP1 and MCM7 (Fig. 2A). Together the results indicate that HERC2 is a component of the DNA replication complex.

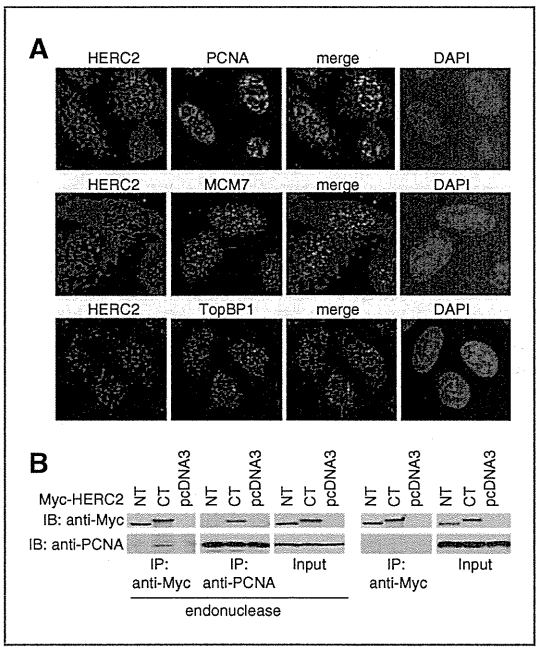

Figure 2. HERC2 localizes at DNA replication foci. A, HeLa cells were immunostained with anti-HERC2 antibody in combination with indicated antibodies. The nucleus was counterstained with DAPI. B, HEK-293T cells were transfected with Myc-HERC2-NT or CT fragment, or parental pcDNA3 vector, and lysed with or without endonuclease. Interaction between Myc-HERC2 fragments and PCNA were assessed by IP followed by IB with indicated antibodies.

HERC2 suppresses DNA replication progression in the absence of Claspin

S-phase progression is regulated by 2 main mechanisms: replication origin firing and elongation. Claspin and Chk1 stabilize replication fork and maintain elongation while they suppress excess origin firing (5, 17). Therefore the observed effect on S-phase progression in cells depleted of Claspin and/or HERC2 (Fig. 1) could result from the 2 contradictory factors. To analyze this mechanism more precisely, we used DNA combing experiments. The nascent DNAs were labeled with BrdUrd followed by IdUrd and the DNA lengths were analyzed with immunofluorescent detection. The reliability of the procedure was first verified by proportionate increase of the DNA length and labeling time length (Supplementary Fig. S2). HCT116 cells were then transfected with Claspin- or HERC2-specific siRNAs. The inhibition of HERC2 and/or Claspin expression was verified by Western blot (Fig. 3A, Supplementary Fig. S1). The lengths of the nascent DNAs in each sample were determined (Fig. 3B–D). Consistent with the previous report (4, 18), inhibition of Claspin shortened the DNA by approximately one third of that in control cells (41.4 vs. 14.0 or 14.7 kbp). Importantly, depletion of HERC2 can alleviate the slow replication fork progression in the Claspin-deficient cells (14.0 vs. 19.1 or 17.9 kbp). Interestingly, however, single HERC2 knockdown slightly shortened, rather than lengthened, the DNA lengths (41.4 vs. 35.6 or 36.5 kbp). The observed effect of HERC2 likely did not rely on its E3 activity for protein degradation, because proteasome inhibitor MG132, instead of HERC2 deletion, did not affect the nascent DNA length shortened by Claspin inhibition (Supplementary Fig. S3).

HERC2 enhances origin firing in the absence of Claspin and facilitates MCM2 phosphorylation in response to replication stress

Inhibition of Claspin leads to uncoupling of MCM helicase complex and polymerase complexes, resulting in enhanced single-strand DNA (SSD) during replication elongation (5). Therefore, the observed complement effect of HERC2 depletion on the slow replication fork progression in the Claspin-deficient cells could be explained by a direct role of HERC2 on the fork stabilization. However, HERC2 depletion in the Claspin-deficient cells did not show an obvious complementary effect on the foci formation of a SSD-binding protein RPA70 (Supplementary Fig. S4), suggesting that HERC2 is not directly involved in the fork stabilization.

