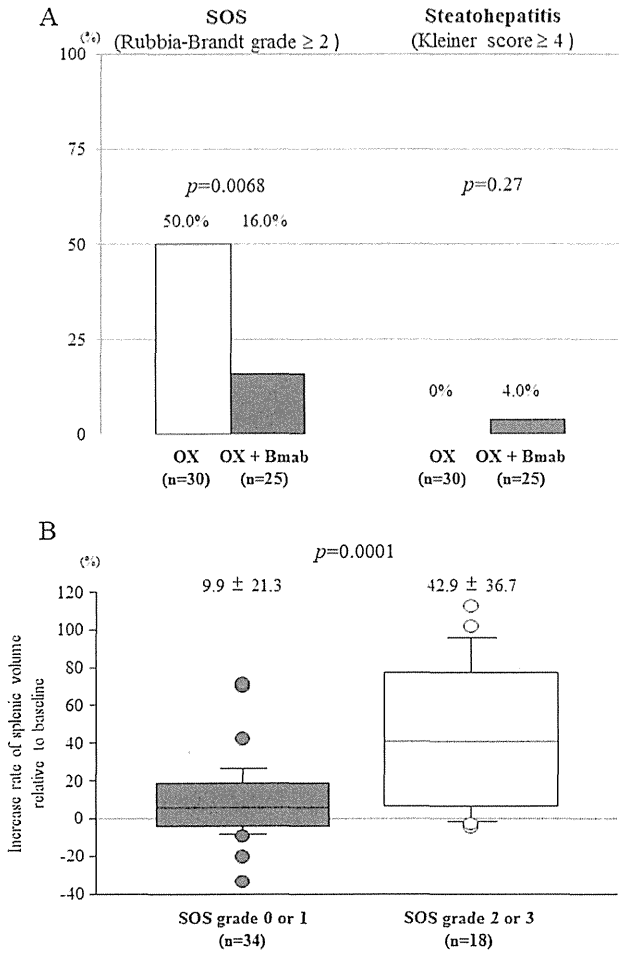

Figure 3. (A) The incidence of SOS (Rubbia-Brandt grade 2 or 3) and steatohepatitis (Kleiner score ≥ 4) after preoperative chemotherapy in the OX and OX + Bmab groups. (B) The increase in the splenic volume relative to baseline according to the presence or absence of SOS.

received irinotecan-based regimens, and a previous report identified irinotecan-induced steatohepatitis as an independent risk factor for the 90-day mortality after surgery for CRLM.[14] The SOS induced by OX-based chemotherapy in the non-tumorous specimens of patients undergoing hepatic resection was first reported by Rubbia-Brandt et al.,[13] and an association between OX and SOS has been confirmed in the other studies.[14,15] There is evidence to suggest that OX-based chemotherapy could be associated with postoperative morbidities.[15,23] In the phase III EORTC 40983 study, postoperative complications occurred more often in the patients who received perioperative OX-based chemotherapy (25%) than in the patients who had surgery alone (16%), specifically showing a doubling in the biliary fistula and hepatic failure rates.[23]

Several studies have identified OX-induced SOS as a risk factor for some adverse events after surgery for CRLM. For example, Nakano et al. reported that SOS was significantly associated with a higher morbidity rate and longer hospital stay in patients undergoing major hepatic resection, and that SOS resulted in an impaired liver functional reserve.[16] Soubrane et al. reported that there was a significant correlation between the occurrence of severe SOS lesions and postoperative morbidity, including a large amount of postoperative liver insufficiency.[24] They also described that both intraoperative bleeding and the transfusion rate were increased in cases of SOS after major hepatic resection, although the increase was not significant. Furthermore, another study demonstrated that SOS could lead to early recurrence and decreased survival in the long term.[17] As a result, OX-induced SOS may not only compromise the perioperative outcome, but also impair the long-term outcome. Being able to predict the severity of SOS in a non-invasive manner would lead to a reduction in perioperative adverse events.

Many previous studies have identified predictive factors for OX-induced SOS, such as the administration of six or more cycles of chemotherapy,[16] the preoperative AST value,[16] the preoperative indocyanine green retention rate at 15 min (ICG-R15),[16] a low platelet count,[25] a high AST to platelet ration index,[25] an enlarged splenic volume[12] and the findings of superparamagnetic iron oxide-enhanced T2-weighted gradient echo imaging.[26] In the current study, an increase in the splenic volume relative to baseline > 25% was the only independent predictor of the development of SOS, and other factors, such as the number of chemotherapy cycles, decreases in the platelet count and the interval between completion of chemotherapy and surgery were not significant in the multivariate analysis (Table 3).

Recently, several studies demonstrated that, when it was added to OX-based chemotherapy for CRLM, Bmab might have a protective effect against the development of SOS in the non-tumorous liver parenchyma.[19,20,25,27] Rivero et al. reported that Bmab reduced the incidence of SOS of any grade from 53.5% (5-FU/OX) to 27.4% (5-FU/OX + Bmab), and of moderate or severe SOS from 27.9% to 8.1%.[19] Klinger et al. also reported that the incidence of high grade (severe) SOS was markedly reduced from 23.9% in the non-Bmab group to 1.9% in the Bmab-treated group.[27] The current study also demonstrated that moderate or severe SOS (Grade 2 or 3) was reduced in patients who received OX + Bmab (16.0%) compared to those who received OX alone (50.0%) ($p = 0.0068$, Fig. 3(A)). There is also evidence that the addition of Bmab to standard chemotherapy, including OX, before hepatic resection for CRLM does not increase the postoperative morbidity.[25,28–30] The current study also showed that the addition of Bmab to OX-based chemotherapy before hepatectomy had no impact on the postoperative morbidity, requirement of a blood transfusion or postoperative hospital stay (Supplement Table 1). These findings suggest that Bmab should be added to OX-based chemotherapy prior to surgery for CRLM from the perspective of not only the anti-tumor effect, but also the

Table 3
The results of the univariate and multivariate analyses of the preoperative factors contributing to the development of SOS.

	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p* value	HR	95%CI	*p* value
Gender (male)	0.83	0.25–2.80	0.77			
Age >65[a]	1.72	0.56–5.29	0.34			
Site of primary tumor (rectum)	1.65	0.52–5.24	0.39			
Synchronous vs. metachronous	1.40	0.41–4.81	0.59			
Largest tumor size >36[a] mm	1.11	0.37–3.38	0.85			
Number of metastases >1	1.46	0.42–5.03	0.55			
Number of chemotherapy cycles >6[a]	2.52	0.80–7.95	0.11			
Decrease in the platelet count >7.3[a] ($\times 10^4$/mL)	2.69	0.86–8.49	0.091	1.62	0.36–7.26	0.53
Bmab use	0.19	0.05–0.69	0.012	0.65	0.12–3.52	0.62
Interval between completion of chemotherapy and surgery>34[a] days	1.72	0.56–5.29	0.34			
Splenic volume before chemotherapy >112[a] mL	0.90	0.29–2.84	0.86			
Splenic volume after chemotherapy >129[a] mL	1.58	0.50–5.00	0.43			
Increase in splenic volume relative to baseline >25%	20.7	4.44–96.2	0.0001	14.4	2.48–83.3	0.0029

Bmab, bevacizumab; 95% CI, 95% confidence interval.
[a] Median.

prevention of the development of SOS and decrease in the postoperative morbidity.

In the current study, the splenic volume increased significantly in the OX group, however, the addition of Bmab prevented the splenic enlargement (Fig. 2). In addition, the increase in the splenic volume was significantly higher in patients with SOS than in those without SOS (Fig. 3(B)). These results suggest that the improvement of splenic enlargement might be a useful indicator of the protective effect of Bmab against OX-induced SOS. Although it remains unclear whether some of the patients treated with Bmab still developed OX-induced SOS in the liver parenchyma, preoperative and non-invasive prediction of the protective effects of Bmab against OX-induced SOS will facilitate the appropriate management of patients with CRLM in terms of the selection of hepatic resection after chemotherapy.

There are some limitations to this study which consisted of two groups, the patients treated in the Kumamoto University, and the patients enrolled in KSCC 0802 study. First, the sample size was relatively small. Second, the patients enrolled in the KSCC 0802 study received only the mFOLFOX6 with Bmab regimen, and in principle, 5 cycles of mFOLFOX6 + Bmab will be administered. Therefore, some bias could be present between the groups. In fact, there was a significant difference in the total amount of chemotherapy cycles between the groups. In this study, postoperative morbidity did not differ between the groups, in spite of the high incidence of SOS in the OX group. This result disagreed with the previous reports,[15,16] and may be influenced by its small sample size and some bias.

In conclusion, the present study showed that the development of SOS induced by OX-based chemotherapy was reduced when Bmab was added, and the inhibition of splenic enlargement might be a useful indicator of the protective effect of Bmab against OX-induced SOS.

Conflict of interest disclosure

None of the authors has any conflict of interest.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ejso.2013.12.009.

