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Multi-Institutional Phase II Study of S-1 Monotherapy in Advanced Gastric Cancer with Pharmacokinetic and Pharmacogenomic Evaluations

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ABSTRACT

This study describes the first phase II study of S-1, a novel oral fluoropyrimidine, in a non-Japanese Asian population with advanced gastric cancer. S-1 was administered twice daily for 28 days every 6 weeks. A pharmacokinetic study was performed on day 28 of cycles 1 and 3. Genomic DNA from peripheral mononuclear cells was analyzed using a cDNA microarray-based comparative genomic hybridization (CGH) method. Thirty-one patients were initially given a dose of 35 mg/m^2 twice daily (bid) (group 1); then, the protocol was amended by increasing the dose to 40 mg/m^2 bid for an additional 31 patients (group 2) because of good tolerability to S-1. The overall response rate was 19.3% (95% confidence interval, 9.2%–29.5%). Over a median follow-up duration of 265 days, the median time to progression and overall survival time were 126 and 264 days, respectively. The 1-year survival rate was 34%. There was no grade 4 toxicity and the major adverse event was anemia. Pharmacokinetic parameters were similar to those of the previous Japanese reports. Microarray CGH identified 18 genes with copy number changes that were associated with hemoglobin reduction with S-1 treatment. A logistic regression analysis, integrating one clinical parameter (initial hemoglobin level) combined with three genetic copy number variations (*HIST1H2BL*, *C10orf127*, and *XPNPEP2*), provided a predictive model for the development of severe hemoglobin reduction. In conclusion, this study showed the feasibility of using S-1 at 35 mg/m^2 bid in gastric cancer. We suggest that the pharmacogenomic markers identified in this study may be potential candidates for predicting anemia after S-1 treatment. ***The Oncologist*** 2007;12:543–554

Disclosure of potential conflicts of interest is found at the end of this article.

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Introduction

Systemic chemotherapy is tried in advanced gastric cancer in order to improve patient survival and quality of life [1–3]. 5-Fluorouracil (5-FU) has served as a mainstay of chemotherapy in this setting. Bolus injection of 5-FU showed a 13%–20% response rate while its protracted continuous infusion (PCI) showed an 18%–26% response rate [4–8]. Although few full-scale trials have been conducted to directly compare these two schedules of 5-FU, PCI is regarded as an acceptable reference treatment in gastric cancer, with less myelosuppression and diarrhea.

S-1 is a fourth-generation oral fluoropyrimidine that was developed to mimic PCI of 5-FU. A high 5-FU level was maintained both in plasma and in tumor without increasing gastrointestinal toxicity by combining tegafur (FT) with two biochemical modulators, 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate (Oxo) [9, 10]. With S-1 monotherapy, early and late phase II trials in Japan achieved promising efficacy rates of 54% and 45%, respectively [11–14]. However, the dose administered was not the same between these studies. Derived from the result of a phase I study, the early phase II study adopted a fixed dose of 75 mg twice a day (bid). However, skin rash and diarrhea hindered further progress of the study with this dose, and the dose had to be reduced to 50 mg bid. After reconsideration of the safety profile of S-1, the dose for the late phase II study was modified, ranging from 64 mg/m^2 per day to 80 mg/m^2 per day according to body surface area (BSA)—80 mg for BSA $<$1.25 m^2, 100 mg for BSA 1.25–1.50 m^2, 120 mg for BSA $\geq$1.50 m^2. The safety profile was better, and based on this dosage S-1 has been a preferred oral agent in gastric cancer in Japan [15]. However, such a high initial tumor response for S-1 has not been reproduced beyond Japan [16, 17]. A phase I U.S. trial had to restrict the clinically recommended dose to 60 mg/m^2 a day because of dose-limiting toxicities (DLTs) of diarrhea and hyperbilirubinemia; and in one European phase II trial as well, diarrhea caused immediate dose reduction of the initial daily dose of 80 mg/m^2 after a few patients were enrolled [16, 18]. These findings suggest that some kind of ethnic differences may contribute to treatment outcome with S-1.