Published OnlineFirst July 20, 2011; DOI:10.1158/0008-5472.CAN-11-0385

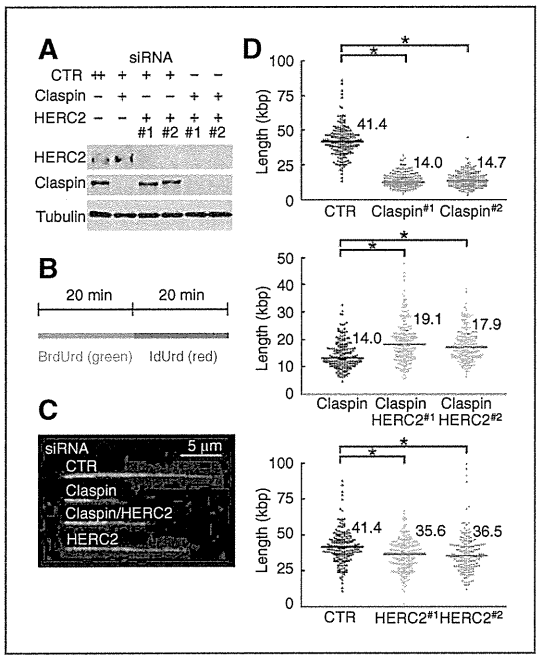

Figure 3. HERC2 suppresses DNA strand elongation in the absence of Claspin. A and B, HCT116 cells transfected with the indicated siRNA were subjected to immunoblot (A) or sequentially treated with BrdUrd and IdUrd for 20 minutes each to label the nascent DNAs (B). C, representative images of labeled DNA fibers from cells with indicated siRNA measured with confocal microscopy. D, distributions of replication fork length during the entire labeling period in cells with the indicated siRNA are shown with the mean percentages (bars). Significance was analyzed by Student *t* test. *, $P < 0.0001$.

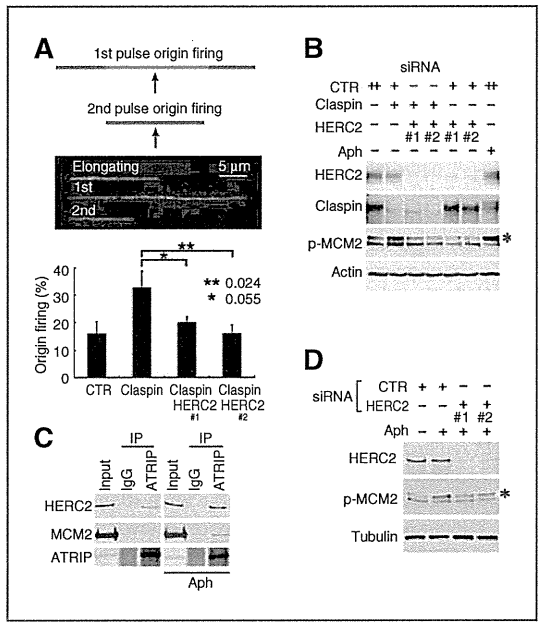

Figure 4. HERC2 enhances origin firing and MCM2 phosphorylation in replication stress. A, the nascent DNAs were labeled as in Fig. 3. Representative images of the labeled DNA fibers of each origin firing or elongation pattern from control cells are shown. Arrows indicate the position of replication initiation. Bottom, quantification of origin firing in cells with the indicated siRNA. Summation of number of first label origins (red-green-red), second label origins (red only), and interspersed patterns (repeated red-green-red) are shown as percentage of all labeled tracks. Data represent the mean of triplicate experimental samples and error bars indicate SD. *, $P = 0.055$; **, $P = 0.024$ by Student *t* test. B, HCT116 cells were transfected with the indicated siRNA and whole-cell lysates were IB with indicated antibodies. Aphidicolin (Aph, 4 μmol/L) was added for 3 hours as a positive control for MCM2 phosphorylation. C, Hela cells untreated or treated with 4 μmol/L aphidicolin for 3 hours were IP in the presence of endonuclease, and IB with indicated antibodies. D, HCT116 cells were transfected with the indicated siRNA, untreated or treated with 1 μmol/L aphidicolin for 3 hours, and whole-cell lysates were IB with indicated antibodies. *, phosphorylated MCM2.