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Liver Resectability of Advanced Liver-limited Col Liver Metastases Following mFOLFOX6 with Bevacizumab (KSCC0802 Study)

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Abstract. *Background/Aim: The Kyushu Study group of Clinical Cancer (KSCC) conducted phase II trials (KSCC0802 - UMIN000001308) concerning liver resectability after first-line treatment of advanced liver-limited colorectal metastases (CRLM) by a prospective, multi-center study. Patients and Methods: Patients received 6 cycles of mFOLFOX6 with bevacizumab followed by evaluating liver resectability. The primary end-point was liver resection rate. Results: The 40 patients enrolled from September 2008 to August 2010. The median number of administration cycles was 6 (range=1-7). The liver resectability cases were 16/40 (40.0 %) and the number of R0 cases was 10 patients (25.0%). An overall response rate was 30.0% (95% CI=15.2%-44.8%). Median progression-free and overall survival of all patients was 9.7 months and 33.0 months), respectively. Conclusion: mFOLFOX6 with bevacizumab regimen is safe and effective for advanced liver-limited CRLM and might lead to high liver resectability.*

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Key Words: Colorectal liver metastases, hepatic resection, chemotherapy, oxaliplatin, fluorouracil, leucovorin, bevacizumab.

Although the liver is most common metastatic organ from colorectal cancer, complete resection rate of colorectal liver metastases (CRLM) has been unsatisfactory due to the greater tumor number and size, complicated tumor location or the presence of extrahepatic metastases. According to the nation-wide registration database maintained by the Japanese Society for Cancer of the Colon and Rectum (JSCCR), the incidences of

0250-7005/2014 $2.00+.40

Abbreviations: CRLM, Colorectal liver metastases; JSCCR, Japanese Society for Cancer of the Colon and Rectum; 5-FU, 5-fluorouracil; FOLFOX, Folinic acid, 5-fluorouracil and oxaliplatin; EGFR, epidermal growth factor receptor; KSCC, Kyushu Study group of Clinical Cancer; university hospital medical information (UMIN); ECOG, Eastern Cooperative Oncology Group; PS, performance status; PFS, progression-free survival; OS, overall survival; RR, response rate; RECIST, Response Evaluation Criteria In Solid Tumors; CT, computed tomography; MRI, magnetic resonance imaging, CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; CTCAE, Common Terminology Criteria for Adverse Events; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable; MST, median survival time; CI, confidence interval.

CRLM were 10.7% of synchronous metastases and 7.1% of the first metachronous metastases (1). The goal of treatment for CRLM patients depends on the initial resectability of the metastases and, according to most known treatment guidelines and algorithms, patients may be categorized in accordance with whether they have 'upfront' resectable metastases, borderline resectable metastases or unresectable disease (2). When complete resection was performed successfully for patients with liver-limited CRLM, a 5-year survival of 40% to 50% could be achieved (3-6). However even for curatively resected patients, the recurrence was frequently observed without perioperative modern chemotherapy.

Folinic acid, 5-fluorouracil (5-FU) and oxaliplatin (FOLFOX) is one of the standard chemotherapy for the patients with advanced CRLM. The mFOLFOX6 regimen can lead to tumors being downstaged in some patients with initially unresectable CRLM and, in a previous study, has allowed hepatic resection in 16-38% of the patients (6). A phase III study of the European Organization for the Research and Treatment of Cancer (EORTC), intergroup Trial 40983, identified that perioperative chemotherapy with FOLFOX4 improved progression-free survival (PFS) after hepatic resection of initially resectable CRLM (7). In recent years, great anti-tumor effects have been demonstrated with targeted agents, such as bevacizumab (anti-vascular endothelial growth factor (VEGF) antibody) or anti-EGFR (anti-epidermal growth factor receptor) antibody. Additional use of bevacizumab on chemotherapy for CRLM can result to increased response rate, prolonged progression-free and overall survival and decreased blue liver (8,9). In Japan, there have been few data concerning liver resectability following mFOLFOX6 with bevacizumab as the first-line treatment of non-resectable liver-limited CRLM.

It is quite difficult to divide CRLM patients to be initially resectable or unresectable, especially in a multi-center study. Limitations of hepatic resection or resection skills are different by each center. Therefore, in the present study, we decided to select H-factor categories according to the issues by JSCCR; H1, four or less metastases with the largest diameter being 5 cm or less; H2, other than H1 or H3; H3, five or more metastases with the largest diameter being more than 5 cm (10). Patients with H2 and H3 have been reported to have significantly poor prognosis compared to H1 (3, 11). The aim of this prospective multicenter study was to evaluate the resectability and safety of mFOLFOX6 plus bevacizumab on H2 and H3 liver-limited CRLM.

Patients and Methods

H2 or H3 liver-limited CRLM patients were enrolled to a multicenter phase II trial of Kyushu Study group of Clinical Cancer (KSCC) 0802 study. The study was registered with a national review board; university hospital medical information (UMIN) 000001308. Institutional Review Board (IRB) approval was obtained from all institutions participating in this study. Written informed consent was obtained from all patients prior to enrollment.

Eligibility criteria. Patients with histologically proven colorectal cancer and at least one measurable lesion in the liver (with no non-hepatic distal metastasis/relapse) were eligible for this study if they met all of the following criteria: H2 or H3 CRLM (either synchronous or metachronous); age ≥20 and ≤ 75 years; no prior chemotherapy except adjuvant chemotherapy if ended ≥6 months before study entry; no prior radiotherapy for advanced/recurrent colorectal cancer; Eastern Cooperative Oncology Group performance status (PS) 0 to 1; life expectancy estimated ≥ 3 months; adequate bone marrow and renal function.

Study design. This is a single-arm study based on the Southwest Oncology Group (SWOG's) standard two-stage design. Assuming null and alternative liver resection rates as 20% and 40%, respectively, 35 patients would be required to achieve 80% power at binomial test with one-sided alpha <0.05. To allow for a 10% drop-out, the number of patients was set to 39. After examining the primary endpoint of the first 20 patients in the first stage, the study was planned to stop if less than 3 patients of liver had been resectable because of low expectation to achieve the alternative liver resection rate. After the second stage, the null liver resection rate was tested for all patients eligible for the analysis.

In principle, 5 cycles of preoperative chemotherapy (mFOLFOX6: oxaliplatin 85 mg/m^2, leucovorin 200 mg/m^2 d1 followed by 400 mg/m^2 bolus 5-FU and a 46-h 2,400 mg/m^2 5-FU infusion every 2 weeks+bevacizumab (5 mg/kg)) was administered within 2 weeks after enrollment. Patients who appeared to be amenable to curative resection after completion of mFOLFOX6+bevacizumab received 1 cycle of mFOLFOX6, in principle, and then underwent liver resection after additional reassessment of liver resectability. Liver resection should be performed between 6 and 9 weeks after the final use of bevacizumab and at least 2 weeks after the final use of mFOLFOX6.

End-points. The primary endpoint was the proportion of patients who underwent liver resection. Secondary endpoints were the percentage of H2 patients and percentage of H3 patients who underwent liver resection, 3-year PFS rate, 3-year overall survival (OS) rate, the objective response rate (RR) according to the

Response Evaluation Criteria In Solid Tumors (RECIST ver. 1.0) criteria (12), safety (adverse events, percentage of patients who completed preoperative chemotherapy, incidence of liver disorder, incidence of postoperative complication, postoperative duration of hospital stay), percentage of R0 resection and histological response. The operative indication was decided according to the policy of each institution.

Assessment of efficacy and adverse events of chemotherapy. During protocol treatment, computed tomography (CT) or magnetic resonance imaging (MRI) and tumor markers (CEA and CA19-9) were basically performed every month. A physical examination, blood counts and blood chemistry were performed at every cycle. Patients were assessed before starting and at each 2-week cycle according to the National Cancer Institute-Common Toxicity Criteria (CTCAE ver. 3) (13).

Liver resection. After 5 cycles of preoperative mFOLFOX6+ bevacizumab, surgical resection in curative intent must be performed as soon as possible after 1 cycle of additional mFOLFOX6. After recognition of enough function of the future liver remnant, hepatic resection was conducted. The operative procedure, as to whether anatomical or non-anatomical hepatic resection is performed, is not instructed and is decided by each surgeon. Treatment of the disappeared lesions is not provided and observation of such lesions is acceptable. Preoperative portal vein embolization was authorized to this study but concomitant use of intraoperative radiofrequency ablation or two-stage hepatectomy was prohibited.

Pathological effects. The pathological response was depended on the criteria of JSCCR (10) as follows: grade 0: with no necrosis or cellular or structural change; grade 1a: with necrosis or disappearance of tumor in <1/3 of the entire tumor; grade 1b: with necrosis or disappearance of the tumor in <2/3 of the entire tumor; grade 2: with necrosis or disappearance of the tumor in >2/3 of the entire tumor but with viable tumor cells remaining; and grade 3: with the entire tumor presenting necrosis and/or fibrosis and no viable tumor cells identified.

Follow-up. Subsequent therapy, unless decided by each clinician, after this experimental treatment is not stipulated regardless of hepatic resection. As a rule, we followed-up patients by screening of metastases or recurrence with CT or MRI and tumor markers at a 3-month 1-year and 3-year period. The survival and recurrence was estimated every 6 or 12 months.

Statistical considerations. One-sided test on the liver resection rate was conducted with the binomial test. The confidence interval (CI) for the proportion was estimated by the exact method. The duration of survival was measured from the day of entry into the study. PFS and OS were calculated by using the Kaplan-Meier method and were compared by using the log-rank test. All statistical analyses were performed with the Stata version 12 (Stata Corp., College Station, TX, USA). A two sided $p<0.05$ was considered as statistically significant, except at the test of the primary endpoint which adopted one-sided test.