The present study describes a phase II trial of S-1 monotherapy that is the first conducted in a non-Japanese Asian population. The primary objectives were to determine the efficacy and safety of S-1 in previously untreated advanced gastric cancer. As part of the secondary objectives, we also performed a pharmacokinetic study and pharmacogenomic evaluations using cDNA microarray-based comparative genomic hybridization (CGH) to find adverse event–related biomarkers.

Materials and Methods

Patient Selection

This study was designed as an open-labeled multi-institutional phase II trial. Patients were required to have: histologically confirmed gastric adenocarcinoma with inoperable or metastatic disease; age $\geq$18 years; a performance status score $\leq$2 according to the Eastern Cooperative Oncology Group criteria; a life expectancy of $\geq$3 months; no prior chemotherapy for advanced disease (adjuvant chemotherapy had to have been completed at least 6 months before enrollment); bidimensionally measurable lesions; and adequate organ function (WBC $\geq$4,000/μl, hemoglobin (Hb) $\geq$ 9.0 g/dl, platelets $\geq$100,000/μl, serum creatinine $\leq$1.5$\times$ upper limit of normal (ULN), total bilirubin $\leq$1.25$\times$ ULN, and serum aminotransferase $\leq$2.5$\times$ ULN). Patients must not have had other active malignancies, brain metastasis, or severe comorbid conditions. After the protocol was approved by the institutional review board (IRB), written informed consent with ICH Guidelines was obtained from patients according to each institution's regulations.

Treatment Protocol

The initially planned dose of S-1 for this trial was 35 mg/m^2 bid (Table 1). The patients were assigned to take S-1 according to BSA, which was rounded off, as in Table 1. S-1 was administered within 1 hour after meals (breakfast and supper) for 28 consecutive days, followed by a 2-week rest. The schedule was repeated until the occurrence of disease progression, unacceptable adverse events, or the patient's withdrawal. If grade $\geq$3 adverse events were shown in the previous course, the dose for the next cycle was reduced by 5 mg/m^2, which corresponds to 30 mg/m^2 bid. Patients who required $\geq$4 weeks of rest for recovery from any toxicity other than alopecia, nausea, vomiting, and anemia, or who required a dose reduction of more than two steps (i.e., 10 mg/m^2, corresponding to 25 mg/m^2 bid), were withdrawn from the study.

Evaluation of Patients

Pretreatment evaluations included a complete medical history, performance status assessment, physical and radiological examinations, electrocardiography, and clinical laboratory tests including CBC, serum biochemistry, and urinalysis. During treatment, patients were evaluated with a weekly CBC. The physical examination, performance status evaluation, imaging studies for tumor measurement, and serum biochemistry tests were re-evaluated prior to each cycle. Imaging studies for tumor response were performed after each cycle. Tumor response was measured bidimen-

Table 1. Dose schedule of S-1 according to body surface area (BSA)

Dosage schedule	Group 1 35 mg/m^2		Group 2 40 mg/m^2		Japanese	
BSA (m^2)	Calculated dose (mg)	Actual dose (mg)	Calculated dose (mg)	Actual dose (mg)	BSA (m^2)	Actual dose (mg)
<1.36	<47.6	45	<54.4	50	<1.25	40
1.36–1.57	47.6–55.0	50	54.4–62.8	60	1.25–1.50	50
1.58–1.78	55.3–62.3	60	63.2–72	70	≥1.50	60
1.79–1.92	62.6–67.2	65	71.6–76.8	75		
1.93–2.07	67.5–72.4	70	77.2–82.8	80		
≥2.08	≥72.8	75	≥83.2	80		

The patients were assigned to take S-1 twice a day for 28 consecutive days followed by a 2-week rest. The Japanese dose schedule is from Sakata et al. [14].

sionally according to the World Health Organization criteria. Patients were considered as evaluable for response if they had received a minimum of one cycle of treatment with at least one follow-up tumor measurement. Primary gastric mass, peritoneal thickening, or ascites were considered nonmeasurable, and follow-up gastroduodenoscopy was planned for patients with a complete response (CR). Adverse events were recorded every week and graded according to the National Cancer Institute Common Toxicity Criteria (Version 2.0).