We next analyzed the effect of HERC2 on origin firing. Origin firing is regulated by 2 contradictory mechanisms. Claspin and Chk1 inhibits excess origin firing while promoting elongation (17, 19). In contrast, ATR-mediated phosphorylation of MCM2 recruits Plk1, which upregulates origin firing as a compensatory mechanism for survival of replication blocks (5, 20). Supporting this mechanism, Claspin-deficient cells showed enhanced origin firing (Fig. 4A). Significantly, additional depletion of HERC2 resuppressed the origin firing to the level of that in control cells. In addition, depletion of Claspin enhanced the MCM2 phosphorylation and additional inhibition of HERC2 dramatically suppressed the MCM2 phosphorylation in the Claspin-deficient cells (Fig. 4B). To test the effect of HERC2 on replication stress in physiologic condition, we treated the cells with aphidicolin and ATRIP was immunoprecipitated. In addition to MCM2, HERC2 was coprecipitated from endonuclease-treated cell lysates (Fig. 4C; Supplementary Fig. S5). Importantly, the aphidicolin treatment increased the amount of HERC2 in the ATRIP immunocomplex (Fig. 4C). In addition, HERC2 depletion suppressed the MCM2 phosphorylation in the aphidicolin-treated cells (Fig. 4D), in similar fashion to that in the Claspin-deficient cells (Fig. 4B).

In summary, our results show that HERC2, an E3 ligase critical for DNA damage repair pathways, also regulates DNA replication progression and origin firing by facilitating MCM2 phosphrylation (Supplementary Fig. S6). Because HERC2 targets BRCA1 for degradation, it is possible that HERC2, Claspin, and BRCA1 cooperate on activation of Chk1 and Plk1. HERC2 in maintenance of DNA stability warrants further study into its potential roles in cancer development and therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Anindya Dutta for helpful discussions and critical reading of the manuscript, and Shinko Hanaki for secretarial assistance. This study was supported by grants from the Ministry of Education, Science, Sports, Culture, and Technology of Japan.

Received February 8, 2011; revised June 17, 2011; accepted June 29, 2011; published OnlineFirst July 20, 2011.

Published OnlineFirst July 20, 2011; DOI:10.1158/0008-5472.CAN-11-0385

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Int J Clin Oncol
DOI 10.1007/s10147-011-0301-8

ORIGINAL ARTICLE

Leptomeningeal carcinomatosis associated with gastric cancer

Hideharu Tomita · Hirofumi Yasui · Narikazu Boku · Yoko Nakasu · Koichi Mitsuya · Yusuke Onozawa · Akira Fukutomi · Kentaro Yamazaki · Nozomu Machida · Keisei Taku · Akiko Todaka · Hiroya Taniguchi · Takahiro Tsushima

Received: 15 February 2011 / Accepted: 20 July 2011

Abstract
Background Leptomeningeal carcinomatosis (LMC) is a rare but devastating complication of gastric cancer.
Methods The subjects were 12 gastric cancer patients who were diagnosed as having LMC at the Shizuoka Cancer Center between October 2002 and March 2009. We conducted a retrospective survey of the medical records of the study subjects and collected data on the clinical features, treatment modalities employed/outcomes, and survival of the patients.
Results Of the 12 patients, 9 (75%) were male, and the median age was 63 years. Histopathologically, the majority of the patients (83%) had diffuse-type adenocarcinoma. At the time of diagnosis of the LMC, the other major sites of metastasis were the peritoneum (75%) and lymph nodes (50%). The median duration from the diagnosis of gastric cancer to the diagnosis of LMC was 15.6 months. While the treatment strategy changed with time, intrathecal chemotherapy ($n = 10$), followed by whole brain irradiation ($n = 7$) and subsequent ventriculo-peritoneal shunt ($n = 3$) was performed in 10 of the patients. Improvement of neurological functions was observed in 6 of the 10 patients. The median overall survival time from the diagnosis of LMC in all the 12 patients was 60 days. One patient survived for a considerably long period of 532 days.
Conclusions Multidisciplinary treatment, including ventriculo-peritoneal shunt for LMC secondary to gastric cancer, may benefit selected patients, but further accumulation of clinical cases is necessary.

