improvement in overall survival (primary endpoint) with the addition of lapatinib to capecitabine plus oxaliplatin (CapeOx) as the first-line treatment of advanced or metastatic HER2+ gastric or gastroesophageal adenocarcinoma (12.2 vs. 10.5 months; HR: 0.91 (95 % CI 0.73, 1.12, $p = 0.35$)). However, pre-specified subgroup analyses showed significant improvements in OS in Asian patients (HR = 0.68) and those under 60 years (HR = 0.69). With regard to toxicity, lapatinib in combination with CapeOx showed an increased rate of grade 3 diarrhea (12 vs. 3 %) and a higher rate of skin toxicity. The next steps in HER2 blockade for GC may follow the developments in breast cancer, with evaluation of TDM-1, a conjugate molecule combining trastuzumab with an antimicrotubule agent; the combination of trastuzumab and lapatinib; and first-line integration of pertuzumab in metastatic disease and the investigational use of HER2 inhibitors in the neoadjuvant setting. RTOG trial 1010 is currently evaluating preoperative chemoradiotherapy in esophageal and GEJ cancers that are HER2+, randomizing patients to receive chemoradiotherapy alone or chemoradiotherapy plus trastuzumab followed by adjuvant trastuzumab after surgery.

In contrast to the success obtained with trastuzumab in advanced GC, monoclonal antibodies that target HER1 (epidermal growth factor receptor, EGFR) have failed to improve outcome in biologically unselected GC patients [36, 59]. It remains to be elucidated from tumor tissue analyses if a small proportion of GC patients may benefit from anti-EGFR targeted therapy, e.g., in the case of EGFR gene amplification [60]. The negative results obtained with cetuximab (EXPAND study) and panitumumab (REAL3 study) emphasize the need to have a biologically meaningful target before studying targeted agents in larger populations. The importance of combining targeted agents with an appropriate chemotherapy backbone is also highlighted. As the REAL3 study taught us, the combination of a triplet chemotherapy (EOX) regimen with panitumumab is suboptimal and associated with inferior survival, probably due to the excessive toxicity experienced when using this chemotherapy backbone.

Other potential targets, including hepatocyte growth factor receptor (c-Met), insulin-like growth factor receptor 1 (IGF-1R), fibroblast growth factor receptor (FGFR), proteins involved in cell cycle regulation, the proteasome, chaperone proteins, matrix metalloproteinases, histone deacetylases, and other structures, are under evaluation. Novel drugs directed against those specific targets are under clinical investigation.

With regards to angiogenesis, the phase III AVAGAST trial [35] could not demonstrate a survival benefit with the addition of bevacizumab, an inhibitor of the ligand for vascular endothelial growth factor receptor 2 (VEGFr2), VEGF-A, to chemotherapy, but it did show improved PFS and RR. However, looking at the American and European patients, there appears to be a modest benefit of using bevacizumab, which highlights how the biology of gastric cancer varies in different parts of the world. A subsequent report suggested that high serum levels of VEGF-A and low tumor neuropilin expression were correlated with the enhanced benefit resulting from treatment with bevacizumab, but only in Western patients treated in the trial [61]. Recently, ramucirumab, a monoclonal antibody directed against VEGFR2 [62], has been shown to prolong survival when used as a monotherapy compared with the best supportive care in the second-line treatment of advanced GC [63]. The phase III RAINBOW study, which is investigating ramucirumab in combination with paclitaxel in the second-line setting (http://clinicalTrials.gov/show/NCT01170663), has completed recruitment and results are awaited.

Another key regulator of cell proliferation, growth, survival, metabolism, and angiogenesis is the PI3K/Akt/mTOR pathway, which is dysregulated in 50–60 % of gastric cancers. Everolimus, an oral mTOR inhibitor, failed to improve OS in patients treated with 1 or 2 lines of systemic chemotherapy when given as monotherapy, but did improve PFS [64]. A phase III trial of the German Arbeitsgemeinschaft Internistische Onkologie (AIO-STO 0111) is currently evaluating paclitaxel with and without everolimus in patients with GC after initial treatment with a fluoropyrimidine-containing regimen (http://clinicalTrials.gov/show/NCT01248403).

The published randomized controlled trials involving molecularly targeted drugs in advanced gastric cancer are delineated in Table 4.

Another target in GC is the receptor tyrosine kinase MET. Met receptor overexpression is associated with poor prognosis for gastric cancer patients. Preliminary results of a randomized phase II trial with rilotumumab, a fully human monoclonal antibody against the Met receptor ligand hepatocyte growth factor, showed improved OS and PFS in patients with high MET expression when combined with ECX [65]. Phase III studies evaluating the clinical benefit of MET inhibitors are under underway.

Post-progression treatment

Post-progression chemotherapy is effective in advanced gastric cancer. Three randomized controlled trials showed superior survival of patients on either irinotecan or docetaxel monotherapy compared with those receiving best supportive care [16, 17, 66]. In the smallest study, which was performed in Germany, it was reported that post-progression chemotherapy not only prolonged survival but also led to better symptom control. Reports of appropriate quality-of-life measurements are, however, missing in

Table 4 Reported phase III trials investigating biologically targeted agents in advanced gastric cancer

Study	Phase/ line	Target	Regimen	*N*	PFS (months)	OS (months)	Primary endpoint	Comment
ToGA [10]	III/1st line	HER2 (MoAb)	Trastuzumab–CF	298	6.7	13.8	OS: positive	HER2+ (IHC 3+ or FISH+)
					5.5	11.9		
			CF	296	HR 0.71	HR 0.74		
					$P = 0.0002$	$P = 0.04$		
AVAGAST [61]	III/1st line	VEGF-A (MoAb)	Bevacizumab–XP	387	6.7	12.1	OS: negative	Bevacizumab–XP is superior in terms of RR and PFS
					5.3	10.1		
			Placebo–XP	387	HR 0.80	HR 0.87		
					$P = 0.0037$	$P = 0.10$		
REAL-3 [59]	III/1st line	EGFR (MoAb)	Panitumumab–EOX	278	6.0	8.8	OS: negative	Lower doses of chemotherapy in the experimental arm
					7.4	11.3		
				275	HR 1.22	HR 1.37		
			EOX		$P = 0.068$	$P = 0.013$		
EXPAND [36]	III/1st line	EGFR (MoAb)	Cetuximab–XP	455	4.4	9.4	PFS: negative	Similar response rates with 29 % (experimental) and 30 % (control)
					5.6	10.7		
				449	HR 1.09	HR 1.0		
			XP		$P = 0.316$	$P = 955$		
Granite 1 [64]	III/2nd or 3rd line	mTOR	Everolimus	439	1.7	5.4	OS: negative	Similar response rates with 4.5 % (everolimus) and 2.1 % Placebo
					1.4	4.3		
					HR 0.66	HR 0.90		
			Placebo	217	$P < 0.0001$	$P = 0.124$		
REGARD [63]	III/2nd line	VEGFR-2	Ramucirumab (IMC-1121B)+BSC	238	2.1	5.2	OS: positive	
					1.3	3.8		
			placebo+BSC	117	HR 0.48	HR 0.78		
					$p < 0.0001$	$p = 0.047$		

BSC best supportive care, *CF* cisplatin/fluorouracil, *XP* capecitabine/cisplatin, *EOX* epirubicin/oxaliplatin/capecitabine, *MoAB* monoclonal antibody, *PFS* progression-free survival, *OS* overall survival

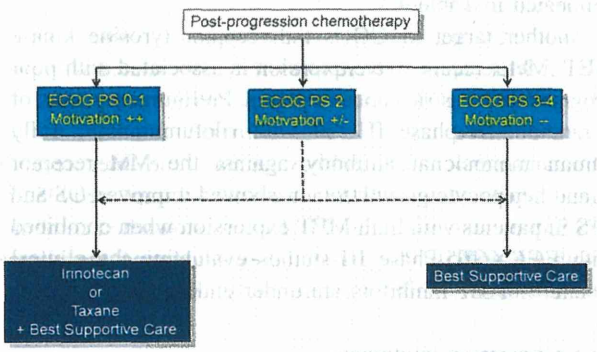

Fig. 3 Indication for post-progression chemotherapy. *ECOG PS* Eastern Cooperative Group performance status

these studies. A study from the West Japan Oncology Group has recently shown that weekly paclitaxel may also be used in second-line advanced GC [67]. Assessment of palliative treatment goals such as general health status, clinical benefit, and quality of life must be included in further studies [68].

