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).

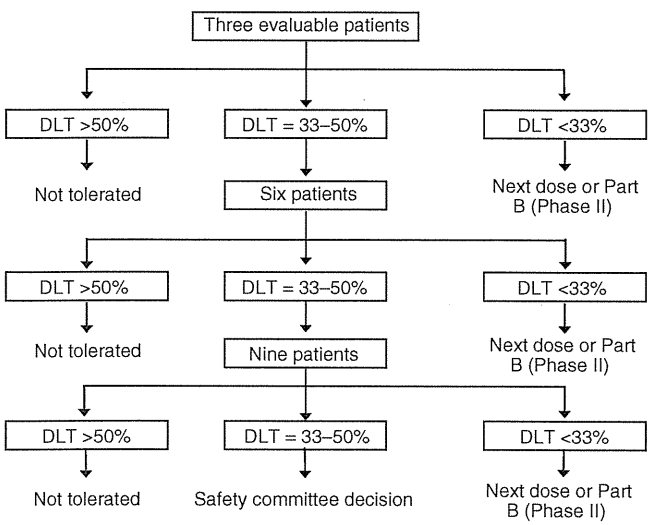

Fig. 1 Study design

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

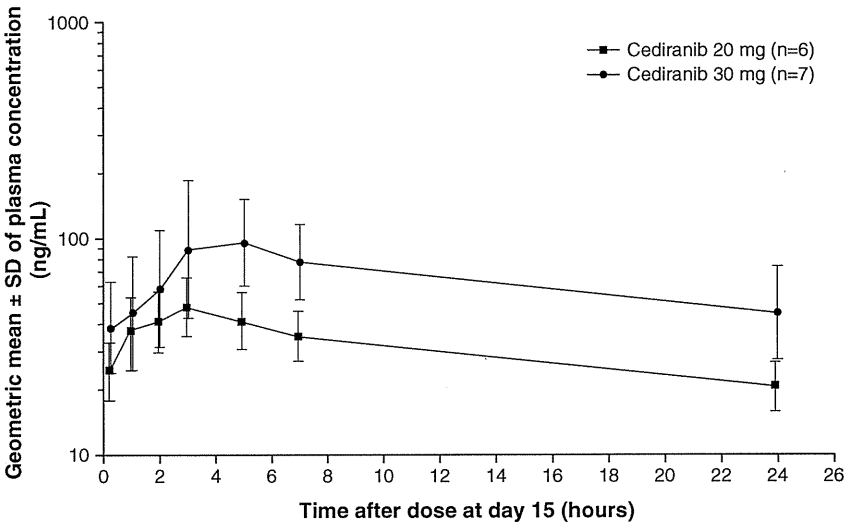

Fig. 2 Geometric mean plasma concentration of cediranib in combination with mFOLFOX6, log scale

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.

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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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Priority Report

Cancer Research

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

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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

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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′-GGAAAUAC-CUGGAGGAUGA-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

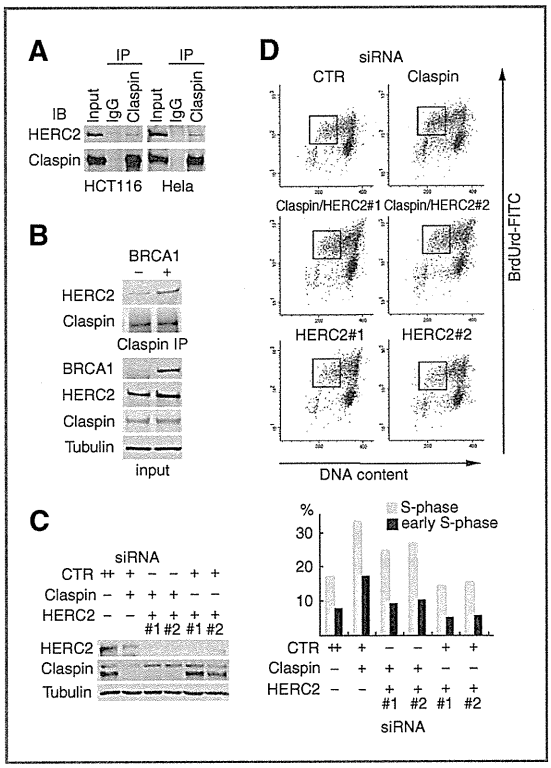

Figure 1. HERC2 interacts with Claspin and affects S-phase progression. A and B, lysates prepared from HeLa, HCT116 (A), and BRCA1-negative and positive UWB 1.289 (B) cells were immunoprecipitated (IP) and immunoblotted (IB) with indicated antibodies. Inputs (1.5%) were also loaded. C, HCT116 cells transfected 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.