Results

Patients' characteristics. From May 2008 to April 2010, a total of 40 patients with H2 or H3 liver-limited CRLM were enrolled in the study from 19 Institutions. No patients were declared ineligible. The patients' characteristics at study entry are shown in Table I. There were 30 patients for H2 and 10 for H3. Median size and number of CRLM were 52.5 and 5 mm, respectively. The median number of cycles of chemotherapy was 6 (range=1-7).

Table I. *Patients' characteristics at study entry.*

Parameter	No. of patients	%
Age (years)		
median (range)	63 (37-74)	
M/F		
Male	29	72.5
Female	11	27.5
Performance status (ECOG)		
0	38	95.0
1	2	5.0
Primary tumor sites		
Colon	25	62.5
Rectum	15	37.5
Unknown	0	0
Primary lesion		
Yes	35	87.5
No	5	12.5
lymph node		
NX	3	7.5
N0	8	20.0
N1	16	40.0
N2	12	30.0
N3	1	2.5
Histology of primary tumor		
Adenocarcinoma		
Well	13	32.5
Moderately	24	60.0
Poorly	1	2.5
Mucinous carcinoma	0	0
Others	1	2.5
Liver metastatic lesion		
tumor size (range)	52.5 (10-135)	
number of liver metastases (range)	5 (1-20)	
Extent of liver metastatic lesion		
H2	30	75.0
H3	10	25.0
Bilateral lobe/unilateral lobe		
Bilateral lobe	28	70.0
Unilateral lobe	12	30.0
Synchronous/metachronous		
Synchronous	33	85.5
Metachronous	7	17.5
Postoperative complication		
Yes	5	25.0
No	15	75.0
Adjuvant therapy		
Yes	4	20.0
No	16	80.0

ECOG, Eastern Cooperative Oncology Group; N and H staging were according to the Japanese Society for Cancer of the Colon and Rectum (JSCCR).

Table II. *Relative dose intensities.*

	n	Median	5%tile	95%tile	mean	SD	min	max
Oxaliplatin	40	90.1	51.8	100.0	82.8	19.7	0.0	100.0
Levofolinate	40	90.1	59.9	100.0	87.1	12.7	52.0	100.6
5-FU (bolus)	40	90.9	54.8	100.0	84.5	15.6	44.3	100.4
5-FU (continuous)	40	90.8	56.7	100.0	85.5	15.0	41.1	100.0
Bevacizumab	40	88.6	47.4	100.0	82.8	17.4	44.8	101.9

5-FU, 5-fluorouracil; SD, standard deviation.

Table III. *Hematologic toxicities (n=40).*

	All grade	%	Grade 3/4	%
White blood cell	25	62.5	4	10.0
Neutrophil	32	80.0	13	32.5
Hemoglobin	31	77.5	0	0
Platelet	13	32.5	0	0
Total bilirubin	7	17.5	0	0
AST	22	55.0	1	2.5
ALT	17	42.5	0	0
ALP	27	67.5	1	2.5
Creatinine	3	7.5	0	0

AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

Table IV. *Non-hematologic toxicities (n=40).*

	All grades	%	Grade3/4	%
Pyrexia	5	12.5	0	0
Febrile neutropenia	3	7.5	2	5.0
Fatigue/malaise	20	50.0	1	2.5
Diarrhea	8	20.0	1	2.5
Constipation	6	15.0	0	0
Nausea	12	30.0	0	0
Vomiting	5	12.5	0	0
Appetite loss	21	52.5	1	2.5
Alopecia	9	22.5	0	0
Hand and foot syndrome	7	17.5	0	0
Taste alteration (taste disorder)	4	10.0	0	0
Hypersensitivity	3	7.5	0	0
Nerve disorder (CTCAE v3.0)	16	40.0	0	0
Neurological symptom (DEB-NTC)	16	40.0	0	0
Hypertension	9	22.5	0	0
Thrombosis/thrombus/embolism	0	0	0	0
Proteinuria	2	5	0	0
Gastrointestinal hemorrhage	0	0	0	0
Bleeding	1	2.5	0	0
Gastrointestinal perforation	0	0	0	0

CTCAE, Common Terminology Criteria for Adverse Events; DEB-NTC, Neurotoxicity Criteria of Debiopharm.

Liver resection rate (primary end-point). One patient who did not undergo liver resection after laparotomy because no hepatic lesion was detected was excluded from the resected patients. The overall resection rate was 40.0% (16/40) and the percentage of H2 and H3 patients who underwent liver resection was 46.7% (14/30) and 20% (2/10), respectively.

Treatments administered and toxicities. The median relative dose intensities of oxaliplatin, levofolinate, 5-FU (bolus), 5-FU (continuous infusion) and bevacizumab were 90.1%, 90.1%, 90.9%, 90.8% and 95.3%, respectively (Table II). Two patients withdrew before the end of the study due to personal reasons (5%) and five received fewer than six cycles of therapy due to toxicity (12%). The combination of bevacizumab plus mFOLFOX6 was relatively well tolerated (Tables III and IV). The most commonly reported hematological toxicity was neutropenia. Grade 3 and 4 neutropenia were observed in 32.5% of patients, however, febrile neutropenia was rare (5.0%). Serious adverse events (Grade 3 and 4) that may be associated with bevacizumab were nil, including thromboembolic events, hypertension and gastrointestinal hemorrhage and perforation. There was no treatment-related death.

Tumor response. All 40 patients were evaluated for their tumor response. No complete response (CR) was observed. The overall objective RR was 30.0% with a 95% CI from 16.6% to 46.5%. Stable disease (SD) was achieved in 55.0% of patients. The tumor control rate (partial response (PR) + SD) was 85.0%. Only three patients (7.7%) experienced progressive disease (PD) during neoadjuvant therapy.

Progression-free and overall survival. The median potential follow-up time from commencement of treatment was 28.9 months (range=9.9-52.3 months). The starting point to calculate survival was the day of registration to this study. The median PFS of all patients (n=40) was 9.7 months (95% CI=6.2-11.8 months). The estimated 1-, 2- and 3-year PFS

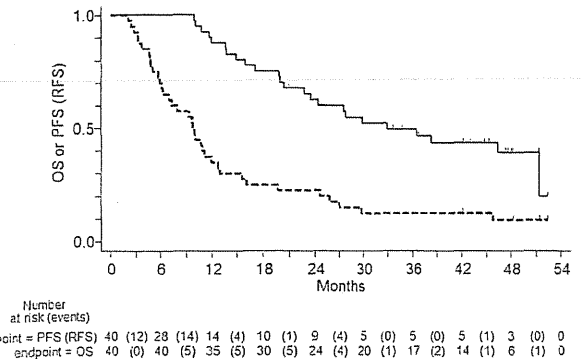

Figure 1. *Cumulative overall survival (OS) and progression-free survival (PFS) rates in all patients with colorectal liver metastases (CRLM). Solid line, OS; dotted line; PFS.*

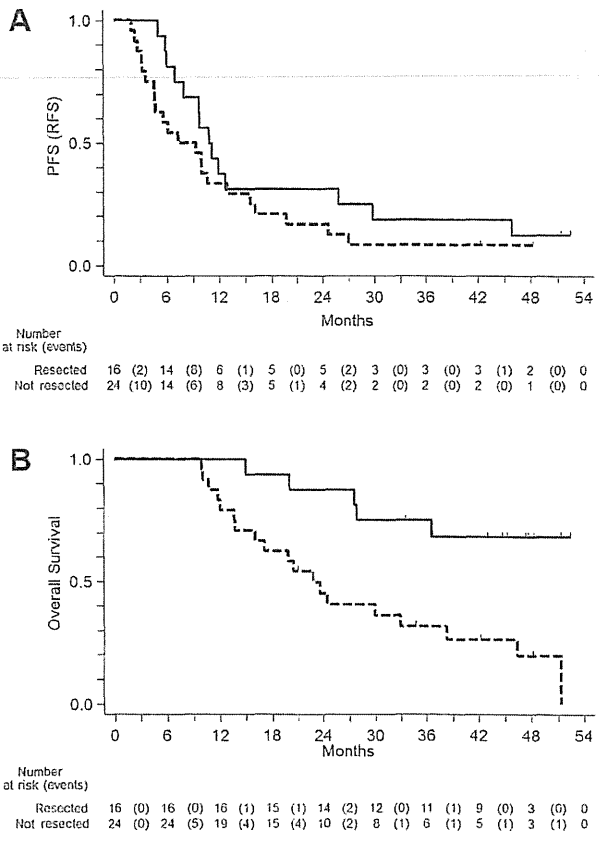

Figure 2. *Cumulative progression-free survival (PFS) (A) and overall survival (OS) (B) curve in patients with initially unresectable liver metastases according to the existence or nonexistence of hepatic resection. Solid line: patients with hepatic resection, dotted line: patients without hepatic resection.*

were 35.0% (95%CI=20.8%-49.6%), 22.5% (95%CI=11.2%-36.3%) and 12.5% (95%CI=4.6%-24.6%), respectively (Figure 1). The median survival time (MST) was 33.0 months (95%CI=22.8 months –not reached). The estimated 1-, 2- and 3-year OS were 87.5% (95%CI=72.5%-94.6%), 62.3% (95%CI=45.4%-75.3%) and 49.3% (95%CI=33.1%-63.7%), respectively (Figure 1).