Second-line chemotherapy is now considered a standard therapy option for patients who progress during or after first-line chemotherapy, who are defined as having Eastern Cooperative Group (ECOG) performance status 0–1(2), and who have motivation to be further treated with chemotherapy. Irinotecan administered every 2 or 3 weeks, docetaxel given once every 3 weeks, or weekly paclitaxel are potential options (Fig. 3). Ramucirumab has also been shown to prolong the survival time with a very reasonable side-effect profile [59], but this anti-angiogenic antibody is not yet on the market.

Future outlook

The more we learn about the biological heterogeneity of gastric cancer, the more we can see that there is no single medical treatment that is the best option for all types of gastric cancers. Even with classical cytotoxic treatment, different sensitivities to specific agents may exist in intrinsic GC subtypes [9]. Histologically, distal gastric cancer is classified into intestinal, diffuse, and signet-ring types, and

adenocarcinoma of the esophagogastric junction is now classified into three unique subsets of upper gastrointestinal adenocarcinoma. With regards to targeted therapy, the development of trastuzumab in HER2-overexpressing gastric cancer raises hope that further progress may be achieved. New targeted agents are under investigation, and some look promising; with better genetic or epigenetic characterization of GC, new and improved treatment options may become available in the future. The identification of biomarkers is essential in order to target the appropriate populations in the trials. Therefore, the collaboration between basic science and clinical research and the performance of well-designed bench-to-bedside studies will be key to achieving further progress in the treatment of advanced gastric cancer.

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ORIGINAL ARTICLE

Comparison of advanced adenocarcinomas of esophagogastric junction and distal stomach in Japanese patients

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Abstract
Background There have been no reports on the incidence, characteristics, treatment outcomes, and prognosis of inoperably advanced or recurrent adenocarcinoma of the esophagogastric junction (AEGJ) in Japan.
Methods We investigated the clinicopathological characteristics, treatment outcomes, and prognosis for 816 patients with esophagogastric junctional and gastric adenocarcinoma who received first-line chemotherapy between 2004 and 2009.
Results Of 816 patients, 82 (10 %) had AEGJ. The patients with AEGJ had significantly more lung and lymph node metastasis, but less peritoneal metastasis, than those with gastric adenocarcinoma (GAC). The objective response rate to first-line chemotherapy was 23.3 % for patients with AEGJ and 22.6 % in patients with GAC ($p = 0.90$). The median survival was 13.0 months in AEGJ and 11.8 months in GAC ($p = 0.445$). In no patient was tumor site a significant prognostic factor ($p = 0.472$). In patients with AEGJ, ECOG PS ≥ 2, presence of liver metastasis, and absence of lung metastasis were significantly associated with poor prognosis.
Conclusions No significant differences were observed in treatment outcomes between advanced AEGJ and GAC. Therefore, the same chemotherapy regimen can be given as a treatment arm in future Japanese clinical trials to both patients with inoperably advanced or recurrent AEGJ and those with GAC.

Keywords Siewert classification · Esophagogastric junction · Adenocarcinoma · Chemotherapy · Prognosis

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Introduction

Adenocarcinoma of the esophagogastric junction (AEGJ) or lower esophagus is one of the most rapidly increasing malignant diseases in the West and appears to have a different etiology from distal gastric cancer [1–4]. In contrast, the incidence of AEGJ is unchanged or only gradually increasing in the East [5–7], and its clinicopathological features have not yet been elucidated, especially in advanced, nonresectable tumors.

From clinical trials for advanced cancers of the esophagus and the stomach, the current status of AEGJ is variable; it may be treated as either esophageal or gastric cancer, or be

excluded from the trial altogether. Chau et al. [8] studied 1,775 patients with advanced esophagogastric cancer, including 457 with AEGJ, who had been treated with chemotherapy in four Western randomized trials. That study found no difference in overall survival (OS), response rates, or toxicities by tumor location.

In Japanese randomized trials for advanced esophageal or gastric cancer, AEGJ has not been specifically examined because of its rarity [9–13]. There is currently no standard chemotherapy regimen for AEGJ, and it is usually treated as a gastric cancer with fluoropyrimidine and platinum.

In this study, we retrospectively investigated clinicopathological features and treatment outcomes associated with advanced AEGJ in Japanese patients treated at a high-volume cancer center, examining whether AEGJ warrants a separate clinical approach in future clinical trials.

Patients and methods

Patients

We retrospectively analyzed patients with inoperably advanced or recurrent gastric and esophageal cancer who had received palliative therapy between January 2004 and December 2009 at the National Cancer Center Hospital in Tokyo. The eligibility criteria for this study were as follows: (1) histologically confirmed adenocarcinoma; (2) treatment with first-line chemotherapy in our hospital; and (3) availability of clinicopathological data at the beginning of the first-line chemotherapy. Carcinomas in remnant stomach after partial gastrectomy were excluded. Of 1,395 patients who received palliative therapy in our hospital between 2004 and 2009, 816 patients were enrolled in this study (Fig. 1). All endpoints were updated in March 2011. Median follow-up time was 11.1 months (range, 0.8–82.0 months), and median follow-up time for the surviving patients was 19.0 months.

Clinicopathological data

Performance status (PS) at the beginning of first-line chemotherapy was evaluated according to the Eastern Cooperative Oncology Group criteria. Clinical tumor response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST ver. 1.0). The histological type of the primary tumor was evaluated by using a biopsy specimen of inoperably advanced cases and the surgical specimen of recurrent cases. Histological type was determined according to the Japanese classification for gastric carcinoma for the predominant histological type [14]. Papillary, well- or moderately differentiated adenocarcinoma was defined as the intestinal type, whereas poorly differentiated

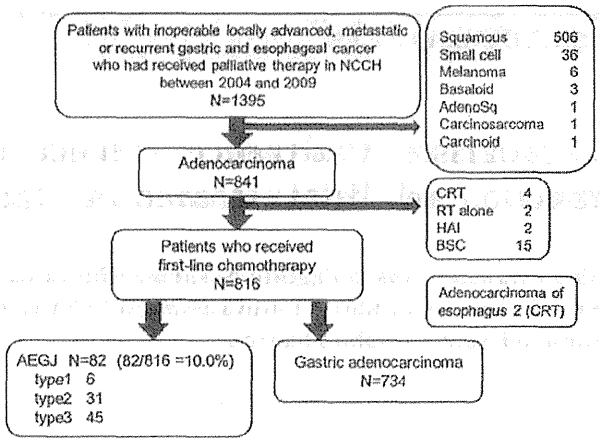

Fig. 1 Trial profile. *NCCH* National Cancer Center Hospital, *AdenoSq* adenosquamous carcinoma, *CRT* chemoradiotherapy, *RT* radiotherapy, *HAI* hepatic arterial infusion therapy, *BSC* best supportive care, *AEGJ* adenocarcinoma of esophagogastric junction

adenocarcinoma or signet-ring cell carcinoma was defined as the diffuse type. Mucinous adenocarcinoma was classified as intestinal or diffuse depending on the secondary predominant histological type.

Baseline characteristics at the beginning of first-line chemotherapy such as age, sex, PS, and laboratory data were evaluated. The following clinicopathological factors were also evaluated: disease status (inoperably advanced or recurrent), histopathology (intestinal or diffuse), metastatic site at the beginning of first-line chemotherapy (liver, peritoneum, lung, bone, abdominal lymph node, mediastinal lymph node, and cervical lymph node), number of metastatic sites, and response to first-line chemotherapy.