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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.

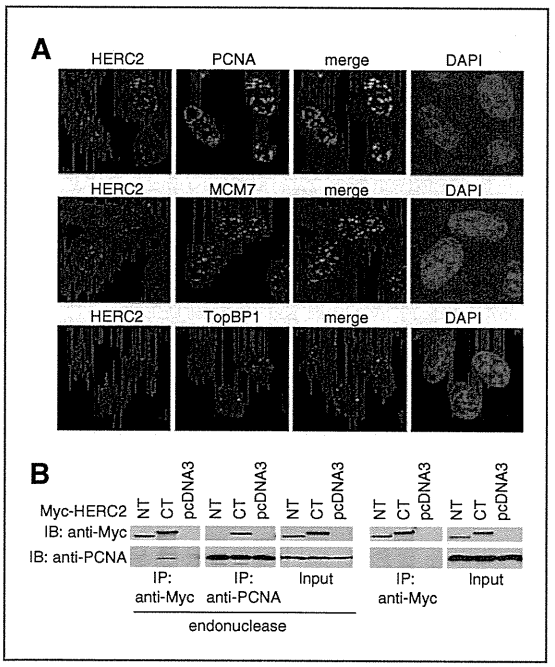

Figure 2. HERC2 localizes at DNA replication foci. A, HeLa cells were immunostained with anti-HERC2 antibody in combination with indicated antibodies. The nucleus was counterstained with DAPI. B, HEK-293T cells were transfected with Myc-HERC2-NT or CT fragment, or parental pcDNA3 vector, and lysed with 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.

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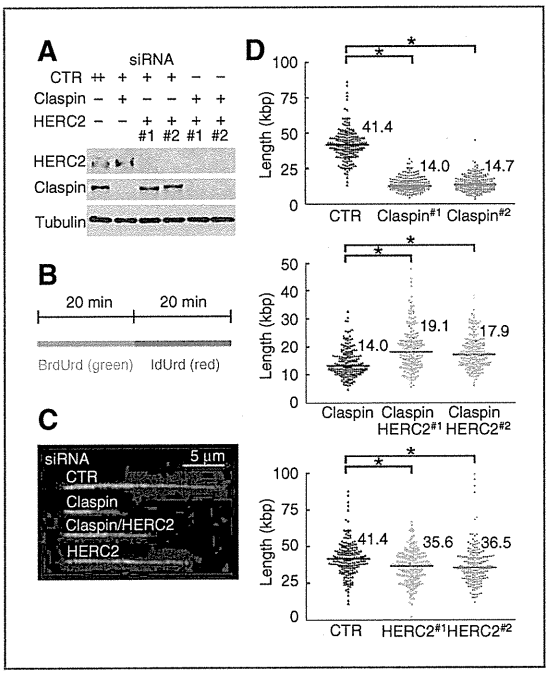

Figure 3. HERC2 suppresses DNA strand elongation in the absence of Claspin. A and B, HCT116 cells transfected with the indicated siRNA were subjected to immunoblot (A) or sequentially treated with BrdUrd and IdUrd for 20 minutes each to label the nascent DNAs (B). C, representative images of labeled 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. *, $P < 0.0001$.