Statistical Consideration of the Phase II Trial

With reference to the previous results for PCI of 5-FU, S-1 was considered active if the response rate exceeded 20%. The hypothesis was: H0, $p < p_0$ (0.10) versus H1, $p > p_1$ (0.20), with $\alpha = 0.05$ and $\beta = 0.10$ (90% power). According to Simon's optimal design and considering a 10% dropout rate, 49 patients who met the criteria for response evaluation were required [19].

However, after the first 31 patients were enrolled, investigators decided to escalate the dose of S-1 because most of the patients tolerated S-1 well and showed favorable compliance. After protocol amendment and IRB reapproval, the study proceeded with this dose increase in another 31 patients. Thus, the initially enrolled 31 patients (group 1) continued the study with dosage based on 35 mg/m^2 bid, while the 31 newly enrolled patients were treated with an S-1 dosage based on 40 mg/m^2 bid (group 2), resulting in 62 patients enrolled overall (Table 1). The dose-modification strategy and schedule for group 2 were the same as for group 1, except for the lowest permitted dosage (25 mg/m^2 bid for group 1 and 30 mg/m^2 bid for group 2). The data were analyzed according to all enrolled patients and for each dose group as well. Statistical analysis was done using the SPSS program (version 12.0, SPSS, Inc., Chicago, IL). Time-dependent variables were estimated using the Kaplan-Meier method.

Pharmacokinetic Study

A pharmacokinetic study was performed in the first and third cycles for three patients in each dosing group. On day 28, peripheral blood was collected before and 1, 2, 3, 6, 8, 10, 14, 24, and 48 hours after the final administration of S-1. Plasma was isolated and stored at −80°C. Urine samples were collected 12 hours before the last dose of S-1 and for the periods of 0–6, 6–12, 12–18, and 18–24 hours after S-1 treatment. After estimation of the total urine volumes, 10-ml samples were stored at −80°C until analysis.

Analyses of FT, 5-FU, CDHP, and Oxo were conducted according to the method described by Matsushima et al. [20, 21]. Briefly, FT was extracted with dichloromethane from each sample and analyzed using HPLC equipped with a UV absorption spectrophotometer. 5-FU and CDHP were extracted with ethyl acetate, and Oxo was separately extracted using a solid extraction column. They were analyzed using a negative ion chemical ionization-gas chromatography mass spectrophotometer. The lower measurable limit of plasma levels for FT, 5-FU, CDHP, and Oxo were 10, 1, 2, and 1 ng/ml, respectively.

cDNA-based Microarray CGH

We used 17K cDNA microarray containing 15,723 unique genes for the cDNA-based microarray CGH. The whole experiment was performed according to the protocol of the Cancer Metastasis Research Center, Yonsei University College of Medicine, Korea, in a sex-matched design [22, 23]. Briefly, 8 μg of genomic DNA isolated from patients' peripheral mononuclear cells was labeled with Cy3- or Cy5–2′-deoxyuridine 5′-triphosphate, using a Bioprime labeling kit (Invitrogen, Carlsbad, CA). The labeled probes

were then mixed with human Cot-1 DNA (GIBCO-BRL, Gaithersburg, MD), yeast tRNA (GIBCO-BRL), and poly-A RNA (Sigma, St. Louis, MO). After concentration and denaturation, the probe mixture was applied to the microarray and hybridized in a hybridization chamber at 65°C for 16 hours.

After hybridization, slides were scanned using a GenePix 4000B scanner (Axon Instruments, Foster City, CA) and TIFF images were analyzed. The signal intensity of each spot was transformed as the $\log_2$ red to green (R/G) ratio. Whole microarray spots were mapped for their chromosomal location, using the software SOURCE (http://genome-www5.stanford.edu/cgi-bin/source/sourceSearch) and DAVID (http://apps1.niaid.nih.gov/david/). Within-slide global normalization was applied, which subtracted the median intensity ratio of the $\log_2$(R/G) from the $\log_2$-transformed data.

Pharmacogenomic Study of S-1–Associated Anemia

Patients were categorized according to the degree of hemoglobin (Hb) reduction per treatment cycle into two groups: the mild reduction group (MRG) and the severe reduction group (SRG). To identify genetic changes that could discriminate between the two groups, we selected genes showing: (a) copy number variations, defined as an amplification ($\log_2$(R/G) >0.68) or a deletion ($\log_2$(R/G) < -0.68); and (b) a frequency difference $\geq$30% between the MRG and SRG [24]. Using the binary outcomes of the MRG and SRG as the dependent variable, a best logistic regression model was identified by performing stepwise selection. The diagnostic accuracy of this model with regard to the severity of Hb reduction was quantified via prediction accuracy and receiver operating characteristic (ROC) analysis.