AEGJ classification

The tumor location of AEGJ was defined in accordance with Siewert's classification [15]. The Siewert subtypes were retrospectively determined by the following method. In recurrent patients, pathologists recorded the relationship between the tumor center and EGJ according to Siewert's classification, when diagnosing the surgically resected specimen. In inoperably advanced patients, two endoscopists retrospectively determined the relationship between the EGJ and the tumor center independently of each other.

Statistical analysis

Statistical analysis was performed using SPSS 17.0 statistical software (SPSS, Chicago, IL, USA). Comparison of categorical variables was tested by the Chi square test. OS was calculated from the date of the first diagnosis of inoperably advanced or recurrent gastric cancer to death from any cause that was scored as an event. Patients who were

Table 1 Clinical findings of all patients

Factor	AEGJ $N = 82$	GAC $N = 734$	p value
Median age, years (range)	62 (24–85)	63 (19–84)	0.085
Gender			<0.001
Male	70 (85 %)	458 (62 %)	
Female	12 (15 %)	276 (38 %)	
Performance status			0.217
0	38 (46 %)	273 (37 %)	
1	40 (49 %)	411 (56 %)	
≥2	4 (5 %)	48 (6 %)	
Unknown	0	2	
Disease status			0.030
Inoperable	47 (57 %)	506 (69 %)	
Recurrent	35 (43 %)	228 (31 %)	
Tumor differentiation on histopathology			<0.001
Intestinal	39 (48 %)	195 (27 %)	
Diffuse	44 (52 %)	437 (77 %)	
Not classified	0	21	
Number of metastatic sites			<0.001
1	45 (55 %)	563 (77 %)	
≥2	37 (45 %)	171 (23 %)	
Metastatic/recurrent sites			
Liver	23 (28 %)	196 (27 %)	0.794
Peritoneum	21 (27 %)	402 (55 %)	<0.001
Lung	21 (27 %)	40 (5 %)	<0.001
Bone	4 (5 %)	44 (6 %)	0.684
Abdominal LN	34 (41 %)	197 (27 %)	0.010
Mediastinal LN	17 (21 %)	22 (3 %)	<0.001
Cervical LN	7 (9 %)	30 (4 %)	0.066
First-line chemotherapy regimen			NA
F alone	31 (38 %)	333 (45 %)	
F + P	24 (29 %)	128 (17 %)	
F + P ± anti-angiogenetic agent	2 (2 %)	32 (4 %)	
F + taxane	11 (13 %)	34 (5 %)	
Irinotecan regimen	9 (11 %)	94 (13 %)	
Taxane alone	5 (6 %)	25 (3 %)	
Best overall response			NA
Complete response	0	9 (1 %)	
Partial response	17 (21 %)	142 (20 %)	
Stable disease	32 (39 %)	323 (44 %)	
Progressive disease	24 (29 %)	193 (26 %)	
Not evaluable	9 (11 %)	67 (9 %)	
Best overall response rate[a]	23.3 %	22.6 %	0.90
Disease control rate[b]	67.1 %	71.1 %	0.48

AEGJ adenocarcinoma of the esophagogastric junction, *GAC* gastric adenocarcinoma, *LN* lymph node, *F* fluoropyrimidine (5-fluorouracil, S-1, capecitabine), *P* platinum (cisplatin, oxaliplatin), *NA* not available

[a] Best overall response rate = (complete response + partial response)

[b] Disease control rate = (complete response + partial response + stable disease)

alive were censused at the last follow-up date. Survival curves were derived from Kaplan–Meier estimates, and the curves were compared by log-rank tests. A prognostic model was established by searching all variables that significantly influenced OS at a level of p values <0.05 in the univariate analysis. Multivariate analysis for OS was performed using stepwise Cox's proportional hazard regression model (entry probability 0.05, removal probability 0.1). All the tests were two sided, and p values <0.05 were considered significant.

Results

Figure 1 shows the screening process of this study; 816 patients were finally enrolled and analyzed. Eighty-two (10 %) patients had AEGJ and 734 patients had gastric adenocarcinoma (GAC). Among the 82 patients with AEGJ, 6 (7 %) were classified as Siewert type I, 31 (38 %) as type II, and 45 (55 %) as type III. Table 1 shows the baseline clinicopathological characteristics. There were significantly more males ($p < 0.001$), recurrent status ($p = 0.03$), intestinal differentiated tumors on histopathology ($p < 0.001$), lung metastasis ($p < 0.001$), and lymph node metastasis ($p < 0.001$) in patients with AEGJ than in those with GAC. On the other hand, there was significantly more peritoneal metastasis in patients with GAC than in those with AEGJ ($p < 0.001$). There was no difference among the two groups in the first-line chemotherapy regimen. The objective response (complete and partial response) rate was 23.3 % for patients with AEGJ and 22.6 % in patients with GAC ($p = 0.90$). The disease control (complete and partial response plus stable disease) rate was 67.1 % for patients with AEGJ and 71.1 % in patients with GAC ($p = 0.48$). In the patients treated by the F + P regimen, the objective response rate was 27.3 % in patients with AEGJ and 29.2 % in patients with GAC, and the disease control rate was 72.7 and 88.3 %, respectively. There were also no significant differences between two groups.

At the time of data cutoff, 668 (82 %) patients had died. Median survival was 13.0 months [95 % confidence interval (CI), 9.0–16.9 months] in AEGJ and 11.8 months (95 % CI, 10.9–12.7 months) in GAC. Figure 2 shows that there were no significant differences in OS between AEGJ and GAC (log-rank, $p = 0.445$). In the patients treated by the F + P regimen, which is the standard therapy for gastric and esophageal cancer, the survival time was not significantly different between the patients with AEGJ and those with GAC (log-rank, $p = 0.352$).

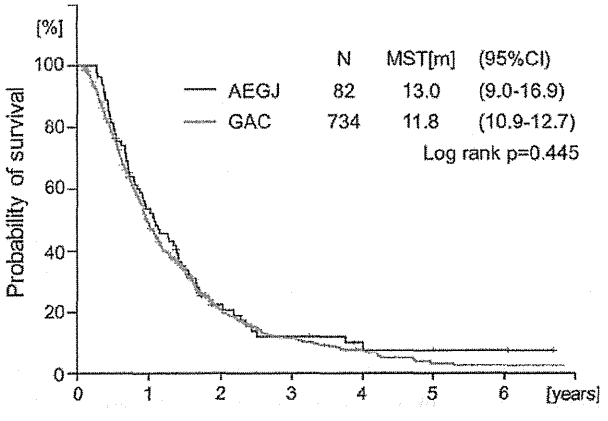

Fig. 2 Overall survival curves according to tumor site. *AEGJ* adenocarcinoma of esophagogastric junction, *MST* median survival time, *CI* confidence interval

In no patient was tumor site (EGJ or gastric) a significant prognostic factor ($p = 0.472$). The results of univariate analysis of clinicopathological variables for prognostic factors in patients with AEGJ and GAC are shown in Table 2. In univariate analysis, four variables were significantly associated with poor survival time in patients with GAC: ECOG PS ≤ 2, inoperably advanced disease status, diffuse histopathology, and two or more metastatic sites. On the other hands, three variables were significantly associated with poor survival time in those with AEGJ: ECOG PS ≥ 2, the presence of liver metastasis, and absence of lung metastasis. The independent prognostic factors identified by the multivariate analysis are all significant prognostic factors identified by univariate analysis. The results of multivariate analysis for prognostic factors in patients with AEGJ and GAC are shown in Table 3. Poor PS was an independent prognostic factor in patients with both AEGJ and GAC. However, there were some differences in prognostic factors between AEGJ and GAC. In patients with GAC, inoperably advanced disease status, diffuse histopathology, and increasing number of metastatic sites influenced survival. The presence of liver metastasis and absence of lung metastasis were also associated with poor prognosis in those with AEGJ.

Discussion

This study first reported from Asia that the clinicopathological characteristics of inoperably advanced or recurrent AEGJ, including sex, tumor location, and histological type, were similar to those of operable AEGJ previously reported in Japan and also in Western countries [8, 16–20].