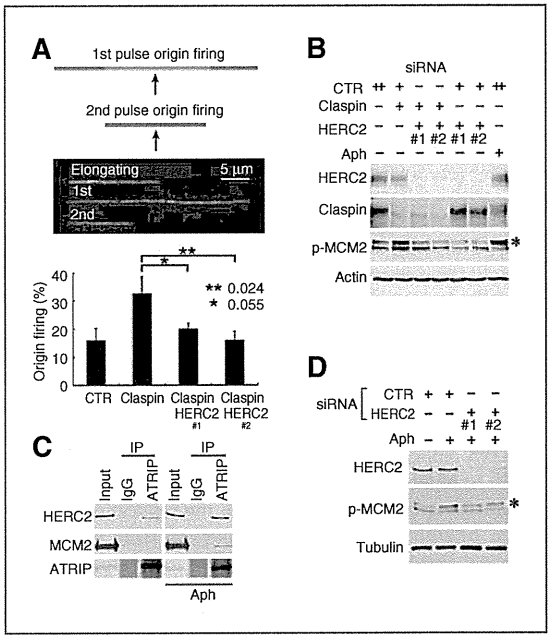

Figure 4. HERC2 enhances origin firing and MCM2 phosphorylation in replication stress. A, the nascent DNAs were labeled as in Fig. 3. Representative images of the labeled DNA fibers of each origin firing or elongation pattern from control cells are shown. Arrows indicate the position of replication initiation. 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.

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ORIGINAL ARTICLE

Comparison of safety and efficacy of S-1 monotherapy and S-1 plus cisplatin therapy in elderly patients with advanced gastric cancer

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Abstract
Background Although S-1 plus cisplatin (SP) therapy is recognized as the standard treatment for advanced gastric cancer (AGC) in Japan, its safety and efficacy in elderly patients have not been investigated sufficiently.
Methods We retrospectively reviewed the data of 58 patients with AGC selected from 82 consecutive patients who were ≥70 years old and were treated with SP or S-1 monotherapy as the first-line therapy. In SP, S-1 (40 mg/m^2, bid) was administered for 3 weeks and cisplatin (60 mg/m^2) on day 8, every 5 weeks. In S-1 monotherapy, S-1 (40 mg/m^2, bid) was administered for 4 weeks, every 6 weeks.
Results SP and S-1 was administered in 21 and 37 patients, respectively. There were some differences in patient characteristics between the treatment groups, such as histological type ($P = 0.16$); the presence of liver metastasis ($P = 0.07$); and the presence of peritoneal metastasis ($P = 0.02$). The incidences of grade 3/4 hematological toxicities were 57% (12/21) in the SP and 35% (13/37) in the S-1 group ($P = 0.17$). Those of non-hematological toxicities were 14% (3/21) and 14% (5/37) for anorexia, 10% (2/21) and 14% (5/37) for fatigue, and 5% (1/21) and 5% (2/37) for nausea in the SP and S-1 groups, respectively. Median progression-free survival and median overall survival in the SP and S-1 groups were 5.0 and 5.2 months, and 14.4 and 10.9 months, respectively.
Conclusion SP and S-1 therapy were both feasible in elderly patients, though there is the risk of a high incidence of hematological toxicities.

Keywords S-1 · Cisplatin · Elderly · Feasibility · Efficacy

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Introduction

With more than 800,000 new cases per year reported globally, gastric cancer is the second most common cause of cancer death [1, 2]. Systemic chemotherapy prolongs survival and improves quality of life in patients with advanced gastric cancer (AGC), compared to the best supportive care provided alone [3–5]. In Japan, the combination chemotherapy of S-1 plus cisplatin (SP) is recognized as a standard treatment for AGC from the results of pivotal phase III studies [6–8].

The population of elderly patients is increasing rapidly all over the world, and age is the most significant risk factor for the survival of various kinds of cancer patients [9]. However, it is uncertain whether evidence on the safety and efficacy of treatments from clinical trials is also

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applicable to patients who are 70 years or older, because the proportion of elderly patients included in most clinical trials is small: patients over 70 years old accounted for less than 25% in the Japan Clinical Oncology Group (JCOG) 9912 trial [6] and only 17% in the S-1 plus cisplatin versus S-1 alone for first-line treatment of AGC (SPIRITS) trial, which compared SP therapy to S-1 monotherapy alone [7]. The subset analysis of the SPIRITS trial showed that the hazard ratio for overall survival in elderly patients between 70 and 74 years old was 0.95, while that in the whole study population was 0.77. Therefore, a different treatment strategy might be necessary for elderly cancer patients.

In the present single-institution retrospective study, we assessed the safety and efficacy of SP therapy and S-1 monotherapy in elderly patients with AGC.