Keywords Gastric cancer · Leptomeningeal carcinomatosis · Intrathecal methotrexate therapy · Whole brain irradiation · Ventriculo-peritoneal shunt

Introduction

Leptomeningeal carcinomatosis (LMC) complicating solid tumors is most often seen in patients with breast and lung cancer and melanoma, and it is a rare complication in patients with gastric cancer. While LMC is reportedly diagnosed clinically in 2–4% of all cancer patients [1], the prevalence of LMC in gastric cancer patients is as low as 0.14–0.24% [2–4]. However, irrespective of the primary site of cancer, LMC is a devastating complication. There have only been a few reports of LMC complicating gastric cancer.

No standard therapy for LMC has yet been established. The poor general condition of patients with LMC, especially in the presence of consciousness disturbance associated with hydrocephalus, convulsions, etc., makes satisfactory treatment of LMC very difficult. On the other hand, the prognosis of the condition is extremely poor

H. Tomita (✉) · H. Yasui · Y. Onozawa · A. Fukutomi · K. Yamazaki · N. Machida · A. Todaka · H. Taniguchi · T. Tsushima
Division of Gastrointestinal Oncology, Shizuoka Cancer Center, 1007 Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan
e-mail: mbhtmt08.g88@auone.jp

N. Boku
Division of Oncology, St. Mariannna School of Medicine, Kanagawa, Japan

Y. Nakasu · K. Mitsuya
Division of Neurosurgery, Shizuoka Cancer Center, Shizuoka, Japan

K. Taku
Division of Medical Oncology, Shizuoka General Hospital, Shizuoka, Japan

Published online: 17 August 2011

without treatment. Recently, the efficacy of multidisciplinary treatment for LMC associated with breast and lung cancer, such as intrathecal chemotherapy (ITC) with methotrexate, cytarabine or liposomal cytarabine [5–7], whole-brain irradiation (WBI) [8] and ventriculo-peritoneal shunt (VP shunt) [9], has been reported. However, there are currently no published reports of case series of LMC complicating gastric cancer.

At the Shizuoka Cancer Center, the treatment for gastric LMC has changed with time. ITC alone was administered initially, followed subsequently by the addition initially of WBI, and then more recently of VP shunt performed by neurosurgeons, when indicated, to control the cerebrospinal fluid pressure.

We have encountered some cases of LMC complicating the clinical course in patients with gastric cancer. In the present retrospective study, we report on the clinical features of gastric LMC and also on the outcomes of treatment for LMC secondary to gastric cancer.

Subjects and methods

The subjects were 12 gastric cancer patients who were diagnosed as having LMC at the Shizuoka Cancer Center between October 2002 and March 2009, and were selected for this study on the basis of the following inclusion criteria: (1) histologically confirmed gastric cancer; (2) LMC confirmed by cerebrospinal fluid (CSF) cytology and/or by magnetic resonance imaging (MRI); (3) no history of other/concurrent malignancies. Patients with LMC caused by direct meningeal invasion from the skull base were excluded. We conducted a retrospective survey of the medical records of the subjects to collect data on the clinical features, treatment modalities employed/outcomes, and survival of the patients.