The cumulative PFS (Figure 2A) and OS (Figure 2B) curve in patients with finally resectable (n=16) and unresectable (n=24) CRLM were investigated. The median PFS was 10.7 months (95%CI=6.9-25.8) and 7.2 months (95%CI=4.6-12.9) in finally resectable and unresectable CRLM, respectively, while the cumulative PFS was equivalent (p=0.21) in the two groups. In contrast MST was longer than 51 months (95%CI=27.8–not reached) and 22.8 months (95%CI=13.7-38.3) in finally resectable and unresectable patients, respectively, whereas the cumulative OS with resectable CRLM was significantly greater than that of CRLM without hepatic resection (p=0.002).

The cumulative PFS (Figure 3A) and OS (Figure 3B) curve in patients with H2 (n=30) and H3 (n=10) CRLM were studied. The median PFS was 10.6 months (95%CI=6.9-16.1) and 6.0 months (95%CI=2.4-9.9) in H2 and H3 patients, respectively, while the cumulative PFS was marginally better (p=0.055) in H2 patients compared to H3 patients. MST was 51.4 months (95%CI=24.4–not reached) and 20.4 months (95%CI=10.0-33.0) in H2 and H3 patients, respectively, whereas the cumulative OS with H2 patients was significantly greater than that of H3 (p=0.015).

Median PFS and OS in patients with responder (n=12) and non-responder (n=28) CRLM, by the RECIST criteria, were equivalent (both, p=0.11); 10.6 months (95%CI=6.2–not reached) and 7.2 (95%CI=4.8-11.0) in PFS compared to not reached (95%CI=19.8–not reached) and 27.5 months (95%CI=17.2-46.4), respectively.

Intra- and postoperative findings. Hepatic resection was performed solely for all 16 patients without synchronous resection of primary tumor. Hepatectomy included hemi-hepatectomy in 4, sectionectomy in 5 and segmentectomy or partial resection in 7. There were no intraoperative complications. Postoperative complications were observed in 5 patients (31.3%), namely biliary leakage in 2 patients (12.5%), intra-abdominal abscess in 1, wound infection in 1 and wound dehiscence in 1. No patient required further surgery. The median postoperative hospital stay was 18 days (range=9-66). Adjuvant chemotherapy after hepatic resection was performed in 7/16 (44%) of the patients (FOLFOX in 2 patients, FOLFOX+bevacizumab in 2, folinic acid, 5-FU and irinotecan (FOLFIRI)+bevacizumab, uracil and tegafur/leucovorin in 1, and S-1 in 1).

Pathological findings. Histological curability was consisted of R0: 10 (62.5%), R1: 2 (12.5%) and R2: 4 (25.0%). The

degree of pathological response of CRLM was classified as Grade 0 in 1, Grade 1a in 2, Grade 1b in 4, Grade 2 in 7 and no Grade3. Pathological examination of background liver showed sinusoidal obstruction (Rubbia-Brandt Grade≥2) (14) in 2 patients (12.5%) and steatosis in 3 (18.8%).

Discussion

The primary end-point of this clinical study was the percentage of CRLM patients who underwent liver resection. To confirm conversion, the rate of initially unresectable CRLM after induction chemotherapy is a completely fascinating issue. However, in a multi-center study it is difficult to analyze the resectability rate because the resection criteria of each Institute differ. Therefore, we decided to enroll H2 or H3 CRLM patients who had poor surgical outcome compared to H1 (3, 11, 15). It is known that patients in the synchronous group demonstrate significantly lower resection rates compared to the metachronous group (5, 16). In the current study, 33 of the 40 patients (82.5%) had synchronous CRLM. Nevertheless, the resection rates were 46.7% for H2 patients and 20% for H3. A similar multi-center study of preoperative chemotherapy using capecitabine, oxaliplatin (CAPOX) plus bevacizumab for patients with poor-risk liver-only CRLM has been initially introduced (17). Of the 30 initially technically unresectable patients in that study, 12 (40%) were considered potentially suitable for resection. Recently, we have reported that conversion rates in a cohort of initially or marginally unresectable 137 CRLM patients were 54.7% for H2 patients and 18.2% for H3 (18).

According to a consensus statement (19) for downsizing of CRLM, while FOLFOX and FOLFIRI represent two chemotherapy backbones of equivalent efficacy, there is a possibility that the three-drug regimen 5-FU, leucovorin, oxaliplatin and irinotecan (FOLFOXIRI) may provide a higher likelihood of a response. Recently FOLFOXIRI plus bevacizumab was introduced in the European Cancer Congress 2013 and updated efficacy/safety findings from a randomized, phase II study in patients with initially unresectable CRLM (OLIVIA study) was demonstrated (20). A total of 80 patients were divided to FOLFOXIRI+ bevacizumab (n=41) and mFOLFOX6+bevacizumab (n=39) group. In the FOLFOXIRI+bevacizumab group, increased resection rate (61% *vs.* 49%) and significantly greater R0 rate (48.8% *vs.* 23.1%, p=0.017) was observed. FOLFOXIRI+bevacizumab may be an extremely promising regimen for unresectable CRLM. To calculate the true conversion rate of unresectable CRLM in the current study, we have performed a central review to assess the resectability by checking paired baseline and post-chemotherapy scans being blinded to patient clinical information like CELIM study (21).

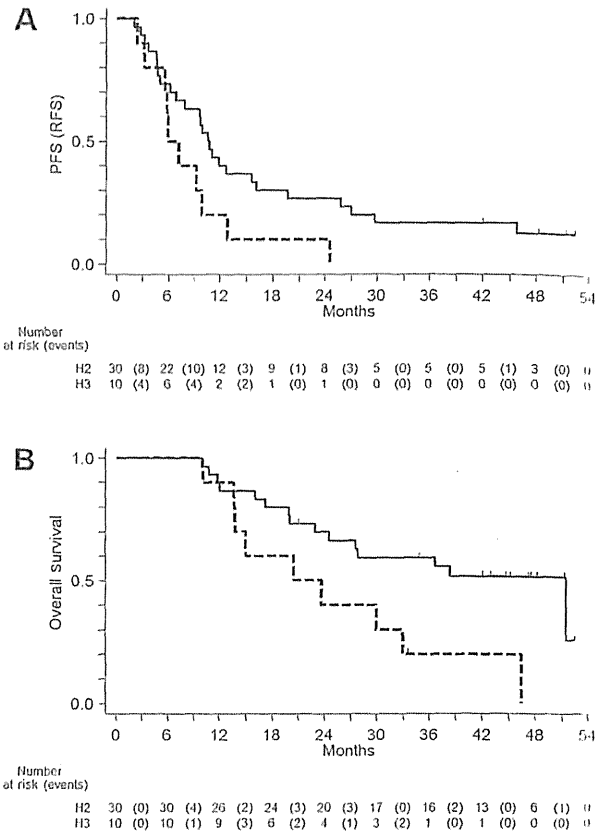

Figure 3. *Cumulative progression-free survival (PFS) (A) and overall survival (OS) (B) curve in patients with H2 and H3 colorectal liver metastases (CRLM). Solid line, H2 CRLM (n=30); dotted line; H3 CRLM (n=10).*

In our study, 40 CRLM patients were safely treated with the preoperative setting of mFOLFOX6 and bevacizumab. The relative doses of the drug administered were satisfactory. Serious adverse events (Grade 3 and 4) were not observed; limited side effects associated with bevacizumab. Maximal 6 courses of FOLFOX and 5 courses of bevacizumab might make this study safer. A total of 6 cycles of preoperative chemotherapy significantly increased the postoperative complication rates (16% to 25%) (7). The incidence rates of biliary fistula or intra-abdominal infection were higher in the patients treated with FOLFOX followed by hepatic resection. In our study, the complication rates were 31.3%, including 2 biliary leakage and 2 infectious complications, however, they were immediately recovered. Median postoperative hospital stay was 18 days enough to the early restart of adjuvant chemotherapy.

In this study, a high disease control rate of 85.0% was obtained, however, the overall objective RR was 30.0%. Bevacizumab can protect against sinusoidal obstruction

syndrome but does not increase the response rate in neoadjuvant XELOX/FOLFOX therapy of CRLM (22, 23). Median PFS and OS in patients with responder and non-responder CRLM were equivalent in our study. In the EORTC 40983 study (7) and in our study PD with RECIST's criteria was observed in 12/182 (7%) and 3/40 (7.5%), respectively. An important finding was the appearance of new intra- and extra-hepatic lesions; it is likely that these new lesions would have occurred immediately after hepatic resection, a presence that might be considered a diagnostic advantage before an unnecessary operation.

Pathological responses to neoadjuvant chemotherapy in CRLM have been shown to correlate with improved survival (24). Bevacizumab in combination with oxaliplatin/fluoropyrimidines was associated with the highest major pathological response rate (25). Extensive pre-operative chemotherapy does not improve the pathological response but can increase the risk for post-operative liver insufficiency (26). In this study, the rate of patients with Grade1b and Grade 2 was 68.8%. Non-invasive CT morphological criteria had also recently been developed that correlated the CT appearances of CRLM after bevacizumab treatment with pathological response and OS (27).

In the present study, pathological sinusoidal obstruction (Rubbia-Brandt Grade≥2) in the background liver was demonstrated only in 2 patients (12.5%) and steatosis in 3 (18.8%). Concomitant use of bevacizumab and oxaliplatin can decrease sinusoidal obstruction (23, 28). We have reported that splenic volume enlargement might be a useful indicator for the protective effect of bevacizumab against oxaliplatin-induced sinusoidal obstruction (28). The presence of sinusoidal obstruction has been shown to impair hepatic regeneration in a rat model (29). Furthermore, sinusoidal obstruction was reported to result in poor recurrent-free and overall survival (30). According to these viewpoints, combination use of bevacizumab and FOLFOX appears reasonable to use before hepatic resection.