Materials and methods

Patients

The subjects of this study were patients with unresectable or recurrent gastric cancer who received SP therapy or S-1 monotherapy at the Shizuoka Cancer Center between September 2002 and March 2008. The patient selection criteria were as follows: age 70 years or older; Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–2; histologically proven adenocarcinoma; absence of history of prior chemotherapy; adequate oral intake; adequate bone marrow, renal, and hepatic functions (absolute neutrophil count of 1,500/μl or more, platelet count 10,000/μl or more, serum creatinine of 1.5 mg/dl or less, serum transaminase levels less than 100 IU/l or less than 200 IU/l if hepatic metastasis existed); presence of at least one non-curative factor other than positive peritoneal washing cytology; and absence of concomitant malignancy. A measurable lesion was not mandatory.

Treatment dose and schedule

In SP therapy, S-1 was administered orally at a dose of 40 mg/m^2 bid on day 1 through day 21 followed by 14 days of rest, with cisplatin 60 mg/m^2 being administered intravenously on day 8. This regimen was repeated every 35 days until detection of disease progression, appearance of unacceptable toxicities, or the patient's refusal to continue treatment. In S-1 monotherapy, S-1 40 mg/m^2 bid was administered on day 1 through day 28, followed by 14 days of rest, until any of the above-mentioned events occurred. In each treatment group, the dose of S-1 was determined according to the body surface area (BSA), as follows: 40 mg bid for BSA less than 1.25 m^2; 50 mg bid for BSA 1.25–1.5 m^2; 60 mg bid for BSA over 1.5 m^2.

These treatments were administered according to standard clinical practice. All physicians generally adhered to the following treatment modification criteria. If a grade 3 or higher adverse event, grade 2 increase of creatinine, or grade 2 infection occurred, treatment was suspended during the cycle or the start of the subsequent cycle was delayed until recovery of non-hematological toxicities grade 1 or lower, the neutrophil count reached more than 1,500/μl, and the platelet count reached more than 7.5 $\times$ 10^4/l. The dose of S-1 and cisplatin was reduced if any of the following adverse drug reactions occurred during the previous cycle: grade 4 leukocytopenia, anemia, or thrombocytopenia; or grade 3 or higher non-hematological toxicities.

Efficacy and toxicity evaluation

We retrospectively obtained all the clinical data from the medical records. Physical examinations and laboratory tests were repeated at least once every 3 weeks. Data on adverse events were collected until 30 days from the last administration or initiation of the subsequent chemotherapy, whichever occurred earlier. We evaluated adverse events on the basis of the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Response evaluation was repeated at least once every 2 months. Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0.

Statistical analysis

Differences in the distribution of variables were evaluated using the Fisher exact test or Mann–Whitney *U* test, as appropriate. Patients who did not have a target lesion were excluded from the response analysis.

Overall survival (OS) was defined as the period from the date of the first administration of S-1 to the date of death from any cause or to the last date of confirmation that the patient was alive in the census. Progression-free survival (PFS) was defined as the period from the date of the first administration of S-1 to the earliest date of detection of tumor progression by imaging, or symptomatic deterioration clinically judged to be caused by disease progression, or the last date that the patient was confirmed to be alive without disease progression in the census. Survival curves were drawn by the Kaplan–Meier method.

The following variables were examined in the univariate analysis of OS and PFS: treatment, age, sex, PS, presence of complications, prior gastrectomy, creatinine clearance, histological type, presence of target lesions, number of metastatic sites, presence of liver metastasis, peritoneal metastasis, and lymph node metastasis. Multivariate analysis included potentially predictive variables for the risk of

disease progression or death in univariate analysis. $P < 0.05$ was considered significant.

All statistical analyses were performed using Dr. SPSS II (SPSS Japan Inc., Japan). Written informed consent was obtained from all the patients before starting the chemotherapy.

Results

Patient characteristics

A total of 82 consecutive patients with gastric cancer who were 70 years or older received SP therapy or S-1 monotherapy between September 2002 and March 2008 at the Shizuoka Cancer Center. Among them, 24 patients were excluded for the following reasons: absence of non-curative factor other than positive peritoneal washing cytology (10 patients), organ dysfunction (7 patients), absence of histological confirmation of adenocarcinoma (6 patients), and concomitant malignancy (1 patient). Therefore, 58 patients were included as subjects in this study; of them, 21 patients were treated with SP therapy and 37 were treated with S-1 monotherapy.