We administered intraventricular injections of methotrexate at 2 mg/body with prednisolone at 10 mg for 5 consecutive days in all the patients who met the following criteria: (1) age $\leq$75 years; (2) no bleeding tendency; (3) no rapid progression of the lesions other than LMC. Initially, for the first three patients, we undertook no additional therapy after the intraventricular injection of methotrexate. Subsequently, for the next 7 patients who had no previous history of WBI, we added WBI at a total dose of 30 Gy, administered in 10 fractions. For the last three patients, we also performed VP shunt after completion of the WBI. The indication for VP shunt was determined based on the following criteria: (1) improvement of clinical symptoms such as headache, vomiting, and consciousness disturbance after drainage of CSF by a subcutaneous reservoir or lumbar puncture; (2) Radiation Therapy Oncology Group Neurologic Functional Classification (RTOG-NFC) of $\leq$3 (Table 1) [10]; (3) low or moderate cell counts and protein level in the CSF, associated with a reduced risk of shunt obstruction.

Table 1 Radiation Therapy Oncology Group neurologic function classification

RTOG neurologic function classification	Description
1	Able to work or perform normal activities; neurologic findings minor or absent
2	Able to carry out normal activity with minimal difficulties; neurologic impairment does not require nursing care or hospitalization
3	Seriously limited in performing normal activities; requiring nursing care or hospitalization; patients confined to bed or wheelchair or have significant intellectual impairment
4	Unable to perform even minimal normal activities; requiring hospitalization and constant nursing care feeding; Patients unable to communicate or in coma

Statistical analysis

The clinical course from the diagnosis of gastric cancer was counted from the date of the initial endoscopy confirming gastric cancer or, in the two cases for whom the date of the initial endoscopy was not available, the date of surgical resection. Overall survival was calculated from the date of diagnosis of the LMC by CSF cytology or MRI to the date of death. The median overall survival was calculated by the Kaplan–Meier method, using StatView software, version 5.0.0 (SAS Institute, Cary, NC, USA).

Results

Patients' characteristics

Between October 2002 and March 2009, 14 gastric cancer patients were diagnosed as having LMC. Two of these patients with direct meningeal invasion from a skull base metastasis were excluded. The remaining 12 patients were enrolled as the subjects of this retrospective study.

The characteristics of the subjects are shown in Table 2. Of the 12 patients, 9 (75%) were male, and the median age was 63 years old (range 30–73 years). All patients had neurological symptoms caused by the LMC, and nine patients (75%) had a poor RTOG-NFC of 3 or 4 (Table 1) [10]. All but one patient had diffuse type adenocarcinoma or small cell carcinoma. At the time of diagnosis of the

LMC, other metastatic disease was also observed in 9 patients, including peritoneal dissemination ($n = 8$), lymph node metastasis ($n = 6$), brain metastasis ($n = 2$), bone metastasis ($n = 2$), and liver metastasis ($n = 1$); the remaining three patients showed no evidence of metastasis other than LMC.

Of the 12 patients, 9 had received chemotherapy for gastric cancer prior to the diagnosis of the LMC. At the onset of LMC, the efficacy of the previous chemotherapy was rated as partial response or stable in 7 (78%) patients, progressive disease in one patient, and unevaluated in one patient. Of the remaining 3 patients who had no history of previous chemotherapy, one developed LMC immediately after the gastric cancer diagnosis, and in the other two patients LMC was detected simultaneously with other recurrence(s) after curative surgery. The median duration from the diagnosis of gastric cancer to the diagnosis of LMC was 15.6 months (range 1.0–91.1 months).

Table 2 Patients' characteristics

Characteristics	
Categories	Number of patients
Sex	
Male/female	9/3
Age, years, median (range)	63 (30–73)
RTOG-NFC	
2/3/4	2/7/3
Primary tumor	
Yes/no	5/7
Histological type	
Intestinal/diffuse/small cell	1/10/1
Metastatis sites	
Peritoneum/lymph nodes/brain/bone/liver/lung	8/6/2/2/1/1
Number of metastatic sites (except for LMC)	
0/1/2/3/4	3/2/2/4/1
Prior chemotherapy	
Yes/no	9/3
Number of prior chemotherapy regimen	
1/2/3/4	2/2/4/1
Response to prior chemotherapy at LMC diagnosis	
Response or stable/progression/not evaluated	7/1/1

Clinical symptoms and LMC diagnosis

The most frequent symptom of LMC was headache, and various clinical neurological signs were noted, including consciousness disturbance, cataplexy, vomiting, convulsion, and cerebellar ataxia.