Lung metastasis was diagnosed in 7–41 % of the patients with advanced esophageal cancer, and its frequency was high compared with about 5 % of patients diagnosed with advanced gastric cancer [12, 21–23]. Because of invasion to the esophagus, AEGJ may have the same drainage system of a vein as lower esophageal cancer. Additionally, it was reported that the mediastinal lymph node metastasis rate of gastric cancer depended on the length of esophageal invasion, and a length of more than 2–3 cm was a risk factor [22, 24]. On the other hand, patients with AEGJ had little peritoneal metastasis because of its anatomical location and histology. Most type I and II AEGJs are not fully covered by the peritoneum. Moreover, peritoneal metastasis was more frequent in patients with diffuse-type histopathology compared to those with intestinal type, the primary histopathological type of AEGJ.

Our data showing that patients with AEGJ have significantly more lung metastasis, more mediastinal lymph node

Table 2 Univariate analyses on survival

Factor	*N*	HR	95 % CI	*p* value	*N*	HR	95 % CI	*p* value
Age								
<60	36	1			270	1		
≥60	46	1.13	0.70–1.82	0.630	464	1.16	0.99–1.38	0.074
Sex								
Male	70	1			458	1		
Female	12	1.62	0.82–3.19	0.166	276	1.09	0.93–1.29	0.293
Performance status								
0–1	78	1			681	1		
≥2	4	16.3	4.09–65.2	<0.001	53	3.30	2.44–4.47	<0.001
Disease status								
Inoperable	47	1			506	1		
Recurrent	35	0.77	0.47–1.26	0.300	228	0.73	0.61–0.87	<0.001
Pathology								
Intestinal	39	1			195	1		
Diffuse	43	1.57	0.97–2.54	0.067	521	1.29	1.07–1.56	0.008
Number of metastases								
1	42	1			546	1		
≥2	40	1.42	0.88–2.28	0.153	188	1.47	1.23–1.77	<0.001
Liver metastasis								
Absent	59	1			538	1		
Present	23	1.97	1.19–3.27	0.009	196	1.16	0.97–1.39	0.104
Peritoneal metastasis								
Absent	61	1			332	1		
Present	21	1.22	0.71–2.08	0.467	402	1.00	0.85–1.18	0.991
Lung metastasis								
Absent	61	1			694	1		
Present	21	0.37	0.20–0.68	0.001	40	0.90	0.63–1.29	0.563
Bone metastasis								
Absent	78	1			690	1		
Present	4	2.07	0.49–8.78	0.322	44	1.35	0.97–1.89	0.076
Lymph node metastasis								
Absent	36	1			518	1		
Present	46	1.16	0.71–1.89	0.543	216	1.15	0.97–1.37	0.118
Abdominal lymph node								
Absent	48	1			537	1		
Present	34	0.92	0.57–1.50	0.737	197	1.14	0.95–1.37	0.145
Mediastinal lymph node								
Absent	65	1			712	1		
Present	17	1.29	0.71–2.34	0.400	22	1.28	0.81–2.03	0.286
Cervical lymph node								
Absent	75	1			704	1		
Present	7	2.32	0.98–5.47	0.055	30	1.16	0.77–1.73	0.480

AEGJ adenocarcinoma of esophagogastric junction, *GAC* gastric adenocarcinoma, *N* number of patients, *HR* hazard ratio, *CI* confidence interval

metastasis, and less peritoneal metastasis than patients with gastric cancer are consistent with those of previous reports.

The median survival time of patients with advanced AEGJ was 13.0 months, and there was no significant difference in survival between the patients with AEGJ and those with GAC ($p = 0.445$) in our analysis. In the patients treated with the F + P chemotherapy regimen, the OS was not significantly different between AEGJ and GAC ($p = 0.352$). These survival data for the patients receiving F + P is almost the same as those for inoperable gastric cancer patients who were enrolled and received F + P in Japanese phase III trials [11–13].

Table 3 Multivariate analysis on survival

Factor	Patients with AEGJ			Patients with GAC		
	HR	95 % CI	*p* value	HR	95 % CI	*p* value
Performance status						
0–1	1			1		
≥2	10.56	2.68–41.86	0.001	3.15	2.32–4.27	<0.001
Disease status						
Inoperable				1		
Recurrent			NE	0.76	0.63–0.91	0.002
Pathology						
Intestinal				1		
Diffuse			NE	1.32	1.09–1.59	0.004
Number of metastases						
1				1		
≥2			NE	1.45	1.21–1.75	<0.001
Liver metastasis						
Absent	1					
Present	2.22	1.31–3.78	0.003			NE
Lung metastasis						
Absent	1					
Present	0.33	0.18–0.63	0.001			NE

AEGJ adenocarcinoma of esophagogastric junction, *GAC* gastric adenocarcinoma, *N* number of patients, *HR* hazard ratio, *CI* confidence interval, *NE* not evaluated

We identified poor PS, the presence of liver metastasis, and absence of lung metastasis as baseline prognostic factors in patients with inoperably advanced or recurrent AEGJ. Several studies have identified prognostic factors for patients with metastatic gastric cancer who received first-line chemotherapy: poor PS, the presence of liver, peritoneal, or bone metastases, microscopically scirrhous type tumors, and number of metastatic sites [25, 26]. Chau et al. [27] also elucidated that poor PS and the presence of liver or peritoneal metastases was associated with poor prognosis for patients with advanced esophageal, EGJ, and gastric cancer. The prognostic factors in AEGJ identified in our report are compatible with the prognostic factors reported in EGJ and gastric cancer.

Chau et al. [8] reported that the survival curves of patients with advanced AEGJ and GAC almost overlapped and so it might not be necessary to distinguish patients with advanced esophagogastric adenocarcinoma according to primary tumor origin. Our results were consistent with this report. We consider that the same chemotherapy can be given to both patients with inoperably advanced or recurrent AEGJ and those with GAC in the clinical practice in Japan, and Japanese future trials on gastric cancer chemotherapy can include both subgroups.

This study had several limitations because it was a retrospective, single-institution study. First, because the selection of chemotherapy regimen in patients with AEGJ was not standardized, the study included several chemotherapy regimens and tumor location itself might have influenced regimen selection, although differences were not statistically significant. Second, disease progression was judged by the investigators in this study.

In conclusion, we identified that the incidence, characteristics, treatment outcomes, and prognosis for patients with AEGJ showed no significant differences compared with those for patients with GAC. We consider that Japanese future trials on gastric cancer chemotherapy can include both subgroups.

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ORIGINAL ARTICLE

A phase I study of sorafenib in combination with S-1 plus cisplatin in patients with advanced gastric cancer

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Abstract
Background Sorafenib inhibits several receptor tyrosine kinases involved in tumor progression and angiogenesis. S-1, an oral fluorouracil antitumor drug, plus cisplatin (CDDP) is the standard regimen for advanced gastric adenocarcinoma (AGC) in Japan. The purpose of this phase I study was to evaluate the safety, pharmacokinetics, and preliminary efficacy of sorafenib in combination with S-1 plus CDDP.
Methods Patients with histologically confirmed previously untreated AGC were evaluated for eligibility and treated with sorafenib (400 mg bid, days 1–35), S-1 (40 mg/m^2 bid, days 1–21), and CDDP (60 mg/m^2, day 8). Treatment was continued until disease progression or unacceptable toxicity. Pharmacokinetics for sorafenib, 5-FU, and CDDP were investigated in cycle 1.
Results Thirteen patients were enrolled and received at least one dose of the study treatment. No specific or serious adverse event was newly reported in this study. Five patients had partial response and 8 had stable disease as the best response. Pharmacokinetic analysis showed no significant differences in the exposures of sorafenib when administered alone or in combination with S-1 and CDDP.
Conclusions The present phase I study demonstrates the acceptable toxicity and preliminary efficacy of combined treatment with S-1, CDDP, and sorafenib.

Keywords Sorafenib · Phase I · S-1 · Cisplatin · Gastric adenocarcinoma

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Introduction

Gastric cancer is the second leading cause of cancer death worldwide [1]. The incidence of gastric cancer has decreased during the past two decades, but the mortality rate still ranks second among all cancer types in Japan (Vital Statistics Japan, Ministry of Health, Labour and Welfare, 2009, http://ganjoho.ncc.go.jp/public/statistics/pub/statistics02.html). Although clinical outcomes for gastric cancer have improved, the overall prognosis remains poor, especially in patients with locally advanced or metastatic disease.