Patient characteristics are shown in Table 1. There were some differences in background between subjects in the SP and S-1 groups, such as histologically determined intestinal type (48 vs. 62%, respectively; $P = 0.16$); the presence of liver metastasis (57 vs. 32%, respectively; $P = 0.07$); and the presence of peritoneal metastasis (14 vs. 43%, respectively; $P = 0.02$).

Table 1 Patient backgrounds

	SP group	S-1 group	*P* value
Number of patients	21	37	
Age (years), median (range)	73 (70–82)	73 (70–80)	0.51
Age ≤75	17 (81%)	25 (68%)	0.27
Age >75	4 (19%)	12 (32%)	
Sex			0.97
Male	16 (76%)	28 (76%)	
Female	5 (24%)	9 (24%)	
ECOG performance status			0.78
0	7 (33%)	14 (38%)	
1	13 (62%)	21 (57%)	
2	1 (5%)	2 (5%)	
Complications			0.28
+	10 (48%)	23 (62%)	
−	11 (52%)	14 (38%)	
Prior gastrectomy			0.82
+	9 (43%)	17 (46%)	
−	12 (57%)	20 (54%)	
Creatinine clearance			0.60
Median (range) (ml/min)	63.2 (40–125.8)	63.9 (35.9–98.7)	
Histological type			0.16
Intestinal	10 (48%)	23 (62%)	
Diffuse	11 (52%)	14 (38%)	
Tumor status			0.22
Metastatic	14 (67%)	30 (81%)	
Recurrent	7 (33%)	7 (19%)	
Metastatic sites			
Liver	12 (57%)	12 (32%)	0.07
Peritoneum	3 (14%)	16 (43%)	0.02
Lymph node	14 (66%)	21 (57%)	0.46
Target lesions			0.65
+	18 (86%)	30 (81%)	
−	3 (14%)	7 (19%)	
Number of metastatic sites			0.64
0	1 (5%)	0 (0%)	
1	7 (33%)	13 (35%)	
2	11 (52%)	19 (51%)	
≥3	2 (10%)	5 (14%)	

Exposure to treatment

The median number of treatment cycles for SP was 3 (range 1–8) and for S-1 was 4 (range 1–18). Treatment modification was required in 11 SP patients (52%) and in 21 S-1 patients (57%) as follows: dose reduction in 3 patients (14%) and in 14 patients (38%), and delay of the subsequent cycle in 9 patients (43%) and in 14 patients (38%), respectively. Both dose reduction and subsequent cycle delay were required in 1 SP patient and in 7 S-1 patients. The median relative dose intensity per patient of S-1 and cisplatin was 80% (range 42–96%) and 82% (range 55–100%), respectively, in the SP group, and that of S-1 was 86% (range 54–100%) in the S-1 group. The main reason for treatment failure was disease progression in both treatment arms: 76% in SP and 92% in S-1 groups. In addition, 19% of patients in the SP group stopped treatment because of adverse events.

Adverse events

The adverse events are shown in Table 2. The incidences of grade 3 or higher hematological toxicities were greater in the SP group (12/21: 57%) than in the S-1 group (13/37: 35%), although the difference was not statistically significant ($P = 0.10$). Incidences of specific hematological toxicities for the SP and S-1 groups were 33% (7/21) and 5% (2/37) for neutropenia, 43% (9/21) and 32% (12/37) for anemia, and 19% (4/21) and 0% (0/37) for thrombocytopenia, respectively. The incidence of grade 3 or higher

non-hematological toxicities was similar in both treatment groups: 14% (3/21) and 14% (5/37) for anorexia, 10% (2/21) and 14% (5/37) for fatigue, and 5% (1/21) and 5% (2/37) for nausea in the SP and S-1 groups, respectively. The median creatinine clearance calculated by the Cockcroft–Gault equation was 53.4 and 56.0 ml/min, respectively, in the 10 patients of SP and 14 of S-1 who experienced grade 3 or 4 toxicity (excluding that of anemia). The median creatinine clearance was 64.1 and 66.8 ml/min in patients who did not experience grade 3 or 4 toxicity in the SP and S-1 groups, respectively.