The initial LMC diagnosis was made by gadolinium-enhanced MRI in 8 patients and by CSF cytology in the remaining 4 patients. Finally, leptomeningeal enhancement (Fig. 1) was detected in 10 patients, and the CSF cytology was class IV or V in 9 of the 10 patients in whom the examination was performed.

Treatment

Among the 12 patients, best supportive care alone was selected for treatment in 2 patients, including one with a past history of WBI for brain metastasis and another who was comatose and developed disseminated intravascular coagulation immediately after being diagnosed with LMC.

The therapeutic modalities applied for the remaining 10 patients were as follows: ITC alone in 3 patients, ITC plus WBI in 4 patients, and ITC plus WBI plus VP shunt in 3 patients. For the intrathecal administration of methotrexate, which was undertaken in all the 10 patients, a subcutaneous (Ommaya) reservoir was implanted in 8 of the patients.

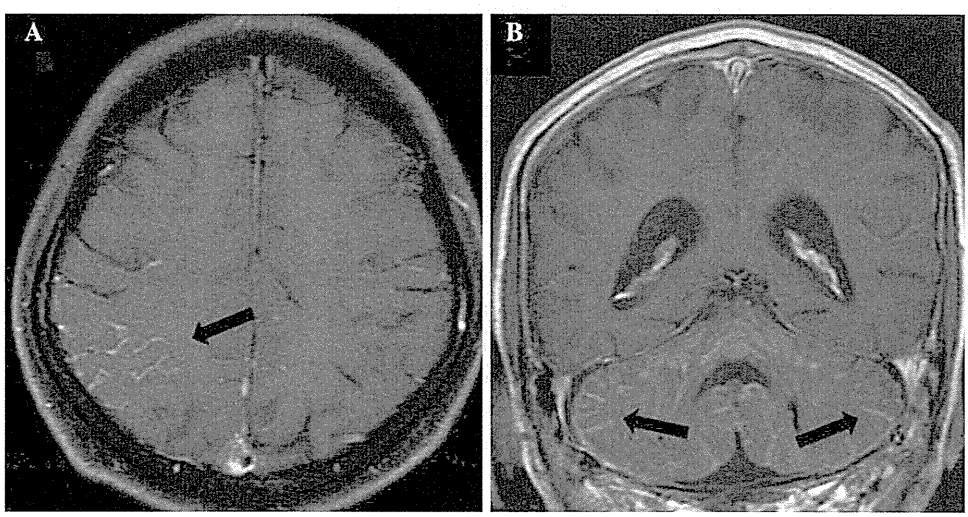

Fig. 1 Magnetic resonance imaging (MRI) scan finding in LMC. MRI scan of the brain shows peripheral contrast enhancement of the cerebellar (*arrows* in **a**) and cerebral (*arrows* in b) sulci

Table 3 Toxicity profiles ($n = 10$)

Toxicity	Grade (NCI-CTC, version 3.0; %)					
	Intrathecal chemotherapy alone ($n = 3$)			Intrathecal chemotherapy + WBI ($n = 7$)		
	3	4	% grade 3 or lower	3	4	% grade 3 or lower
Luekopenia	1	0	33	1	1	29
Neutropenia	0	1	33	0	1	14
Hemoglobin	1	0	33	3	0	43
Thrombocytopenia	2	0	67	0	1	14
AST	0	0	10	1	0	14
ALT	1	0	33	2	0	29
Febrile neutropenia	0	0	0	0	1	14
Headache	0	0	0	1[a]	0	14
Nausea	0	0	0	1[a]	0	14
Appetite loss	0	0	0	1[a]	0	14

Five patients (50%) died within 30 days of the last administration of intrathecal methotrexate

NCI-CTC National Cancer Institute common toxicity criteria

[a] All the grade 3 or lower headache, nausea, and appetite loss were observed at the beginning of the treatment