Currently, the standard chemotherapeutic regimen for advanced gastric cancer in Japan is a combination of fluoropyrimidines plus platinum. S-1 is a fourth generation of oral fluoropyrimidine composed of tegafur, 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate (Oxo) in 1:0.4:1 molar ratio. S-1 is widely used for the treatment of gastric cancer in Japan and an increasing number of clinical trials have been conducted using this drug. A randomized

phase III study comparing S-1 alone with S-1 plus cisplatin (CDDP) as a first-line treatment showed a significant improvement in the median survival time of patients receiving a combination of S-1 and CDDP (The SPIRITS trial [2]). More recently, a study conducted in China [3] demonstrated similar results to the SPIRITS trial. These clinical trials highlight the importance of S-1 plus CDDP as a first-line chemotherapeutic regimen for gastric cancer. However, the benefit of this new combination regimen is still modest, with a median survival time of approximately 13 months. To further improve the overall prognosis of gastric cancer patients, the development of a new combination regimen is desperately needed.

Recent advances in molecular biology have led to a better understanding of differences between cancer and normal cells at a genetic/molecular level and subsequently to the development of novel therapeutic strategies to specifically block the molecules required for cancer growth and metastasis. So far, many preclinical studies and clinical trials have been conducted using molecular-targeting agents such as epidermal growth factor receptor (EGFR)-targeting agents and vascular endothelial growth factor (VEGF)-targeting agents. Sorafenib (BAY 43-9006), identified through a screening for Raf kinase inhibitors, is a potent multikinase inhibitor and one of the promising targeted drugs currently used in the clinical setting. Antitumor efficacy of sorafenib depends on potent inhibition of c-Raf and wild-type and mutant-b-Raf, combined with inhibition of mitogen-activated protein (MAP) kinase signaling pathway and of tumor angiogenesis via vascular endothelial growth factor receptor-2 (VEGFR-2) and platelet-derived growth factor receptor-β (PDGFR-β) [4]. Sorafenib has been approved in many countries for the treatment of advanced renal cell carcinoma and hepatocellular carcinoma.

Activation of the MAP kinase signaling pathway via EGF/EGFR signaling has been reported in human gastric cancer. Moreover, tumor angiogenesis through the VEGF/VEGFR signaling is involved in the progression of gastric cancer [5, 6]. Therefore, these signaling pathways could be candidate targets for molecular-targeted therapy against gastric cancer. Boku et al. [7] examined the correlation between survival and VEGF expression in patients with gastric cancer treated with S-1 or S-1 plus CDDP. Although the survival of patients with VEGF-negative tumors was slightly longer than those with VEGF-positive tumors in a group treated with S-1 alone, patients with VEGF-positive tumors survived remarkably longer than those with VEGF-negative tumors in a group treated with a combination of S-1 and CDDP. The authors suggested that clinical benefit of adding CDDP to S-1 might be more pronounced in patients with VEGF-positive tumors than in those with VEGF-negative tumors [7]. Another study has shown that EGFR expression correlates with worse prognosis in gastric cancer patients [8]. These findings together suggest the potential efficacy of sorafenib for the treatment of gastric cancer. In fact, the combination of sorafenib, docetaxel, and cisplatin was investigated in a phase II study for the treatment of metastatic or advanced gastric cancer and showed promising results [9].

Addition of sorafenib to the standard regimen of S-1 plus CDDP could enhance the therapeutic efficacy in an additive or synergistic manner and further improve the survival rate of patients with gastric cancer. We therefore conducted this phase I study, before a large-scale controlled trial, to investigate the safety, pharmacokinetics, and pharmacodynamics of sorafenib in combination with S-1 plus CDDP for patients with unresectable or recurrent gastric cancer.

Patients and methods

Patient eligibility

The main inclusion criteria were as follows: patients with histologically or cytologically confirmed unresectable or recurrent gastric adenocarcinoma; age between 18 and 74 years; no prior chemotherapy or immunotherapy; Eastern Cooperative Oncology Group performance status (ECOG-PS) of 0–1; an estimated life expectancy of $\geq$12 weeks; and adequate bone marrow, liver, and renal function within 7 days before dosing [hemoglobin $\geq$8.5 g/dl, absolute neutrophil count (ANC) $\geq$1,500/mm^3, platelet count $\geq$100,000/mm^3, total bilirubin $\leq$1.5 $\times$ upper limit of normal (ULN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leq$2.5 $\times$ ULN, creatinine clearance $\geq$60 ml/min). Prior adjuvant therapy/neo-adjuvant therapy is allowed if recurrence occurred 6 months after completion of these therapies. The main exclusion criteria were as follows: patients with brain metastasis; ascites; active bacterial infection; fungal infection; chronic hepatitis B/C; pregnancy; and hemodialysis.

The institutional review board approval was obtained at each participating institution. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. All patients gave written informed consent after receiving a sufficient explanation before study treatment.

Study design

This is a multicenter (Gastrointestinal Oncology Division, National Cancer Center Hospital, Tokyo, Japan; Division of Gastrointestinal Oncology, National Cancer Center Hospital East, Chiba, Japan; Division of Medical Oncology, Kobe University Hospital, Hyogo, Japan), unblinded,

uncontrolled phase I trial. The primary objective is to investigate the safety and pharmacokinetics of sorafenib administered in combination with S-1 plus CDDP to patients with unresectable or recurrent gastric cancer (chemotherapy-naive for advanced disease). The secondary objective is to investigate preliminary efficacy profile.

The phase III trials, the SPIRITS and the FLAGS studies, using S-1 plus CDDP for advanced gastric cancer-based regimen, were referred to for the study design. Initially three cohorts were planned for this study. Treatment schedule and dosage were set based on the SPIRITS study regimen for cohort 1 and on the FLAGS study regimen for cohorts 2 and 3. Cohorts 2 and 3 were canceled because of negative results of the FLAGS study reported during conduct of cohort 1 [10].

The dosages of S-1 and CDDP in cohort 1 were based on the SPIRITS study as follows: S-1, 40 mg/m^2, p.o. twice daily 21 days followed by 14 days rest; CDDP, 60 mg/m^2, i.v. on day 8. Sorafenib was continuously administered orally at 400 mg twice a day. Each cycle was defined as 35 days. In terms of cycle 1, sorafenib was orally administered continuously on days 2–35 for pharmacokinetics evaluation (Fig. 1). In this study, strict dose reduction criteria in a two-step process were defined for each drug. Dose reduction or discontinuation of the drug was determined according to the grades of the adverse events developed during study treatment. The dose reduction levels of S-1 included level 1 (20 % reduction) and level 2 (35 % reduction). The dose reduction levels of CDDP included level 1 (25 % reduction) and level 2 (50 % reduction). The dose reduction levels of sorafenib included level 1 (50 % reduction, 400 mg once daily) and level 2 (75 % reduction, 400 mg once every second day).

Pharmacokinetics (PK)

Patients who have at least one evaluable PK profile were valid for PK analysis. Blood samples (5-ml aliquots) for the determination of plasma concentration of sorafenib were collected at 0 h (pre-morning dose), and 1, 2, 4, and 8 h after morning dose, and before evening dose (~12 h after morning dose) on days 8 and 35 in cycle 1.

Blood samples (10-ml aliquots) for the determination of plasma concentration of 5-FU (for S-1) were collected at 0 h (pre-morning dose), and 1, 2, 4, and 8 h after morning dose and before evening dose (~12 h after morning dose) on days 1, 8, and 15 in cycle 1.

Blood samples (6-ml aliquots) for the determination of plasma concentration of total and free platinum were collected at 0 h (pre-dose), end of infusion, and 2 h after end of infusion on day 8 in cycle 1, at 24 h after starting of infusion, and at any three time points among 48, 72, 96, 120, and 144 h after starting of infusion.