One patient from each treatment group died within 30 days of the last administration of chemotherapy. One was a 74-year-old man from the SP group, who started S-1 at the standard dose after palliative total gastrectomy. After administration of cisplatin on day 8, he received hydration therapy from day 11 to 14 for the treatment of anorexia (grade 2) and diarrhea (grade 1). After recovering from these symptoms, he was discharged from the hospital on day 15. On day 18, he suffered from diarrhea again, and was admitted to another hospital. Despite intensive care, he died on day 27 because of arrythmia. In this case, the possibility of treatment-related death could not be excluded, because dehydration due to severe diarrhea might have caused the arrythmia. The other patient from the S-1 group was a 74-year-old man who presented after gastrojejunostomy for obstruction due to the primary tumor. He received the standard dose of S-1, and visited our hospital on days 15 and 29 in the first cycle without any serious adverse events. However, he was found dead at home on day 38. He had no specific concomitant disease except mild hypertension. The cause of death was diagnosed as acute heart failure, and it is possible that S-1 contributed to his death.

Table 3 Response in patients with target lesions

	SP group ($n = 18$)	S-1 group ($n = 30$)
Best overall response		
CR	1	2
PR	8	12
SD	3	8
PD	6	7
NE	0	1
Response proportion (%)	50.0	46.7

CR complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluated

Response and survival

Eighteen patients in the SP group and 30 in the S-1 group had measurable lesions. The objective response rate was 9/18 (50.0%) in the SP group and 14/30 (46.7%) in the S-1 group. Among the responders, complete response was obtained in one patient in the SP group and 2 in the S-1 group (Table 3).

Twenty-one patients in the SP group and 37 in the S-1 group were involved in the PFS and OS analysis. The curves of PFS and OS for the SP and S-1 groups almost overlapped (Figs. 1, 2). The median PFS was 5.0 months in the SP group and 5.2 months in the S-1 group [hazard ratio (HR): 1.18, 95% confidence interval (CI): 0.68–2.06]. The median survival time (MST) was 14.4 months in the SP group and 10.9 months in S-1 (HR: 0.99, 95% CI: 0.57–1.71). The proportion of patients who received subsequent chemotherapy was similar in the SP and S-1 groups: 62% (13/21) and 65% (24/37), respectively.

Table 2 Adverse events

	SP group ($n = 21$)				S-1 group ($n = 37$)			
	G1/2	G3	G4	≥G3 (%)	G1/2	G3	G4	≥G3 (%)
Hematological								
Leukocytopenia	8	5	1	29	16	1	0	3
Neutropenia	7	5	2	33	8	2	0	5
Anemia	12	5	4	43	24	12	0	32
Thrombocytopenia	12	3	1	19	8	0	0	0
Non-hematological								
Febrile neutropenia	–	0	0	0	–	0	0	0
Fatigue	10	1	1	10	16	5	0	14
Anorexia	16	2	1	14	18	5	0	14
Diarrhea	5	1	0	5	12	0	0	0
Stomatitis	4	0	0	0	13	1	0	3
Nausea	11	1	0	5	9	2	0	5
Vomiting	2	0	0	0	6	0	0	0

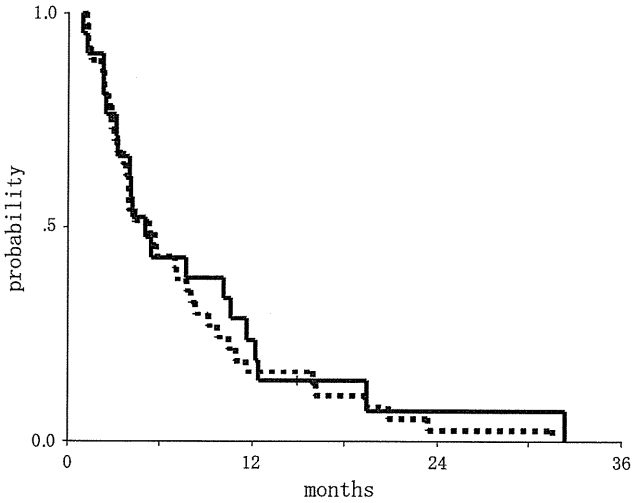

Fig. 1 Progression-free survival (PFS). The median PFS was 5.0 months in the SP group ($n = 21$, *solid line*) and 5.2 months in the S-1 group ($n = 37$, *dotted line*). The hazard ratio was 1.18, and the 95% confidence interval was 0.68–2.06