In conclusion, mFOLFOX6 with bevacizumab is safe and effective and therefore, is recommended as a preoperative chemotherapy regimen for patients with advanced liver-limited CRLM undergoing potentially curative liver resection.

Acknowledgements

We thank the patients who participated in this study and their families. We are indebted to the physicians, all other co-medical staff and Independent Data Monitoring Committee (Dr. Toshiro Kuroiwa, Dr. Yoichi Nakanishi, Dr. Shuji Nakano and Dr. Yasuyo Okada) who contributed to this study. We also thank Ms. Taniguchi, Ms. Sakamoto and the other stuff at the Clinical Research Support Center Kyushu (CReS Kyushu) for their excellent collection and management of data, secretarial assistance and any other support.

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Received July 3, 2014
Revised August 4, 2014
Accepted August 6, 2014

Invest New Drugs
DOI 10.1007/s10637-013-0057-2

PHASE II STUDIES

A phase II trial of a selective c-Met inhibitor tivantinib (ARQ 197) monotherapy as a second- or third-line therapy in the patients with metastatic gastric cancer

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Received: 5 September 2013 / Accepted: 1 December 2013

Summary *Background* Tivantinib is a selective, non-ATP competitive, small-molecule inhibitor of c-Met and is under development in several cancers including non-small cell lung and hepatocellular carcinoma. Activation of c-Met has been frequently found in metastatic gastric cancer (MGC) and is associated with poor prognosis. In this single-arm study, we evaluated the efficacy of tivantinib monotherapy in Asian patients with previously treated MGC. This is the first clinical report from the trials evaluating the efficacy of a selective c-Met inhibitor for MGC. *Patients and methods* Eligibility criteria included: MGC with at least one measurable lesion; 1 or 2 prior chemotherapy regimens; and ECOG PS 0 or 1. Tivantinib was daily administered orally. The primary endpoint was the disease control rate (DCR). Pre-treatment tumor tissue was collected to evaluate the biomarkers related to efficacy. *Results* Thirty patients, including 12 patients with prior gastrectomy, received tivantinib; median age 62.5 years; ECOG PS 0/1 (8/22); 1/2 prior regimen (16/14). No objective response was observed, and DCR was 36.7 %. Median progression-free survival was 43 days (95 % CI: 29.0-92.0). Grade 3 or 4 adverse events occurred in 13 patients (43.3 %), in whom neutropenia (N=4) and anemia (N=4) were recognized as drug-related. c-Met gene amplification was observed in 2 patients (6.9 %). No obvious relationship was identified between efficacy and biomarkers including gene amplification of c-Met, expression of c-Met, p-Met and HGF. *Conclusion* Tivantinib as a monotherapy showed a modest efficacy in previously treated MGC, and further studies taking account of predictive biomarkers and/or combination with other chemotherapy may be needed in MGC.

Keywords c-Met inhibitor · Tivantinib · ARQ 197 · Gastric cancer · Phase II study

Electronic supplementary material The online version of this article (doi:10.1007/s10637-013-0057-2) contains supplementary material, which is available to authorized users.

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Published online: 15 December 2013

Introduction

Gastric cancer is the fourth most common malignancy and the second leading cause of cancer death in the world where 988,000 of new cases and 736,000 deaths are estimated to have occurred annually, with one half of the patients found in Eastern Asia in 2008 [1]. In the first-line setting of metastatic gastric cancer (MGC), combination chemotherapy based on

fluoropyrimidine (fluorouracil or its derivatives) and platinum is currently regarded as the standard treatment. Recently, as the first molecular-targeted therapy for MGC, an anti-HER2 antibody trastuzumab demonstrated a survival benefit in HER2 positive patients, but HER2 positive patients are limited to around 20 % of MGC [2]. In the second-line setting, although irinotecan and taxanes (paclitaxel, docetaxel) are currently used for the palliative chemotherapy, clinical benefit of these agents had not been provided by a large clinical trial at the time of designing this trial [3]. Therefore, a standard second-line or third-line chemotherapy is needed for MGC.

c-Met and its ligand, hepatocyte growth factor (HGF), play important roles in oncogenesis. Aberrant activation of the HGF/c-Met signaling pathway may lead to increased tumor cell proliferation, resistance to apoptosis, invasive growth, and tumor angiogenesis [4]. Gene amplification of c-Met, overexpression of c-Met, or elevation of serum HGF are known to correlate with poor prognosis in MGC [5–9]. In view of the critical role of the HGF/c-Met signaling pathway in gastric cancer progression, several biologics and low-molecular-weight compounds, which interfere with HGF/c-Met axis, are currently under clinical investigation. However, the clinical impact of HGF/c-Met axis inhibition is still unknown [10]. In addition, relevant biomarkers have not been defined to select a MGC patient population potentially responding to HGF/c-Met inhibitors.

Tivantinib (also known as ARQ 197) is a low-molecular-weight compound, and is the first in class, orally available selective inhibitor of c-Met [11]. Tivantinib disrupts c-Met phosphorylation in a non-ATP competitive manner, distinguishing it from other c-Met inhibitors in clinical trials [12]. Growth inhibition in c-Met expressing gastric cancer cell lines was induced by exposure to tivantinib in vitro and in vivo [11]. Tivantinib has been studied in several clinical trials across various tumor types and demonstrated efficacy in two placebo-controlled randomized phase 2 studies in non-small cell lung cancer (NSCLC) and hepatocellular carcinoma (HCC) [13, 14]. The previous phase 1 study in the Japanese patients with solid tumors showed that cytochrome P450 2C19 (CYP2C19) genotype affect the pharmacokinetics and the tolerability of tivantinib. CYP2C19 is known to have genetic polymorphisms leading to enzymatic dysfunction. Thus the recommended phase 2 dose of tivantinib in Asian population was determined by a pretreatment test for CYP2C19 polymorphism: a 360 mg twice daily dose for consecutive days was recommended for patients with at least one wild-type allele of CYP2C19, who are the majority (approximately 80 %) of the Asian population [15].

This is the first clinical trial evaluating the efficacy of a selective c-Met inhibitor in MGC patients. The primary endpoint was to assess the disease control rate (DCR) of tivantinib in the patients with MGC.

Patients and methods

Study design

This was a phase 2, open-label, single-arm, multicenter trial among Korean and Japanese patients with MGC. The primary objective was to assess the DCR, defined as the proportion of patients demonstrating complete response (CR), partial response (PR), or stable disease (SD). Secondary objectives included overall response rate (ORR), progression-free survival (PFS), overall survival (OS), safety profiles, and predictive biomarkers. In addition, the pharmacokinetic (PK) differences between Korean and Japanese or between patients with and without prior gastrectomy were also evaluated. All results were analyzed in the full analysis set (FAS), which included all eligible patients who received at least one dose of tivantinib.

For the PK analysis, 18 patients (9 Korean and 9 Japanese) without any history of gastrectomy were divided into three groups, and the patients received a single dose of 120, 240 or 360 mg tivantinib on the first day of administration (day 1). The remaining 12 patients (6 Korean and 6 Japanese) with gastrectomy were treated with 360 mg on day 1. On day 2 and thereafter, all patients started daily continuous tivantinib at the dose of 360 mg bid between meals until disease progression, symptomatic deterioration, unacceptable toxicity, treatment interruption for any reason more than 14 days, or withdrawal of informed consent. Dose adjustments were gradually allowed from 360 mg bid to 240 mg bid and then 120 mg bid, if grade ≥3 toxicity was observed and patients were able to recover within 14 days of drug interruption.

This study was sponsored by Kyowa Hakko Kirin Co., Ltd. and conducted in accordance with institutional guidelines, Good Clinical Practice guidelines and the Declaration of Helsinki. Documented approvals from the Institutional Review Boards were obtained. All patients provided written informed consent. This trial was registered in ClinicalTrials.gov as NCT01152645.

Patient population

The participating centers included Asan Medical Center (Korea), Shizuoka Cancer Center (Japan), Aichi Cancer Center Hospital (Japan) and Shikoku Cancer Center (Japan).

Patients were eligible if they had a histologically or cytologically confirmed MGC; ≥ 20 years of age with informed consent; previously treated with 1 or 2 prior chemotherapies; CYP2C19 genotype with at least one wild-type allele; at least one measurable lesion per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [16]; ECOG performance status 0 or 1; adequate organ functions; and consent to tumor sample submission for biomarker analysis. Key exclusion criteria were any prior treatment with c-Met inhibitors; surgery for cancer within 4 weeks prior to the first dose of

tivantinib; any concomitant anticancer treatment including chemotherapy, hormone therapy, radiotherapy, immunotherapy, or another investigational drug used within 2 weeks; multiple primary neoplasm within 5 years; inability to take oral tivantinib twice daily; central nervous system metastasis; gastrointestinal disorders that might interfere with the absorption of tivantinib and underlying uncontrolled complication.