Table 4 Leptomeningeal carcinomatosis with gastric cancer: treatment and outcome

Case	Age (years)	Sex	RTOG-NFC at diagnosis of LMC	Treatment of LMC	RTOG-NFC after treatment[a]	Transition to home care	Survival after LMC diagnosis (days)
1	73	M	3	IT MTX	4	No	14
2	63	M	3	IT MTX	2	Yes	92
3	41	F	3	IT MTX	2	Yes	60
4	70	M	3	IT MTX,WBI	4	No	13
5	30	F	3	IT MTX,WBI	2	Yes	89
6	63	M	3	IT MTX,WBI	3	No	61
7	59	M	3	IT MTX,WBI	2	Yes	47
8	59	M	2	IT MTX,WBI, VP shunt	1	Yes	532
9	66	F	4	IT MTX,WBI, VP shunt	3	No	114
10	59	M	2	IT MTX,WBI, VP shunt	2	Yes	104

[a] At the time intrathecal methotrexate was finished (intrathecal methotrexate alone cases) or WBI was finished

IT MTX intrathecal methotrexate

Toxicities

The worst grades of toxicity in each patient during the intrathecal methotrexate therapy and WBI are summarized in Table 3. Grade 3 or 4 neutropenia was observed in two patients and grade 3 or 4 leukopenia in three patients. All of the grade 3 adverse events, namely headache, nausea and appetite loss, were observed at the beginning of the treatment. The main non-hematological adverse events that appeared anew after the beginning of the treatment were headache (2 cases), nausea (2 cases), vomiting (2 cases), and general fatigue (1 cases). All of these adverse events were grade 1 in severity, except for headache, which was grade 2 in severity. Early death within 30 days of the last administration of intrathecal methotrexate occurred in five patients, of whom one patient who developed grade 4 neutropenia, leukopenia and febrile neutropenia died on the day following the last intrathecal methotrexate administration. The major complications caused by VP shunt were not observed.

Efficacy

The treatment and clinical outcomes of the 10 patients who received treatment are summarized in Table 4. RTOG-NFC improvement was obtained in 6 (60%) patients, 5 of whom and another patient could leave the hospital temporarily (Table 4). All the patients had died by the time of this analysis. One patient was considered to have died a treatment-related death, 1 died from progression of peritoneal dissemination, and all of the remaining 10 patients died from the progression of the LMC. The median overall survival time from the diagnosis of LMC was 60 days in the 12 patients (Fig. 2), but 90 days in those who had received any treatment for LMC. The RTOG-NFC class did not become worse with VP shunt in any of the 3

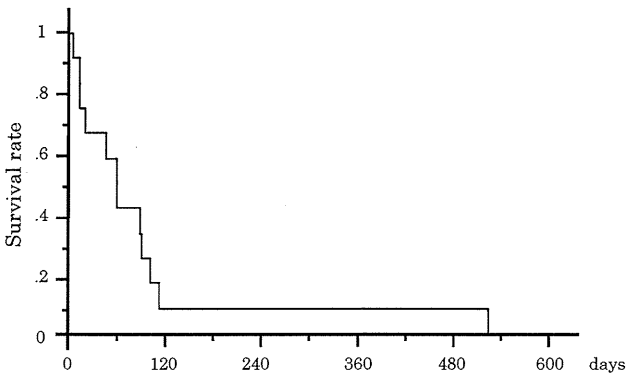

Fig. 2 Overall survival from the diagnosis of LMC

patients in whom VP shunt was performed, and 1 patient in whom ITC, WBI, and VP shunt were administered survived for a considerably long period of 532 days.

Discussion

Although cancer cells may seed the leptomeninges in patients with any type of solid tumors, the highest incidence of LMC has been reported in breast and lung cancer patients (12–34 and 10–26%, respectively); the incidence of LMC complicating gastrointestinal tract cancer is comparatively low (4–14%) [11]. Recently, the advances in systemic chemotherapy brought about by the development of new agents, including molecular-targeted agents, has contributed to a longer survival of cancer patients than that in the twentieth century. The median survival time of advanced gastric cancer patients treated by systemic chemotherapy has exceeded one year. In this retrospective study, the median interval between the initial diagnosis of gastric cancer and development of LMC was 15.6 months. Thus, it is anticipated that the incidence of LMC complicating gastric cancer will increase along with the prolonged survival brought by the advances in systemic chemotherapy.