RESULTS

Patient Characteristics

From September 2003 to November 2004, a total of 62 patients was enrolled. Fifty-seven patients were evaluable for response. Four patients withdrew their consent after the first cycle of treatment, and one patient stopped treatment as a result of rapid symptomatic deterioration during the first cycle of treatment.

The median BSA of all patients was 1.67 m^2 (range, 1.27–2.09). Thirteen patients received prior adjuvant chemotherapy after curative gastrectomy. Liver and abdominal lymph nodes were common sites for measurable lesions, and a primary gastric mass was the main nonmeasurable lesion. The average size of the measurable lesions was 1,200 mm^2 (range, 115–2,392), and the median number of measurable lesions per patient was two (range, 1–5). Baseline characteristics of these patients are presented in Table 2.

Treatment Outcomes

A total of 163 cycles (82 in group 1; 81 in group 2) was administered, with a median number of cycles of two (range, 1–10). The median dose administered was 120 mg in group 1 (range, 90–150) and 140 mg in group 2 (range, 100–150). The planned dose intensity was 327 mg/m^2 per week in group 1 and 373 mg/m^2 per week in group 2. In group 1, only two patients were subjected to a dose reduction because of an adverse event, resulting in a median actual dose intensity (ADI) of 327 mg/m^2 per week (relative dose intensity [RDI], 1.00) (range, 236–327). In group 2, dose reduction occurred in seven patients, and the median ADI was 367 mg/m^2 per week (RDI, 0.98; range, 189–373). The overall median dose intensity was 327 mg/m^2 per week, and no treatment delay was recorded in either group.

If we analyzed dose intensity according to the daily dose administered, there was no significant difference in the median RDI for each dose level (100 mg, 0.97; 120 mg, 0.99; 130 mg, 1.0; 140 mg, 1.0; 150 mg, 0.92). This suggests that S-1 showed favorable compliance throughout all dose levels.

Efficacy

The confirmed overall response rate was 19.3% by intent-to-treat analysis (95% confidence interval [CI], 9.2%–29.5%). All the responses were partial responses (PRs), without a CR (Table 3). The median time to response was 35 days (range, 35–245), and the median response duration was 153 days (range, 68–559+). One patient from group 1 who had a CR of all measurable lesions and stable disease (SD) of a gastric mass underwent curative surgery after four cycles.

There were 13 cases of unconfirmed PRs; initial responses in nine patients were not confirmed at the next cycle, and the remaining four patients failed to proceed to the next cycle, because of symptomatic deterioration, early death, gastrorrhagia, or patient refusal. Abdominal lymph nodes showed a higher response than liver or other lesions (48% versus 32% or 33%). There were no significant differences in response with respect to initial size or number of lesions.

Survival

Over a median follow-up of 265 days (group 1, 270 days; group 2, 264 days), 57 patients showed disease progression, and 50 patients (81%) died. The median time to progression for all patients was 126 days (95% CI, 77–175) (Fig. 1A)—

Table 2. Patient characteristics

Characteristic	Group 1 35 mg/m^2	Group 2 40 mg/m^2	Total
n of enrolled patients	31	31	62
n of evaluable patients	28	29	57
Age in years (median)	28–75 (61)	23–71 (54)	23–75 (57)
Sex (M:F)	27:4	21:10	48:14
Performance status score			
0–1	29	31	60
2	2	0	2
Prior treatment			
None	20	22	42
Gastrectomy only	4	3	7
Gastrectomy plus adjuvant therapy	7	6	13
Histology			
Well differentiated	1	1	2
Moderately differentiated	12	12	24
Poorly differentiated	14	11	25
Signet ring cell	2	6	8
Undifferentiated	2	1	3
Number of involved organs			
One	10	21	31
Two	14	8	22
Three	4	2	6
Four or more	3	–	3
Number of target lesions per patient			
One	11	5	16
Two	8	12	20
Three	7	10	17
Four or more	5	4	9

141 days (95% CI, 88–194) for group 1 and 119 days (95% CI, 65–173) for group 2 (Fig. 1B).