Plasma concentrations of sorafenib and 5-FU were determined by validated liquid chromatography–tandem mass spectrometry (LC/MS/MS) after protein precipitation.

Plasma platinum concentrations were measured by quantifying total and free platinum by validated flameless atomic absorption spectrometry (FAAS) after dilution [11]. Depending on the concentration of the sample, a certain volume of Triton-X solution (1 %) or nitric acid (6.5 %) was added. For analysis of free platinum in plasma, plasma samples were centrifuged with filter devices (Millipore, Centrifree YM-30) to obtain an ultrafiltrate fraction.

The pharmacokinetic parameters of sorafenib, total and free platinum, and 5-FU were calculated using the model-independent (non-compartment) method and the PC program WinNonlin.

Pharmacodynamics (PD)

Plasma protein biomarkers

To measure protein levels in plasma, enzyme-linked immunosorbent assay (ELISA) kits for EGFR (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), HER2/neu (Siemens Healthcare Diagnostics), PDGFR-β (R&D Systems, Minneapolis, MN, USA), VEGF (detecting VEGF-

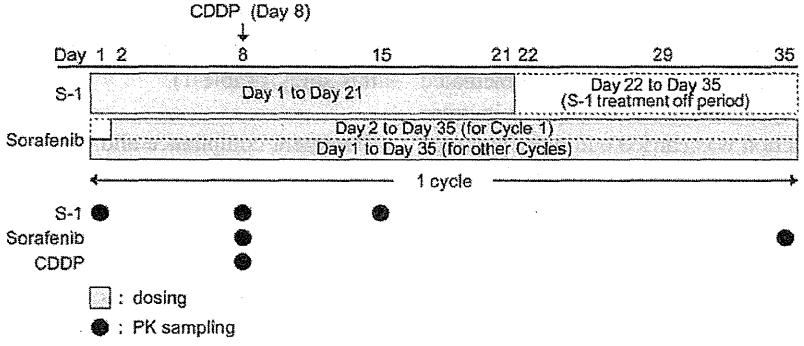

Fig. 1 Treatment cycle [drug administration and pharmacokinetics (PK) sampling schedule]

165; R&D Systems), and sVEGFR-2 (R&D Systems) were used according to the manufacturers' specifications.

Immunohistochemical (IHC) analysis

The anti-VEGFR2 rabbit monoclonal antibody (mAb) (clone 55B11), the anti-AKT rabbit mAb (clone C67E7), the anti-phosphorylated AKT (pAKT) rabbit mAb (clone D9E), the anti-ERK mouse mAb (clone 3A7), and the anti-phosphorylated ERK (pERK) rabbit mAb (clone D13.14.4E) were purchased from Cell Signaling Technology (Danvers, MA, USA). The anti-pAKT rabbit mAb (clone 14-5), was purchased from Dako (Carpinteria, CA, USA). The anti-pERK mouse mAb (clone MAPK-YT) was purchased from Sigma (St. Louis, MO, USA). The mouse IgG isotype control antibody and the rabbit IgG isotype control antibody were purchased from Dako. For AKT, pAKT, ERK, and pERK, the staining intensity of each section was determined by comparing the intensity of a control slide containing an adjacent section stained with an irrelevant, negative control antibody that is species- and isotype matched to the test article. The staining intensity was classified as follows: 0, no staining relative to background; 1+, weak staining; 2+, moderate staining; and 3+, strong staining. The H-score was calculated based on the summation of the product of percent of cells stained at each intensity using the following equation: (3 × % cells staining at 3+) + (2 × % cells staining at 2+) + (1 × % cells staining at 1+). Evaluation of VEGFR2 was performed by pixel analysis. Stained slides were scanned on an Aperio CS Digital Slide Scanner (Vista, CA, USA), and results were evaluated using the Positive Pixel V9 algorithm. The fraction of positive to total stained pixels was determined and reported as the percent positive for each staining threshold.

Microarray analysis of biopsy specimens

RNA was isolated from endoscopic biopsy specimens according to the Qiagen RNeasy Micro Handbook (Qiagen, Hilden, Germany, 2007). An aliquot (100 ng) of total RNA from each biopsy specimen was first reverse transcribed using a T7-Oligo(dT) Promoter Primer in the first-strand cDNA synthesis reaction followed by a RNase H-mediated second-strand cDNA synthesis. The subsequent in vitro transcription (IVT) reaction was carried out in the presence of T7 RNA polymerase and a biotinylated nucleotide analogue/ribonucleotide mix for amplified RNA (aRNA) amplification and biotin labeling. The labeled aRNA was fragmented and hybridized to an oligonucleotide microarray HG-U133 Plus 2.0 array (Affymetrix, Santa Clara, CA, USA). The arrays were washed and stained using the GeneChip Fluidics Station 450, then scanned by GeneChip-3000 Scanner at 570 nm. All reactions were performed using the 3'IVT Express Labeling and Control Reagents from Affymetrix. Data were analyzed using the Affymetrix MAS 5.0 algorithm. Tests for differential expression were performed for each transcript separately and did not take dependencies between transcripts into account. The transcripts were then sorted by the respective measures of statistical significance (p values). Adjustments for multiple hypothesis testing were performed through the use of the false discovery rate (FDR). Statistical analysis was performed by means of the SAS System Version 9.1.3 (SAS Institute, Cary, NC, USA) and the Bioconductor packages [12] from release 2.7 within R software version 2.12.1. A gene ontology (GO) classification of the differentially expressed genes with corrected p values <0.05 was carried out by the GO Term Finder [13].

Clinical assessment

Physical examination, complete blood cell counts, serum chemistries, and urinalysis were carried out at baseline and at least every week for the first two cycles after initiating treatment and three times per cycle during cycle 3 and 4, two times per cycle from cycle 5. All observations on the safety of the treatment were recorded, and patients were routinely monitored for adverse events, which were recorded with severity and relationship to study medication according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Tumor response was assessed every 8 weeks for the first six cycles and every 12 weeks after cycle 6 by Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0 using the same imaging techniques and methods used at baseline.

Results

Patient characteristics

From May 2008 to January 2009, a total of 13 chemo-naive patients (10 male and 3 female patients with a mean age of 61 years) with advanced gastric cancer were enrolled in this study (Table 1).

Treatment compliance and duration

The median number of treatment cycles was 4 (range 1–12 cycles). Of 13 patients, 8 were able to continue at least 4 cycles of treatment, and 6 of them had received 5 cycles or more. The median relative dose intensity (RDI) of sorafenib (50.0 %) was lower than those of S-1 (89.3 %) or CDDP (92.0 %) (Table 2). Focusing on median RDI from

cycle 1 to 4, RDI of sorafenib was gradually decreasing, whereas those of S-1 and CDDP were sustained around 80 % (Table 3); this could be partly because dose reduction criteria were different between these drugs (50 % reduction for sorafenib versus 20 % for S-1 and 25 % for CDDP at level 1). Relatively large numbers of patients required dose reduction or interruption because of adverse events of sorafenib or S-1 (Table 4). Seven of 13 patients discontinued study treatment permanently for reasons of adverse events such as diarrhea, elevation of transaminase, myelosuppression, and hand-foot skin reaction (Table 5). The other 6 patients discontinued treatment permanently because of progressive disease (PD).