Efficacy evaluation

The primary efficacy objective was to assess the DCR, which was defined as the proportion of patients with CR, PR, or SD as the best overall response according to the following criteria based on the RECIST version 1.1. PR or CR was confirmed 4 weeks after first detection of response. Patients were assigned a best overall response of SD if they achieved SD after the 8 weeks treatment with tivantinib. The investigators measured tumor size at baseline, 4 and 8 weeks after the beginning of treatment, and every 6 weeks thereafter. The response and progression was determined by the central radiology review committee for the primary objective evaluation. The DCR was summarized in terms of percentage, with a 95 % confidence interval (CI), and was calculated primarily based on the assessment of the central radiology review. A sample size of 30 patients will provide ≥99 % power to indicate that the lower limit of the 95 % CI of the DCR will exceed the threshold of 20 % (futility rate), if the DCR with tivantinib is expected to be 60 % (targeted rate).

Pharmacokinetics analysis

Blood samples were obtained on day 1 (pre, 1, 2, 4, 6, 10, 12, and 24 h after the first dose of 120 mg, 240 mg or 360 mg), and before dosing on day 15 and day 29. Plasma samples were stored at -20 °C until analysis, and concentration of tivantinib in the plasma was measured by liquid chromatography/tandem mass spectrometry. PK parameters were calculated by noncompartmental analysis using WinNonlin (Pharsight, Mountain View, CA).

Biomarker analysis

Biomarkers tested herein were c-Met expression, phosphorylated c-Met (p-Met) expression, HGF expression and c-Met gene copy number in tumor samples and HGF concentration in serum samples.

Paraffin-embedded tumor samples from fresh or archived tumor specimens were collected from all the patients. Optional re-biopsy after 3 weeks treatment with tivantinib was done in the patients with an additional consent. The immunohistochemistry (IHC) for c-Met, p-Met and HGF, and fluorescence in situ hybridization (FISH) for c-Met and centromere 7 (CEP7, as an assay control) were conducted by a commercial laboratory (SRL Inc., Tokyo, Japan). The following antibodies were used for IHC: anti-c-Met (clone C12, Santa Cruz Biotechnology, Inc., TX, USA), anti-phospho-c-Met (Tyr1349) rabbit mAb (clone 130H2, Cell Signaling Technology, Inc., MA, USA), and anti-human HGFα rabbit IgG (Immuno-Biological Laboratories Co., Ltd., Fukuoka, Japan). The results of the IHC staining were quantified using the H-score which ranges from 0 to 300 on the basis of both the percentage of positive tumor area (0-100) and the staining intensity (0, no staining; 1, weak staining; 2, moderate staining; or 3, intense staining). High c-Met expression was defined if H-score on tumor cell membrane or in tumor cell cytoplasm exceeded 100, which approximates the median score among the tested samples. c-Met copy number per cell was determined by FISH with the use of Vysis D7S522/CEP 7 FISH Probe Kit (Abbott Molecular Inc., IL, USA). c-Met gene amplification was defined as previously reported; a ratio of c-Met/CEP7 of higher than 2.2 [8].

Blood samples for serum HGF were also collected on day 1 (pre-treatment and 12 h after the first dose) and on day 29. The concentration of serum HGF was measured by a commercial laboratory, SRL Inc.

Results

Patient characteristics and treatment

A total of 31 patients were enrolled from July 2010 to June 2011. The FAS population, which is defined as the patients who received at least one dose of tivantinib, included 30 patients; the remaining patient did not receive study medication due to the determination of ineligibility after the registration. Table 1 shows the patient characteristics of the FAS population. Of 30 patients, 80 % of patients were male and the median age was 62.5 years. There were no notable differences in patient characteristics between Korean and Japanese except the distribution of ECOG PS. On the data cut-off date for this report, all patients had discontinued the study treatment.

The median duration of treatment was 56.5 days and the median relative dose intensity was 94.4 %. Ninety-seven percent of the patients discontinued this study due to disease progression.

Efficacy and biomarkers

Of 30 patients in the FAS, 11 achieved disease control (0 CR; 0 PR; 11 SD), resulting in the DCR of 36.7 % (95 % CI: 19.9–56.1 %). The lower limit of its 95 % CI did not exceed the target threshold of 20 % (Table 2). The median PFS was 43.0 days (95 % CI: 29.0–92.0 days), and the median survival time was 344.5 days (95 % CI: 227.0–380.0 days) (Fig. 1). The median duration of stable disease was 98.0 days (95 % CI: 92.0–347.0 days) among the 11 subjects who achieved a best overall response of SD.

Table 1 Patients characteristics

		Overall	Ethnicity	
		n = 30	Japanese *n* = 15	Korean *n* = 15
Sex	Female	6 (20.0 %)	3(20.0 %)	3 (20.0 %)
	Male	24 (80.0 %)	12(80.0 %)	12 (80.0 %)
Age	Median (range)	62.5 (41-70)	63.0(41-70)	59.0 (43-70)
Tumor histology	Well differentiated	3 (10.0 %)	2(13.3 %)	1 (6.7 %)
	Moderately differentiated	10 (33.3 %)	3(20.0 %)	7 (46.7 %)
	Poorly differentiated	14 (46.7 %)	8(53.3 %)	6 (40.0 %)
	Other	2 (6.7 %)	1(6.7 %)	1 (6.7 %)
	Unknown	1 (3.3 %)	1(6.7 %)	0 (0.0 %)
ECOG PS	0	8 (26.7 %)	8(53.3 %)	0 (0.0 %)
	1	22 (73.3 %)	7(46.7 %)	15 (100.0 %)
Prior chemotherapies	1	16 (53.3 %)	9(60.0 %)	7 (46.7 %)
	2	14 (46.7 %)	6(40.0 %)	8 (53.3 %)
Gastrectomy	Yes	12 (40.0 %)	6(40.0 %)	6 (40.0 %)
	No	18 (60.0 %)	9(60.0 %)	9 (60.0 %)
Current cancer diagnosis	Advanced	23 (76.7 %)	11(73.3 %)	12 (80.0 %)
	Recurrent	7 (23.3 %)	4(26.7 %)	3 (20.0 %)
CYP2C19 genotype [a]	*1/*1	12 (40.0 %)	6(40.0 %)	6 (40.0 %)
	*1/*2	13 (43.3 %)	6(40.0 %)	7 (46.7 %)
	*1/*3	5 (16.7 %)	3(20.0 %)	2 (13.3 %)

*1 is the wild type genotype, whereas *2 and *3 are the major polymorphism leading to functional deficiency

[a] CYP2C19 genotype

Archival or fresh biopsy tumor samples and blood samples were collected from all patients for the exploration of the predictive biomarker of tivantinib. One tumor specimen did not contain tumor cells, thus a total of 29 tumor samples was analyzed for IHC and FISH assessment. Regarding the IHC analysis, 11 patients (37.9 %) had high c-Met expression (H-score >100) in tumor cell cytoplasm and only 1 patient (3.4 %) had high c-Met expression on tumor cell membrane. For the FISH analysis, only 2 patients (6.9 %) harbored c-Met gene amplification. As a result, none of these pretreatment biomarkers were found to demonstrate any correlation with best overall response (disease control) or treatment duration in this study (Fig. 2, Supplemental tables).

We conducted post-treatment biopsy in 4 patients, and no apparent change of biomarkers including c-Met, p-Met and HGF expression was observed in the post-treatment samples. Serum HGF concentration was also not changed dramatically from baseline to 12 h or 29 days after treatment (data not shown).

Table 2 Tumor responses

Best overall responses (*n* = 30)					DCR (%) [95 % CI]	ORR (%) [95 % CI]
CR	PR	SD	PD	NE		
0	0	11	19	0	36.7 [19.9-56.1]	0 [0.0-11.6]

Safety and pharmacokinetics

Of the 30 patients, AEs occurred in 29 (96.7 %), and AEs with grade ≥3 according to CTCAE v4.0 occurred in 13 patients (43.3 %). The most common drug-related AEs included nausea in 7 patients (23.3 %) and anemia and decreased appetite

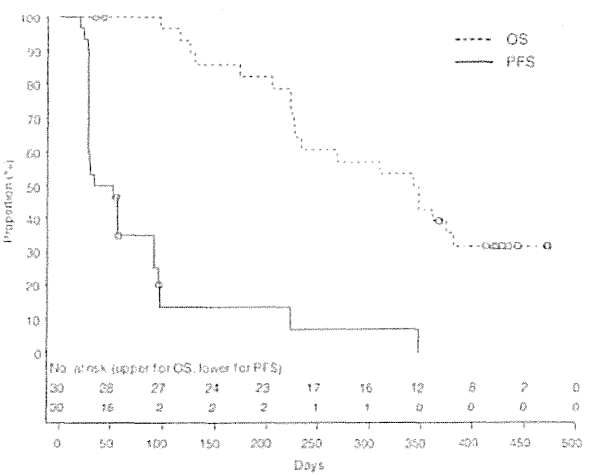

Fig. 1 Kaplan-Meier curves for progression-free survival (PFS) and overall survival (OS) in the MGC patients treated with tivantinib. The *solid* and *dot curves* indicate PFS and OS, respectively. PFS was determined according to RECIST criteria, based on the assessment by the central radiology review committee