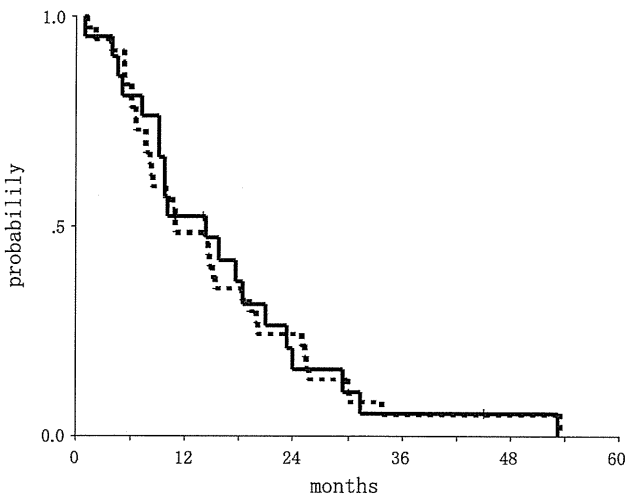

Fig. 2 Overall survival (OS). The median survival time was 14.4 months in the SP group ($n = 21$, *solid line*), and 10.9 months in the S-1 group ($n = 37$, *dotted line*). The hazard ratio was 0.99, and the 95% confidence interval was 0.57–1.71

Multivariate analysis showed that poor PS was the only factor associated with shorter OS (HR: 2.12, 95% CI: 1.37–3.26, $P = 0.001$) among the three potentially predictive variables selected by univariate analysis (age, PS, presence of peritoneal metastasis), while there was no predictive variable for PFS.

Discussion

Following the results of pivotal phase III trials, SP therapy is considered the standard chemotherapy in patients with unresectable or recurrent gastric cancer in Japan [6–8]. In the SPIRITS trial, the survival benefit of SP therapy over S-1 monotherapy was demonstrated with acceptable toxicity levels; however, the subset analysis showed that the hazard ratio in patients who were 70 years or older was 0.95 (95% CI: 0.71–1.27) [7]. However, because the subset analysis contained only 50 patients (17%) who were 70 years or older, there is uncertainty about the superiority of SP therapy over S-1 monotherapy in elderly patients with AGC. Thus, further investigation of SP therapy and S-1 monotherapy in elderly gastric cancer patients is necessary.

Because this study was retrospective, patient backgrounds between the two groups were not well balanced. In the S-1 group, the proportion of patients with peritoneal metastasis was significantly higher than in the SP group. Peritoneal metastasis is generally considered to be one of the unfavorable factors relating to shorter survival time in AGC; the patients included in the prognostic model had radiologically evident peritoneal metastasis or massive ascites [10]. In contrast, in the subset analysis of the JCOG 9912 trial, excluding the patients with severe peritoneal metastasis, patients without measurable lesions, mainly those with mild peritoneal metastasis, survived longer than those with measurable lesions [6]. Furthermore, in a randomized phase II study comparing S-1 and capecitabine, for which eligibility criteria included adequate oral intake, peritoneal metastasis was not a prognostic factor for OS [11]. Therefore, controversy exists over whether or not peritoneal metastasis is a prognostic factor in AGC. In our study, peritoneal metastasis was diagnosed in 10 out of 16 patients in the S-1 group and in 1 out of 3 patients in the SP group by laparotomy, not by radiological assessment. Because all patients had adequate oral intake, the peritoneal metastasis of most patients in this study was not so severe (only one patient had massive ascites), and therefore we consider that the presence or absence of peritoneal metastasis may not have had a major impact on survival. In the present study, the response rates in the SP and S-1 groups were 50.0 and 46.7%, respectively, and MST was 14.4 and 10.9 months, respectively. Though MST seemed longer in the SP group, the Kaplan–Meier curves of both treatment groups almost overlapped, and the hazard ratio was 0.98 (95% CI: 0.57–1.69). This hazard ratio of SP therapy over S-1 monotherapy was very similar to that of the subset analysis of elderly patients in the SPIRITS trial [7].