The median overall survival duration for all patients was 264 days (95% CI, 233–295)—270 days (95% CI, 154–386) for group 1 and 264 days (95% CI, 212–316) for group 2 (Fig. 1C). The overall 1-year survival rate was 34%.

Adverse Events

There was no grade 4 adverse event documented in any of the patients. The incidence of adverse events increased with the increased dose. Although the toxicity profiles were not different between the two dosing groups, the incidence of hyperbilirubinemia was exclusively high in group 2 (Table 4). We experienced a sudden mortality in group 2 after the first cycle of treatment, without evidence of disease progression or any specific event. The most common severe (grade ≥3) hematologic adverse event was anemia without evidence of bleeding, which was documented in eight patients (13%). Three patients received transfusions because of symptomatic anemia. The major nonhematologic adverse event was colicky abdominal pain during treatment, and severe (grade ≥3) diarrhea was recorded in only two patients (3%).

Pharmacokinetics

Pharmacokinetic samples were collected from three patients in each dose level on day 28 of the first and the third cycles. Pharmacokinetic parameters of FT, 5-FU, CDHP, and Oxo at the different dose levels are summarized in Table 5. Plasma levels of FT steadily increased after the administration of S-1. Mean values for the maximum plasma concentration and area under the plasma concentration-

Table 3. Treatment response

	Group 1 35 mg/m^2			Group 2 40 mg/m^2			Total		
n of enrolled patients	31			31			62		
n of evaluable patients	28			29			57		
	n	ITT (%)	PP (%)	*n*	ITT (%)	PP (%)	*n*	ITT (%)	PP (%)
CR	–	–	–	–	–	–	–	–	–
PR (confirmed)	5	16.1	17.9	7	22.6	24.1	12	19.3	21.0
PR (unconfirmed)	5	16.1	17.9	7	22.6	24.1	12	19.3	21.0
SD	11	35.5	39.3	11	35.5	37.9	22	35.5	38.6
PD	7	22.6	25.0	4	12.9	13.8	11	17.7	19.3
Response rate (initial)		32.2	35.8		45.2	48.3		38.6	42.0
Response rate (confirmed)		16.1	17.9		22.6	24.1		19.3	21.1
(95% CI)		(2.4–29.8)			(7.0–38.2)			(9.2–29.5)	
Disease control rate		67.7	75.0		80.6	86.2		74.2	80.7

Abbreviations: CI, confidence interval; CR, complete response; ITT, intent-to-treat analysis; PD, progressive disease; PP, per protocol analysis; PR, partial response; SD, stable disease.

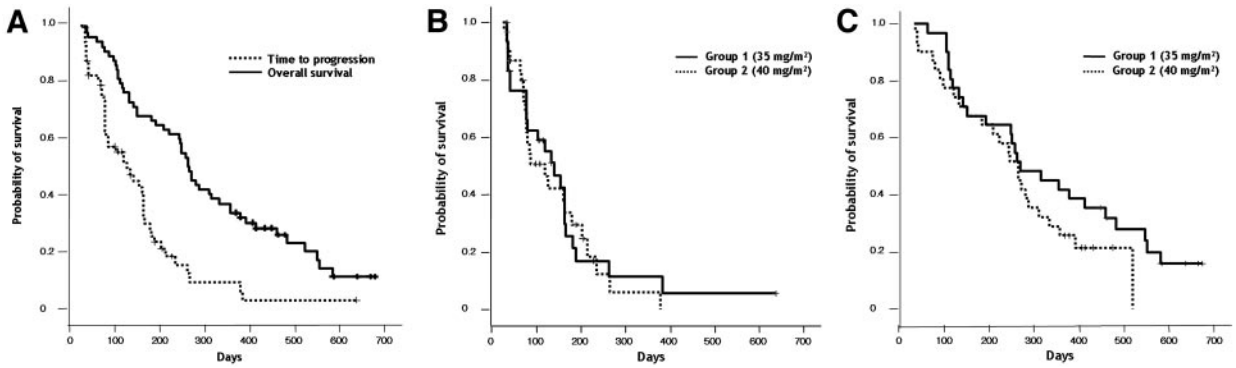

Figure 1. **(A):** Progression-free survival and overall survival curves of all patients with a median follow-up duration of 265 days. **(B):** Progression-free survival. **(C):** Overall survival curves of the patients according to dosing group. The median follow-up duration was 270 days for group 1 (35 mg/m^2 twice daily) and 264 days for group 2 (40 mg/m^2 twice daily).