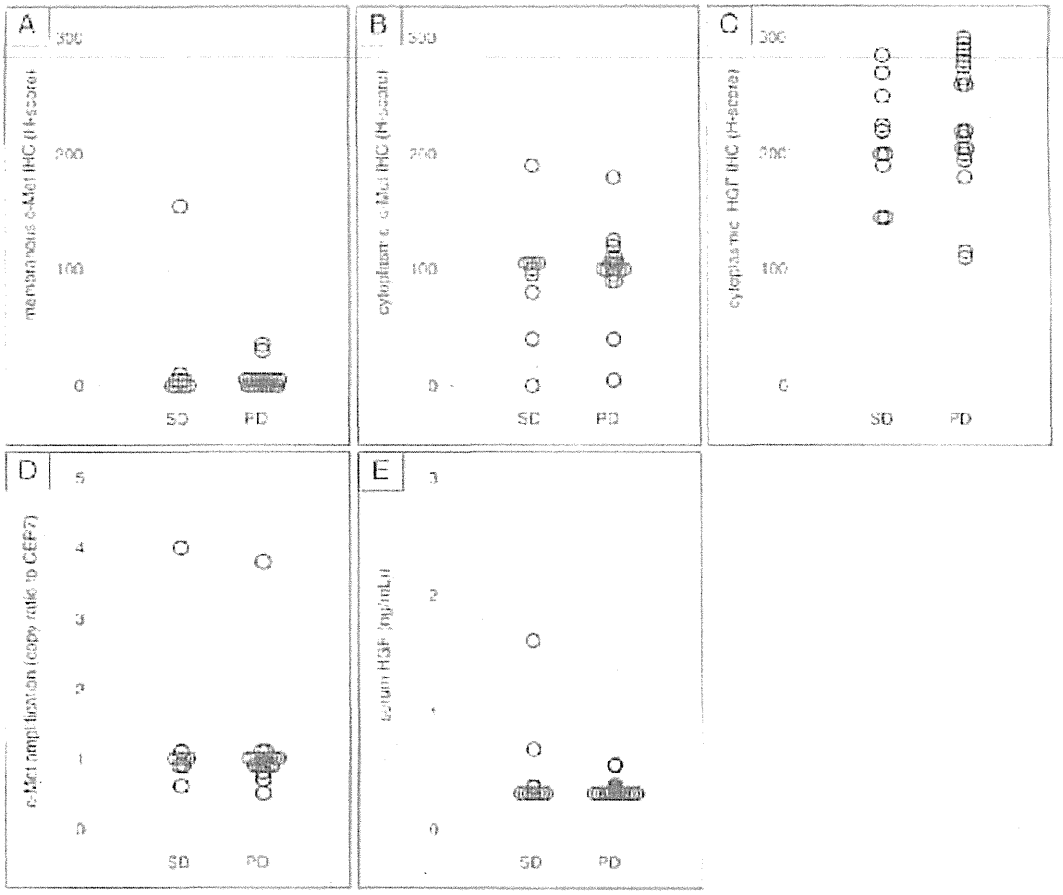

Fig. 2 Relationship between tumor responses and predictive biomarkers. The H-score for membranous c-Met (**A**), cytoplasmic c-Met (**B**) and HGF (**C**) as well as c-Met gene copy number ratios per CEP7 (**D**) and the concentration of serum HGF (**E**) are dotted for each individual with his/her tumor response

in 6 patients (20.0 %) respectively. The drug-related adverse events of grade ≥3 were mainly hematological toxicities including neutropenia, febrile neutropenia, leukopenia and anaemia (Table 3). In the four patients who experienced neutropenia of grade ≥3, plasma trough concentrations on day 15 were 3680, 3680, 4180 and 4600 ng/mL, respectively. Although two patients missed several doses by day 15, their plasma concentrations of tivantinib were relatively high compared to other patients without severe neutropenia ($N = 19$, 1513±1121 ng/mL, mean±SD), being consistent with the preceding report [17]. There was one AE (gastric stenosis) who needed treatment-discontinuation, and no treatment-related death was observed.

No clear difference was seen in the plasma concentration-time profile between Korean and Japanese patients after the first dose of tivantinib at any tested dose (120 mg, 240 mg or 360 mg). There was also no clear difference in pharmacokinetics between patients with and without prior gastrectomy (Fig. 3).

No clear relationship between efficacy and pharmacokinetics parameters of tivantinib was observed (Supplemental figure).

Discussion

This is the first clinical trial evaluating the efficacy of a selective c-Met inhibitor in second- or third-line MGC. The efficacy of tivantinib resulted in a DCR of 36.7 % with 95 % CI: 19.9–56.1 %, in which the lower limit of its 95 % CI did not exceed the target threshold of 20 %. Recently, Korean investigators reported the results of a phase III study demonstrating that the DCR after 6 weeks of treatment with docetaxel and with irinotecan were 59.5 % and 52.0 %, respectively, in patients with MGC with one or two prior chemotherapy regimens [18]. Compared with the Korean phase III trial in similar patients, we concluded that tivantinib has modest efficacy in non-selected MGC patients.

To select patients who may respond to the tivantinib, we assessed c-Met overexpression (IHC) and c-Met amplification (i.e. c-Met copy number), as candidates for predictive biomarkers. IHC analysis showed a broad level of c-Met expression among the tested patients, and 11 of 29 patients (37.9 %) with H-score >100 in either cytoplasm or membrane were

Table 3 Drug-related adverse events occurring in ≥2 patients

Drug-related adverse events (n = 30)	All grades		>Grade 3	
	n	(%)	n	(%)
Any drug-related adverse event	28	(93)	10	(33)
Nausea	7	(23)	0	
Anemia	6	(20)	4	(13)
Anorexia	6	(20)	0	
Fatigue	5	(17)	0	
Lymphocyte count decreased	5	(17)	0	
Neutrophil count decreased	5	(17)	4	(13)
White blood cell decreased	5	(17)	1	(3)
Malaise	4	(17)	0	
Aspartate aminotransferase increased	3	(10)	1	(3)
Diarrhea	3	(10)	0	
Alanine aminotransferase increased	2	(7)	1	(3)
Alkaline phosphatase increased	2	(7)	0	
Blood and lymphatic system disorders	2	(7)	0	
Fever	2	(7)	0	
Hyperuricemia	2	(7)	1	(3)
Mucositis oral	2	(7)	0	
Pain	2	(7)	0	
Pruritus	2	(7)	0	
Vomiting	2	(7)	0	

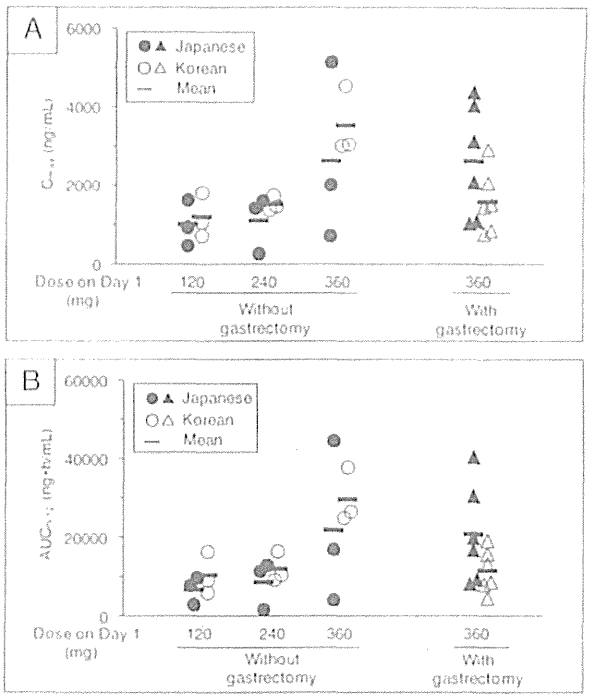

Fig. 3 The plasma exposures to tivantinib on day 1. C_{max} (A) or AUC_{0-12} (B) are dotted for each individual treated with the indicated doses. The *closed* and *open dots* represent exposures of Japanese and Korean patients, respectively. The history of gastrectomy for each patient was indicated at the bottom of the figure

recognized as c-Met high expressers. The c-Met high expression ratio was consistent with previous reports where 40-65 % of gastric cancer was recognized as c-Met high expression [6, 19]. However, our study did not find a particularly favorable efficacy in c-Met high expression population, suggesting that c-Met high expression in our setting may not be suitable for a predictive biomarker of tivantinib. This might be unexpected because two precedent placebo-controlled phase II studies for NSCLC [13, 20] and HCC [14, 21] demonstrated a favorable efficacy in selected population with c-Met high expression using IHC method. We think that one of the reasons for the unexpected results would be due to the immature IHC method using invalidated anti-c-Met antibody clones. Our IHC assay showed that c-Met was predominantly expressed on cytoplasm in the majority of tumor tissue samples, whereas only one patient showed a membranous high expression, although c-Met protein is known as transmembraneous receptor tyrosine kinase. On the other hand, our result showed that two patients (6.9 %) harboring c-Met amplification, and the frequency was consistent with literature showing that c-Met gene amplification was observed in very small population in gastric cancer [8, 22]. Catenacci and colleagues reported a durable complete response with an anti-c-Met antibody onartuzumab in a patient with MGC harboring the c-Met gene polysomy associated with high expression [23]. In this study, the c-Met-amplified patient did not show a notable efficacy, and this small-size phase II could not clearly show the potential of c-Met amplification as a useful predictive biomarker for tivantinib responder.