The relative dose intensity in the SP and S-1 groups was over 80% for each drug. Dose reduction was required in 14% of SP group subjects and 38% of S-1 group subjects. Though there was a higher incidence of grade 3 or 4 hematological toxicities in the SP group than the S-1 group, only one patient of SP needed dose reduction because of hematological toxicity. Most dose modifications were required because of non-hematological toxicities, the incidences of which were similar between the SP group and

Table 4 The incidence of grade 3 or 4 adverse events (%)

	Present study		SPIRITS trial	
	SP group ($n = 21$)	S-1 group ($n = 37$)	SP group ($n = 148$)	S-1 group ($n = 150$)
Hematological				
Leukocytopenia	28.6	2.7	11	2
Neutropenia	33.3	5.4	40	11
Anemia	42.9	32.4	26	4
Thrombocytopenia	19.0	0	5	0
Non-hematological				
Febrile neutropenia	0	0	3	1
Fatigue	9.5	13.5	4	1
Anorexia	14.3	13.5	30	6
Diarrhea	4.8	0	4	3
Stomatitis	0	2.7	0.7	0
Nausea	4.8	5.4	11	1
Vomiting	0	0	4	2

the S-1 group. Chemotherapy in both treatment groups was discontinued due to disease progression in many patients. It was evident that both treatments were feasible even in elderly patients.

The incidence of grade 3 or higher adverse events in the present study was more frequent than in the SPIRITS trial (Table 4), which could possibly be attributed to decreased creatinine clearance. In this study, patients with poor renal function experienced more severe adverse events. The pharmacokinetics of S-1 are dependent on renal function because 5-chloro-2,4-dihydroxypyridine, which is an inhibitor of dihydropyrimidine dehydrogenase [12–17], is eliminated through the kidneys. Organ functions, including renal function in the elderly, are likely to be somewhat impaired, and it has been reported that the glomerular filtration rate generally decreases with age [18]. The decreased creatinine clearance might lead to more frequent and severe toxicities associated with S-1, especially in elderly patients. Therefore, it is necessary to consider renal function before starting S-1-based chemotherapy, especially in elderly patients.

In geriatric oncology, neither the Karnofsky Performance Scale Index nor ECOG PS may be reliable for assessing physical status because comorbidities in elderly patients might affect their physical or mental status [19]. It has been reported that assessment of the condition of elderly cancer patients measured by comprehensive geriatric assessment (CGA) is useful for predicting tolerance to chemotherapy and survival [20–22]. CGA is a multidimensional evaluation scale of an elderly patient's physical performance, comorbidity, cognition, psychological stage, socioeconomic status, nutritional status, and medications [23, 24]. In some clinical trials targeting elderly cancer patients, functional assessment scales were adopted for patient selection in addition to PS and organ function assessments [25, 26]. In this study, PS was the only factor associated with survival. In addition, CGA might interfere with measurement of PS, as demonstrated in previous studies [20, 24, 27]. Thus it is suggested that CGA might also affect the clinical outcomes, especially the survival rates, of gastric cancer patients treated with chemotherapy.

In conclusion, SP therapy and S-1 monotherapy were both feasible in elderly patients with AGC, though the superiority of SP therapy over S-1 monotherapy was not so prominent in this review. Further clinical trials are warranted to establish a new standard care, especially in elderly gastric cancer patients.

Conflict of interest No author has any conflict of interest.

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ORIGINAL ARTICLE

Efficacy of trastuzumab in Japanese patients with HER2-positive advanced gastric or gastroesophageal junction cancer: a subgroup analysis of the Trastuzumab for Gastric Cancer (ToGA) study

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Abstract

Background The Trastuzumab for Gastric Cancer (ToGA) study is the first international trial to include Japanese patients with human epidermal growth factor 2 (HER2) positive advanced/metastatic gastric or gastroesophageal junction cancer. ToGA showed that trastuzumab plus chemotherapy (capecitabine/cisplatin or 5-fluorouracil/cisplatin) improved overall survival in the overall population (hazard ratio 0.74). Regional differences in outcome in favor of Japanese populations were observed in other studies; therefore, subgroup analyses of ToGA may contribute to the evaluation of the potential benefits of this regimen in Japanese patients.

Methods We performed subgroup analyses on 101 Japanese patients enrolled into ToGA (trastuzumab plus chemotherapy, $n = 51$; chemotherapy, $n = 50$).

Results Median overall survival in the Japanese subgroup was 15.9 months (95% confidence interval 12–25) for trastuzumab plus chemotherapy and 17.7 months (95% confidence interval 12–24) for chemotherapy (hazard ratio 1.00; 95% confidence interval 0.59–1.69). After adjusting

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