Table 1 Baseline demographics and disease characteristics

$n = 13$	n (%)		n (%)
Sex		Histological type	
Male	10 (77)	Adenocarcinoma	13 (100)
Female	3 (23)	(tub:por:sig:muc)	(6:5:1:1)
Age (years)		Sites of metastatic lesion	
Median (range)	61 (41–72)	Lymph nodes	10 (77)
ECOG performance status		Liver	7 (54)
0	9 (69)	Peritoneum	1 (8)
1	4 (31)	Lung	1 (8)
Prior gastrectomy		Bone	1 (8)
Yes	3 (23)[a]		
No	10 (77)		
Prior systemic anticancer therapy			
Yes	0 (0)		
No	13 (100)		

ECOG Eastern Cooperative Oncology Group, *tub* tubular adenocarcinoma, *por* poorly differentiated adenocarcinoma, *sig* signet ring cell carcinoma, *muc* mucinous adenocarcinoma

[a] Distal gastrectomy

Table 2 Received dose intensity (%) of each drug

	Sorafenib (%) ($n = 13$)	S-1 (%) ($n = 13$)	CDDP (%) ($n = 13$)
Mean (SD)	53.9 (18.3)	85.2 (13.1)	86.2 (16.7)
Median (range)	50.0 (31.8–96.0)	89.3 (55.0–100.0)	92.0 (50.0–100.0)

SD standard deviation

Safety

The most common adverse events were anorexia (100 %), rash/desquamation (100 %), neutropenia (92 %), thrombocytopenia (92 %), hand-foot reaction and nausea (85 %), leukopenia, fatigue, and elevation of lipase (77 %) (Table 6). Seven (54 %) patients discontinued study treatment because of adverse events such as diarrhea (2 patients), anemia, neutropenia, gastric perforation, hand-foot skin reaction, transaminase increase (1 patient, respectively). Although the ratio of skin rash was high (100 %), all were relatively mild (grades 1 and 2), and only a few patients required dermatologist consultation. No treatment-related death was observed during the study period.

Efficacy

Tumor response was evaluated in 12 patients having a target lesion for assessment according to RECIST 1.0 criteria by the investigator. The overall response rate was 38.5 % [95 % confidence interval (CI), 13.9–68.4] (Table 7). Remarkably, all the 12 patients evaluable for tumor response showed reduction of target lesion after study treatment compared with baseline on one level or another (Fig. 2).

The median change in percentage of target lesions was −22.9 % (range −8.9 to −100 %). In 1 patient, the target lesion was shrunk by 100 % after study treatment, whereas all the non-target legions did not show CR. Therefore, this patient was regarded as PR overall.

One-year survival rate was 76.9 % (95 % CI, 44.2 %, 91.9 %).

Pharmacokinetics (PK)

Sorafenib

Plasma concentration–time profiles of sorafenib were available in 4 patients on day 35 (sorafenib alone) and in 11

Table 3 Relative dose intensity (RDI) of each drug at each treatment cycle

		Sorafenib (%)		S-1 (%)		CDDP (%)
Cycle 1	$n = 13$	77.8 (41.2–100.0)	$n = 13$	100.0 (61.9–100.0)	$n = 13$	100.0 (100.0–100.0)
Cycle 2	$n = 11$	46.4 (28.1–100.0)	$n = 11$	81.0 (45.8–100.0)	$n = 10$	100.0 (0.0–100.0)
Cycle 3	$n = 8$	47.2 (25.0–100.0)	$n = 8$	83.3 (66.7–100.0)	$n = 8$	75.0 (0.0–100.0)
Cycle 4	$n = 8$	36.0 (25.0–74.3)	$n = 8$	83.3 (56.9–100.0)	$n = 7$	75.0 (75.0–100.0)

Values are expressed as median (range)

Table 4 Number of patients who required dose reduction or interruption because of adverse events from each drug

	Sorafenib	S-1	CDDP
Number of subjects with dose reduction			
No	1 (8 %)	3 (23 %)	8 (62 %)
Yes	12 (92 %)	10 (77 %)	5 (38 %)
Number of subjects with dose interruption			
No	0	5 (38 %)	9 (69 %)
Yes	13 (100 %)	8 (62 %)	4 (31 %)

Efficacy analysis set, $n = 13$

Table 5 Adverse events leading to discontinuation of study drug in seven patients

Case number	Adverse event	End of cycle
1	AST	Cycle 1
	ALT	
2	Diarrhea	Cycle 2
	Mucositis	
3	Perforation, GI, Stomach	Cycle 2
4	Hypophosphatemia	Cycle 1
	Diarrhea	
5	Hemoglobin	Cycle 6
6	Hand-foot skin reaction	Cycle 2
7	Neutrophils	Cycle 4

patients on day 8 (in combination of S-1 plus CDDP). There appeared to be no difference in the geometric mean plasma concentration profiles of sorafenib between in patients receiving sorafenib alone and those receiving a combination of three drugs (Fig. 3).

We evaluated geometric means [% coefficient of variation (CV)] C_{max} and AUC_{0-tn} of sorafenib in patients receiving sorafenib alone (day 35) and in those receiving a combined administration of sorafenib, S-1, and CDDP (day 8), as well as the mean ratios of C_{max} and AUC_{0-tn} of sorafenib (value at combination treatment on day 8/value at sorafenib monotherapy on day 35) in four patients obtained on both day 8 and day 35 (Table 8). These pharmacokinetic data showed no significant change in AUC_{0-tn} and C_{max} of sorafenib under a combined administration of S-1 and CDDP.

5-FU

Plasma concentration–time profiles of 5-FU were available in 13 patients on day 1 (S-1 alone) and day 8 (in combination with sorafenib and CDDP).

Geometric means of plasma 5-FU concentrations were slightly higher when 5-FU was administered in combination with the other two drugs (day 8) than that when administered alone (day 1) (Fig. 4).

Mean ratios (day 8/day 1) of C_{max} and AUC_{0-tn} of 5-FU were 1.64 and 1.56, respectively, in 13 patients in whom these measurements were obtained on both day 1 and day 8 (Table 9).

Both AUC_{0-tn} and C_{max} were higher when S-1 was administered in combination with the other drugs (day 8) than that when administered alone (day 1).

CDDP

Because the PK of CDDP administered alone was not examined in this study, the effect of concomitant administration of sorafenib and S-1 on the PK of CDDP could not be evaluated.

Geometric mean (%CV) C_{max} and AUC_{0-tn} of total platinum on day 8 were 3.06 mg/l (14.5 %) and 152.28 mg·h/l (14.8 %), respectively (Table 10). Geometric mean (%CV) C_{max} and AUC_{0-tn} of free platinum on day 8 was 1.25 mg/l (15.8 %) and 4.47 mg·h/l (39.6 %), respectively (Table 10). T_{max} was 1.98 h for both total platinum and free platinum.

Pharmacodynamics (PD)

Blood samples were taken from 12 patients during screening and on day 15 of cycle 1. Changes in plasma concentrations of biomarkers, including VEGFR2, VEGF, PDGFR-β, EGFR, and HER2/neu, were plotted individually and compared before and during treatment (day 15) (Fig. 5). Among these markers, plasma concentration of EGFR tended to increase more during treatment in patients showing PR [median percent change from screening, 55.9 % ($n = 4$)] than in those showing SD [median percent change from screening, −2.0 % ($n = 8$)] classified according to RECIST.

For IHC analysis, six patients giving his/her consent provided two endoscopic biopsy specimens taken at a screening period and during treatment (at the end of cycle 2). In total, 24 formalin-fixed, paraffin-embedded (FFPE) tumor specimens were analyzed. After review of hematoxylin and eosin (H&E)-stained slides, 12 specimens (6 pairs of specimens obtained at a screening period and during treatment) were selected and stained for VEGFR2, AKT, pAKT, ERK, and pERK. IHC analyses for pAKT and pERK were done with two mAbs because both clones indicated adequate specificity and sensitivity for IHC assays in Western blot analysis and FFPE cell pellet IHC experiments (data not shown). Decrease in pAKT staining during treatment was shown by two different antibodies in patient 20001-1003 while total AKT increased. However, change in pAKT was not obvious in another patient. Also, a slight