In this study, the histological type of the tumor in 10 of the 12 patients (83%) was poorly differentiated adenocarcinoma or signet-ring cell carcinoma. This result is consistent with that suggested by previous reports. The majority of gastric cancer patients with LMC have poorly differentiated or signet-ring cell cancer [3, 4, 12]. In the diffuse type of gastric cancer, peritoneal dissemination and lymph node metastasis appear to be the major metastatic sites. Thus, the most frequent sites of concurrent metastasis associated with LMC in patients with gastric cancer were lymph node metastasis and peritoneal dissemination, as corroborated by both this study and previous reports [13].

Once LMC develops, irrespective of the primary cancer site, depressed neurological functions and neurological deficits cause reduction in the activities of daily living and extreme deterioration of the quality of life, and the prognosis is very poor. Because systemic chemotherapy is not effective against LMC, intraventricular chemotherapy and/or radiation have been most commonly employed for its treatment. Although no novel administration method of intrathecal methotrexate has been established, use of a low dose and daily intraventricular administration of methotrexate have been reported to be associated with reduced neurotoxicity [14]. Notwithstanding this treatment, the overall median survival of LMC patients was as low as 0.7–5.8 months [15–17]. In the largest case series of 90 patients with LMC complicating various kinds of cancers at the Memorial Sloan-Kettering Cancer Center who received focal irradiation and intraventricular methotrexate from 1975 to 1980, the overall median survival was reported to be 5.8 months [17]. However, breast cancer (46 patients) was the most commonly documented primary tumor in that study, and no gastric cancer patients were included in the report. As for the breast cancer patients in the case series, 28 patients (61%) showed symptomatic improvement or stabilization, and their overall median survival was 7.2 months. Consistent with the prognosis of LMC complicating gastric cancer being much worse, with an overall median survival ranging from 4 to 6–7 weeks [3, 4], the median overall survival of the 12 patients was about nine weeks (60 days) in our present study. Thus, establishment of an effective treatment strategy for LMC complicating gastric cancer is warranted.

Omuro et al. [9] reported that VP shunt resulted in improvement of the symptoms of intracranial hypertension in 27 (77%) of the 37 patients with LMC. In our study, the treatment strategy changed with the passage of time and accumulation of experience, and VP shunt was applied to the last three patients, one of whom survived for a considerably long period of 532 days. Retrospectively, among the five patients who were actually treated with the two modalities of intraventricular methotrexate plus WBI, two patients would have fulfilled the eligibility criteria for VP shunt; the survival times of these two patients were as short as 61 and 47 days. This study had its limitations, e.g., the small sample size of the study population and the retrospective study design; nonetheless, the results suggest that VP shunt may have potential clinical benefit for selected gastric cancer patients with complicating LMC. Ideally, the survival benefit of VP shunt should be evaluated in future clinical trials, although most patients with LMC are generally poor candidates for clinical trials.

The goals of treatment of LMC include not only prolongation of survival, but also improvement of the neurological symptoms. ITC and/or WBI have been tried in the palliative setting for selected patients. Lee et al. [3] reported that incomplete resolution of neurological symptoms was observed in only one among the 10 patients who

received ITC, and five patients showed no significant changes in clinical symptoms. Once LMC is diagnosed in gastric cancer patients, immediate hospitalization is indicated, because of the severe neurological symptoms. In this study, improvement of RTOG-NFC was seen in 2 of 3 patients by ITC and 4 of 7 by ITC followed by WBI. It is considered a very significant result that 6 of the 10 patients who received multidisciplinary treatment could temporarily leave the hospital.

In conclusion, this study suggests that ITC might be effective for obtaining improvement of neurological functions, and further accumulation of clinical experience is necessary to evaluate the efficacy of multidisciplinary treatment, including WBI and VP shunt.

Conflict of interest No author has any conflict of interest.

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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].