versus-time curve from time 0 to 48 hours (AUC_{0-48}) for 5-FU, CDHP, and Oxo accordingly increased with the dose increase.

Mean AUC_{0-48} values for 5-FU and CDHP were higher in cycle 3 than in cycle 1, while other parameters showed little changes because of the 2-week washout period. This increase for 5-FU and CDHP might imply a correlation between dihydropyrimidine dehydrogenase inhibition by CDHP and 5-FU level after S-1 administration.

Pharmacogenomic Evaluation

We investigated pharmacogenomic classifiers associated with Hb reduction after S-1 treatment in 36 patients. The median values of Hb before treatment and nadir during treatment were 11.3 g/dl (range, 9.2–15.3) and 9.4 g/dl (range, 7.0–13.2), respectively. The mean Hb reduction rate per cycle was 1.0 (range, 0.0–3.1), which was the boundary value for separating the patients into the MRG (ΔHb/cycle $\leq$1.0) and SRG (ΔHb/cycle $>$1.0). Twenty-two patients were categorized into the MRG, and the remaining 14 patients were categorized into the SRG. There were no statistical differences with regard to median age ($p = .83$), sex ($p = .43$), performance status score ($p = .66$), or dose intensity ($p = .72$) between these two groups.

Using microarray CGH, 18 genes with copy number changes (12 amplified, 6 deleted) were identified that discriminated between the MRG and SRG (Fig. 2A; online supplementary Table 1). By univariate analysis, the clinical factor initial Hb level and the copy number variation in eight genes (*HIST1H2BL*, *CLN6*, *C10orf127*, *SPATS2*, *CA11*, *XPNPEP2*, *DJ167A19.1*, and *LOC158257*) were each significantly correlated with Hb reduction. A logistic