Our results demonstrated that tivantinib was also well tolerated at the daily dose of 360 mg bid in MGC patients with at least one allele of wild type CYP2C19. It is consistent with the previous phase I studies which demonstrated the tolerability of a daily continuous dose of 360 mg bid in Western countries [24, 25] and Japan [17]. Of note, tivantinib was tolerated even in the patients with a history of gastrectomy, and this fact was supported by PK analysis showing that tivantinib exposure was mostly constant regardless of prior gastrectomy. This would be a favorable feature of tivantinib as a possible treatment for the gastric cancer, where gastrectomy is the standard care for the early stage. As was the case for previous phase I study [17], this study showed that hematological toxicities including neutropenia, leukopenia and anemia were the most frequent drug-related adverse events to tivantinib, and novel toxicities specific for MGC were not suggested in this study. In addition, this study demonstrated that the incidence of severe neutropenia is related to the high exposure to tivantinib, as shown in the previous Japanese phase I study [17]. It could be concluded that there was no major safety

issue in the use of tivantinib of 360 mg bid for Asian patients with previously treated MGC who had at least one allele of wild type CYP2C19.

As a conclusion, although c-Met is an attractive molecular target for the treatment of MGC, tivantinib monotherapy showed a marginal efficacy for unselected MGC as a single agent. Further studies for the validation of predictive biomarkers and/or combination with chemotherapy or other molecular targeted agents might be warranted.

Acknowledgments We thank the patients, their families, caregivers, and all of the personnel who contribute to patient care and data collection for this study of tivantinib.

We also thank the members of the independent safety monitoring committee: Dr. Kazuo Tamura, Dr. Hiroshi Saito and Dr. Ichinosuke Hyodo, and the members of the central radiology review committee: Dr. Yoshinori Miyata, Dr. Hye-Jin Kang, Dr. Kohki Yoshikawa and Dr. Kunihisa Miyakawa.

Funding This work was sponsored by Kyowa Hakko Kirin Co., Ltd.

Conflict of interest Y. K. Kang received honoraria from Kyowa Hakko Kirin Co., Ltd. Y. Kamiya and S. Akinaga are employees of Kyowa Hakko Kirin Co., Ltd. All remaining have declared no conflict of interest.

Ethical standards This study was conducted in accordance with institutional guidelines, Good Clinical Practice guidelines and the Declaration of Helsinki. Documented approvals from the Institutional Review Boards were obtained. All patients provided written informed consent before study participation.

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Invest New Drugs (2014) 32:261–270
DOI 10.1007/s10637-013-9948-5

PHASE I STUDIES

Phase I study of sunitinib plus S-1 and cisplatin in Japanese patients with advanced or metastatic gastric cancer

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Received: 28 November 2012 / Accepted: 1 March 2013 / Published online: 12 May 2013

Summary *Background* This phase I, dose-finding study evaluated the maximum tolerated dose (MTD), safety, pharmacokinetics, and antitumor activity of sunitinib plus S-1/cisplatin in Japanese patients with advanced/metastatic gastric cancer. *Patients and methods* Patients received oral sunitinib on a continuous daily dosing (CDD) or 2-weeks-on/2-weeks-off schedule (Schedule 2/2; 25 mg/day or 37.5 mg/day), plus S-1 (80–120 mg/day)/cisplatin 60 mg/m^2. *Results* Twenty-seven patients received treatment, including 26 patients treated per protocol (sunitinib 25 mg/day CDD schedule, $n=4$; sunitinib 25 mg/day Schedule 2/2, $n=16$ [dose-limiting toxicity (DLT) cohort, $n=6$ plus expansion cohort, $n=10$]; sunitinib 37.5 mg/day Schedule 2/2, $n=6$). One patient erroneously self-administered sunitinib 12.5 mg/day and was excluded from the analyses. The MTD was sunitinib 25 mg/day on Schedule 2/2. DLTs were reported for: 2/4 patients given sunitinib 25 mg/day on the CDD schedule; 1/6 patients administered sunitinib 25 mg/day on Schedule 2/2 (grade [G] 3 neutropenic infection, G4 thrombocytopenia, and S-1 dose interruption ≥5 days), and 3/6 patients given sunitinib 37.5 mg/day on Schedule 2/2. Results below are for the overall MTD cohort ($n=16$). The most frequently reported G3/4 adverse events were neutropenia (93.8 %) and leukopenia (75.0 %). The objective response rate was 37.5 %; six additional patients experienced no disease progression for ≥24 weeks. Median progression-free survival was 12.5 months. No pharmacokinetic drug–drug interactions were observed between sunitinib/S-1/cisplatin and S-1/cisplatin. *Conclusions* The MTD of sunitinib was 25 mg/day on Schedule 2/2 combined with cisplatin/S-1 in patients with advanced/metastatic gastric cancer. This regimen had a manageable safety profile and preliminary antitumor activity.

Keywords Sunitinib · Gastric cancer · Phase I · Dose-finding

Presented in part on the clinical trial registry located at ClinicalTrials.gov (identification No. NCT00553696) and at:

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Introduction

Gastric cancer is the second most common cause of cancer-related death worldwide, with more than 730,000 deaths estimated to have occurred in 2008 [1]. Globally, the

5-year survival rate for gastric cancer is approximately 20 % [2], and most patients present with advanced, non-resectable disease [3–5].

Despite recent advances in the treatment for gastric cancer [6], a standard chemotherapy regimen has not been established for recurrent or unresectable advanced gastric cancer; combination chemotherapy is associated with significant survival and quality of life advantages, compared with best supportive care [7, 8]. The use of a 5-fluorouracil (5-FU)-based regimen in combination with a platinum analog is the most widely accepted first-line treatment regimen, although combination therapy does have a higher associated toxicity burden compared with single-agent chemotherapy [8].

Blockade of receptors such as vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR) has been shown to inhibit tumor-related angiogenesis and tumor growth [9, 10]. Not only are these receptors expressed in gastric cancers but they are known to have direct effects on the growth and metastasis of this disease [9–14].

Sunitinib malate (SUTENT®; Pfizer Inc., New York, NY, USA) is an oral, multitargeted, tyrosine kinase inhibitor of VEGFRs 1–3, PDGFR-α and -β, and other receptors [15–17]. Sunitinib is approved multinationally for the treatment of unresectable and/or metastatic imatinib-resistant/-intolerant gastrointestinal stromal tumor, advanced/metastatic renal cell carcinoma, and unresectable or metastatic, well-differentiated pancreatic neuroendocrine tumors. Phase II study results in advanced gastric cancer have shown that sunitinib had activity as a single-agent; progression-free survival (PFS) was 2.3 months and overall survival was 6.8 months in the second-line setting [18].

In preclinical tumor models, sunitinib has been shown to enhance the antitumor activity of 5-FU and cisplatin, suggesting that sunitinib might enhance the effect of chemotherapy in cancer patients [19, 20]. In the First-Line Advanced Gastric Cancer Study (FLAGS), the combination of S-1, an oral derivative of 5-FU, and cisplatin was found to be effective when administered as a 3-week on/1-week off regimen (Schedule 3/1) [21]. Therefore, this phase I, dose-finding study was conducted to determine the maximum tolerated dose (MTD) and overall safety profile of sunitinib plus S-1 and cisplatin in Japanese patients with advanced/metastatic gastric cancer. Tolerability, pharmacokinetics (PK), and antitumor activity were also evaluated.

Materials and methods

Study population

Patients (male or female) eligible for inclusion in this study were aged ≥20 years, had an Eastern Cooperative Oncology Group performance status of 0 or 1, adequate organ function, and histologically or cytologically confirmed Stage IV gastric adenocarcinoma or gastroesophageal junction adenocarcinoma not amenable to surgery or radiation. Prior adjuvant therapy was permitted with a recurrence-free interval of >3 months after the completion of adjuvant therapy. Prior chemotherapy in the advanced/metastatic setting was not permitted; one regimen of chemotherapy, such as S-1 monotherapy, without progressive disease was allowed if the duration of treatment was less than 4 weeks.

Exclusion criteria included central nervous system (CNS) metastases, carcinomatous meningitis, or uncontrolled hypertension (blood pressure >150/100 mmHg). Patients with severe/unstable angina, myocardial infarction, coronary artery bypass graft, symptomatic congestive heart failure, cerebrovascular accident, including transient ischemic attack, or pulmonary embolism within 12 months prior to starting study treatment were also excluded.

The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice guidelines, the declaration of Helsinki, and applicable local regulatory requirements and laws. Approval from the institutional review board or independent ethics committee with the appropriate jurisdiction was required for each participating investigator/center. Written informed consent was obtained from all patients.

Study design

This was a phase I, open-label, dose-finding study of sunitinib in combination with S-1 and cisplatin in patients with advanced/metastatic gastric cancer (NCT00553696). Patients received open-label, oral S-1 at a starting dose of 80–120 mg/day (based on body surface area) on Schedule 3/1 and a cisplatin 60 mg/m^2 infusion on day 1 that was repeated every 28 days. Patients were allocated to different doses of oral sunitinib based on a 3+3 design. Initially, sunitinib was planned to be administered on a continuous daily dosing (CDD) schedule or on Schedule 3/1. After four patients received treatment in the CDD arm, the protocol was revised to use a 2-week-on/2-week-off schedule (Schedule 2/2), instead of Schedule 3/1, due to the pattern of adverse events (AEs). Patients received sunitinib 25 mg/day on a CDD schedule, or 25 mg/day or 37.5 mg/day on Schedule 2/2 in 4-week cycles (Fig. 1).

Initially, three patients were enrolled to receive sunitinib 25 mg/day on the CDD schedule in combination with S-1 and cisplatin 60 mg/m^2. If no patients experienced a dose-limiting toxicity (DLT) in cycle 1 then patients would be enrolled to the next highest dose level. If no more than one of the initial three patients experienced a DLT within cycle 1, then the cohort was expanded to a total of six patients. If no more than one of these six patients experienced a DLT,