Table 6 Incidence of adverse events by NCI CTCAE version 3.0

AE term	$n = 13$					
	Grade					
	1	2	3	4	All	≥3
Hematological						
Neutropenia	0	5	4	3	12 (92 %)	7 (54 %)
Thrombocytopenia	5	4	3	0	12 (92 %)	3 (23 %)
Leukocytopenia	2	5	2	1	10 (77 %)	3 (23 %)
Anemia	0	4	3	0	7 (54 %)	3 (23 %)
Lymphopenia	0	2	1	0	3 (23 %)	1 (8 %)
Nonhematological						
Anorexia	7	4	2	0	13 (100 %)	2 (15 %)
Rash/desquamation	9	4	0	0	13 (100 %)	0
Hand-foot skin reaction	2	6	3	0	11 (85 %)	3 (23 %)
Metabolic/lab, other	2	9	0	0	11 (85 %)	0
Nausea	8	2	1	0	11 (85 %)	1 (8 %)
Fatigue	6	1	3	0	10 (77 %)	3 (23 %)
Lipase	0	2	5	3	10 (77 %)	8 (62 %)
Alopecia	8	1	0	0	9 (69 %)	0
Amylase	5	1	3	0	9 (69 %)	3 (23 %)
Diarrhea	4	2	2	0	8 (62 %)	2 (15 %)
Mucositis (functional/symptomatic), oral cavity	3	2	2	0	7 (54 %)	2 (15 %)
Vomiting	6	1	0	0	7 (54 %)	0
Bilirubin (hyperbilirubinemia)	1	4	1	0	6 (46 %)	1 (8 %)
Constipation	5	1	0	0	6 (46 %)	0
Hiccoughs	1	5	0	0	6 (46 %)	0
AST	2	2	0	1	5 (39 %)	1 (8 %)
Hypertension	1	2	2	0	5 (39 %)	2 (15 %)
ALT	3	0	0	1	4 (31 %)	1 (8 %)
Fever	2	2	0	0	4 (31 %)	0
Hyperpigmentation	4	0	0	0	4 (31 %)	0
Hypophosphatemia	0	2	2	0	4 (31 %)	2 (15 %)
Periodontal	3	1	0	0	4 (31 %)	0
Taste alteration	3	1	0	0	4 (31 %)	0
Flu-like syndrome	3	0	0	0	3 (23 %)	0
Hemorrhage, other (specify)	3	0	0	0	3 (23 %)	0
Nail changes	3	0	0	0	3 (23 %)	0
Proteinuria	2	1	0	0	3 (23 %)	0
Weight loss	2	1	0	0	3 (23 %)	0

increase in pERK was observed by two different antibodies while total ERK remained unchanged (Table 11).

For exploratory gene expression analysis, 30 endoscopic biopsy specimens were obtained from 9 patients during a screening period and during treatment (at the end of cycle 2). One sample whose scaling factor was out of range was excluded from statistical analysis. Differential expression analysis identified a total of 624 differentially regulated probe sets in response to treatment. Analysis of the error rates indicated that the differences between differentially and nondifferentially expressed probe sets were not very well resolved. Among 624 probe sets, 469 could be assigned to GO terminologies. Of the 10 transcripts showing the most significant differential expression, 7 fell under GO annotations indicating their involvement in cell structural components (cytoskeleton, cell membranes) or membrane transport processes. Another transcript (AC133561.1), although not annotated in the GO, was identified as a transmembrane transport protein (Table 12).

Table 7 Response rate and best overall response based on investigator assessment

	$n = 13$
Response rate (95 % CI)	38.5 % (13.9–68.4)
Complete response (CR)	0
Partial response (PR)	5 (38.5 %)
Stable disease (SD)	8 (61.5 %)
Progressive disease (PD)	0

CI confidence interval

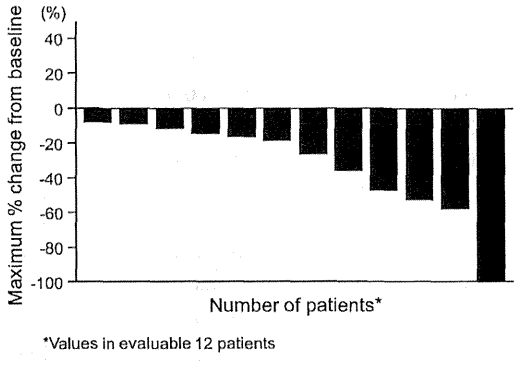

Fig. 2 Maximum percent reduction of target lesions in individual patients

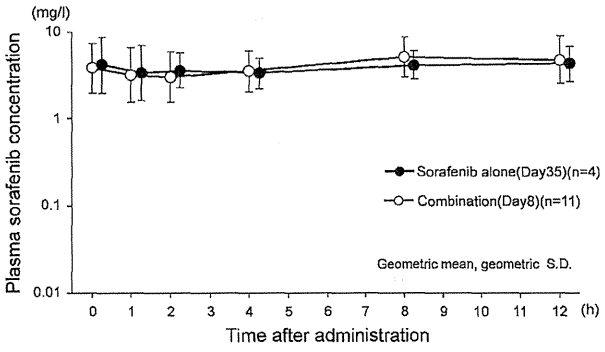

Fig. 3 Serial change in plasma concentrations of sorafenib in patients receiving sorafenib alone (400 mg p.o., twice a day on days 2–35) and in those receiving a combined administration of sorafenib, S-1, and cisplatin (CDDP) (S-1, 40 mg/m^2, p.o., twice a day for 21 days followed by 14 days rest; CDDP, 60 mg/m^2, i.v., on day 8; sorafenib, 400 mg p.o., twice a day on days 2–35 for cycle 1 and days 1–35 for other cycles)

Discussion

In this phase I clinical trial, we evaluated the safety, pharmacokinetics, and efficacy of sorafenib in combination with S-1 and CDDP in Japanese patients with advanced gastric cancer. This combination therapy was tolerated and demonstrated antitumor activity. Pharmacokinetic results suggested that the combination of S-1 and CDDP did not affect the PK of sorafenib. There was no unpredictable adverse event associated with the combination of the three drugs. Analysis of biomarkers suggested a possible link between plasma EGFR level and treatment response.

Sorafenib is a promising antitumor agent against a broad range of tumors, including hepatocellular carcinoma and renal cell carcinoma. Sorafenib has also been tested for its efficacy in gastric cancer in combination with other chemotherapeutic agents. Recently, a phase I study was

Table 8 Pharmacokinetics parameters of sorafenib

Sorafenib		Day 35 (alone)	Day 8 (combination)	Combination/ alone ratio
C_{max}	n	4	11	4
	mg/l	5.582 (51.6)	5.872 (58.7)	1.02 (0.62–1.66)
AUC_{0-tn}	n	4	11	4
	mg·h/l	38.551 (39.2)	37.348 (53.6)	0.90 (0.59–1.39)

Geometric mean (% coefficient of variation)

Combination/alone ratio is given as ratio (90 % confidence interval)

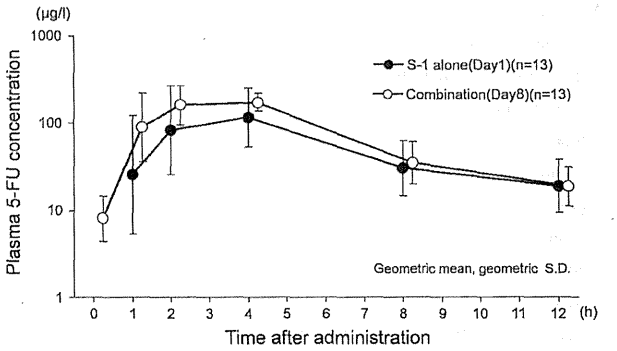

Fig. 4 Serial change in plasma concentrations of fluorouracil (5-FU) in patients receiving S-1 alone (40 mg/m^2, p.o., twice a day) and in those receiving a combined administration of sorafenib, S-1, and CDDP (S-1, 40 mg/m^2, p.o., twice a day for 21 days followed by 14 days rest; CDDP, 60 mg/m^2, i.v., on day 8; sorafenib, 400 mg p.o., twice a day on days 2–35 for cycle 1 and days 1–35 for other cycles)

Table 9 Pharmacokinetics parameters of fluorouracil (5-FU)

5-FU		Day 1 (alone)	Day 8 (combination)	Combination/ alone ratio
C_{max}	n	13	13	13
	mg/l	0.123 (95.6)	0.202 (33.1)	1.64 (1.24–2.16)
AUC_{0-tn}	n	13	13	13
	mg·h/l	0.613 (82.0)	0.955 (24.6)	1.56 (1.19–2.04)

Geometric mean (% coefficient of variation)

Combination/alone ratio is given as ratio (90 % confidence interval)