Table 4. Adverse events

Adverse event	Group 1 35 mg/m^2 (n = 31)			Group 2 40 mg/m^2 (n = 31)			Total (n = 62)		
	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)[a]	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)[a]	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)[a]
Anemia	13 (41.9)	11 (35.4)	3 (9.7)	8 (26.8)	13 (41.9)	5 (16.1)	21 (33.9)	24 (38.7)	8 (12.9)
Leukopenia	5 (16.1)	1 (3.2)	1 (3.2)	12 (38.7)	2 (6.5)	–	17 (27.4)	3 (4.8)	1 (1.6)
Neutropenia	1 (3.2)	1 (3.2)	2 (6.5)	8 (26.8)	2 (6.5)	1 (3.2)	9 (14.5)	3 (4.8)	3 (4.8)
Thrombocytopenia	1 (3.2)	–	1 (3.2)	3 (9.7)	–	–	4 (6.5)	–	1 (1.6)
Mucositis	4 (12.9)	1 (3.2)	–	2 (6.5)	5 (16.1)	–	6 (9.7)	6 (9.7)	–
Diarrhea	5 (16.1)	3 (9.7)	–	7 (22.5)	–	2 (6.5)	12 (19.3)	3 (4.8)	2 (3.2)
Anorexia	10 (24.3)	11 (35.4)	1 (3.2)	7 (22.5)	10 (24.3)	1 (3.2)	17 (27.4)	21 (33.9)	2 (3.2)
Nausea	9 (29.0)	7 (22.5)	1 (3.2)	11 (35.4)	3 (9.7)	2 (6.5)	20 (32.3)	10 (16.1)	3 (4.8)
Vomiting	5 (16.1)	3 (9.7)	–	8 (26.8)	3 (9.7)	–	13 (21.0)	6 (9.7)	–
Abdominal pain	8 (26.8)	5 (16.1)	3 (9.7)	10 (32.3)	9 (29.0)	1 (3.2)	18 (29.0)	14 (22.6)	4 (6.5)
Dyspepsia	8 (26.8)	4 (12.9)	–	8 (26.8)	3 (9.7)	1 (3.2)	16 (25.8)	7 (11.3)	1 (1.6)
Weight loss	2 (6.5)	1 (3.2)	–	3 (9.7)	3 (9.7)	1 (3.2)	5 (8.1)	4 (6.5)	1 (1.6)
General weakness	6 (19.3)	5 (16.1)	–	3 (9.7)	6 (19.4)	1 (3.2)	9 (14.5)	11 (17.8)	1 (1.6)
Skin pigmentation	13 (41.9)	1 (3.2)	–	10 (32.3)	2 (6.5)	–	23 (37.1)	2 (3.2)	–
Skin rash	7 (22.6)	2 (6.5)	–	4 (12.9)	4 (12.9)	–	11 (17.7)	6 (9.7)	–
Itching sensation	4 (12.9)	1 (3.2)	–	5 (16.1)	3 (9.7)	–	9 (14.5)	4 (6.5)	–
Hand–foot syndrome	–	–	–	2 (3.2)	–	–	2 (3.2)	–	–
Hematochezia	–	–	1 (3.2)	–	1 (3.2)	1 (3.2)	–	1 (1.6)	2 (3.2)
Musculoskeletal pain	6 (19.3)	1 (3.2)		4 (12.9)	3 (9.7)	1 (3.2)	10 (16.1)	4 (6.5)	1 (1.6)
Constipation	6 (19.3)	–	–	3 (9.7)	4 (12.9)	–	9 (14.5)	4 (6.5)	–
Serum ALP	11 (35.4)	3 (9.7)	–	9 (29.0)	2 (6.5)	–	20 (32.3)	5 (8.1)	–
Serum AST/ALT	5 (16.1)	1 (3.2)	–	2 (6.5)	1 (3.2)	1 (3.2)	7 (11.3)	2 (3.2)	1 (1.6)
Hyperbilirubinemia	4 (12.9)	–	–	4 (12.9)	3 (9.7)	2 (6.5)	8 (12.9)	3 (4.8)	2 (3.2)
Serum creatinine	1 (3.2)	–	–	–	–	–	1 (1.6)	–	–

Grading according to the National Cancer Institute Common Toxicity Criteria (Version 2.0).
[a] There were no cases of grade 4 adverse events.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

regression model was built, and the combination of the following components provided the best logistic model in predicting Hb reduction: initial Hb level (p = .03), $\log_2$(R/G) ratio of *HIST1H2BL* (p = .05), *C10orf127* (p = .02), and *XPNPEP2* (p = .05). The best logistic regression (Z) was as follows: logit LN(Z) = −18.767 + (1.658 × initial Hb level) + (−10.466 × *HIST1H2BL*) + (10.57 × *C10orf127*) + (−8.67 × *XPNPEP2*). The final model predicted correctly for 33 of 36 patients (92%) and misclassified one patient from the MRG and two patients from the SRG. The AUC was 0.98 for the final regression model (Fig. 2B).

Discussion

Upon designing this phase II study, our first speculation was the dosage of S-1. Japanese trials have adopted a dosing system based on 80 mg/m^2 with a schedule of 6 weeks per cycle that roughly follows BSA, limiting the maximal dosage to 120 mg in patients with a BSA ≥1.50 m^2 [13–15, 25–28]. This may result in an inadvertent underdosage in patients with a high BSA. On the other hand, western trials followed a BSA-based scheme with a schedule of 5 weeks per cycle. However, in one European phase II trial, a daily dose of 80 mg/m^2 was intolerable, with the occurrence of severe nonhematological toxicity [16]. Therefore, in order to procure an adequate dose intensity without impeding safety, we started the initial dosage at 35 mg/m^2 adjusted by BSA with a schedule of 6 weeks per cycle. The resulting planned dose intensity was 327 mg/m^2 per week, and eight patients (26%) were assigned to an S-1 dosage that was higher than the dosage from the conventional Japanese trial —seven patients at 130 mg, one patient at 150 mg.

Table 5. Pharmacokinetic profile

Dose (mg/m^2)	Korea (after 28 days)				U.S. (single dose)	Japan (after 28 days)
	Cycle 1		Cycle 3			
	35 ($n = 3$)	40 ($n = 3$)	35 ($n = 3$)	40 ($n = 3$)	35 ($n = 10$)	35.9 ($n = 10$)
FT						
T_{max} (hours)	1.8 ± 1.9	1.8 ± 1.9	4.0 ± 0.3	1.3 ± 1.1	1.8 ± 0.5	3.0 ± 1.8
C_{max} (ng/ml)	4,484 ± 1,231	5,487 ± 4,078	4,143 ± 257	6,428 ± 2,563	1,943 ± 365	4,166 ± 834
$AUC_{0–48}$ (ng·h/ml)	99,907 ± 38,999	132,606 ± 12,991	103,634 ± 7,918	129,455 ± 60,026	18,863 ± 2,190	80,032 ± 20,993
$T_{1/2}$ (hours)	22.1 ± 5.1	19.4 ± 10.3	17.1 ± 0.3	20.1 ± 6.7	10.8 ± 1.7	16.2 ± 2.4
5-FU						
T_{max} (hours)	3.3 ± 1.2	2.7 ± 1.2	4.0 ± 1.1	2.0 ± 0.3	2.5 ± 1.0	3.4 ± 1.3
C_{max} (ng/ml)	91 ± 23	133 ± 46	84 ± 12	139 ± 15	176 ± 22	114 ± 41
$AUC_{0–48}$ (ng·h/ml)	750 ± 120	767 ± 194	831 ± 116	916 ± 274	1,004 ± 57	609 ± 170
$T_{1/2}$ (hours)	4.2 ± 1.2	3.7 ± 1.6	4.4 ± 0.9	5.6 ± 1.3	2.0 ± 0.2	2.9 ± 1.1
CDHP						
T_{max} (hours)	2.0 ± 0.2	2.0 ± 1.7	4.0 ± 0.2	1.3 ± 1.1	2.3 ± 1.3	2.6 ± 1.8
C_{max} (ng/ml)	191 ± 14	231 ± 147	341 ± 147	524 ± 269	398 ± 196	276 ± 142
$AUC_{0–48}$ (ng·h/ml)	1,359 ± 373	1,683 ± 687	1,488 ± 98	2,216 ± 740	1,784 ± 591	1,364 ± 352
$T_{1/2}$ (hours)	9.4 ± 4.9	3.8 ± 0.8	7.1 ± 4.0	4.3 ± 2.9	4.3 ± 0.3	4.2 ± 1.4
Oxo						
T_{max} (hours)	2.2 ± 1.8	1.8 ± 1.9	6.0 ± 2.8	2.0 ± 0.4	2.5 ± 1.0	2.6 ± 2.1
C_{max} (ng/ml)	33.1 ± 1.0	105 ± 25	24.1 ± 8.6	84.4 ± 4.5	43 ± 29	130 ± 190
$AUC_{0–48}$ (ng·h/ml)	337 ± 80	477 ± 85	328 ± 137	355 ± 16	206 ± 229	550 ± 500
$T_{1/2}$ (hours)	5.3 ± 1.2	3.9 ± 2.4	5.9 ± 0.9	4.1 ± 3.0	3.7	5.0 ± 2.5

U.S. data are from Hoff PM, Saad ED, Ajani JA et al. Phase I study with pharmacokinetics of S-1 on an oral daily schedule for 28 days in patients with solid tumors. Clin Cancer Res 2003;9:134–142; Japan data were obtained from Hirata K, Horikoshi N, Aiba K et al. Pharmacokinetic study of S-1, a novel oral fluorouracil antitumor drug. Clin Cancer Res 1999;5:2000–2005.

In the study by Hirata et al. [28], an average single dose of 35.9 mg/m^2 was given (range, 31.7–39.7 mg/m^2).

Abbreviations: 5-FU, 5-fluorouracil; $AUC_{0–48}$, area under the plasma concentration-versus-time curve from time 0 to 48 hours (last measured plasma concentration); CDHP, 5-chloro-2,4-dihydroxypyridine; C_{max}, maximum plasma concentration; FT, tegafur; $T_{1/2}$, elimination half-life; Oxo, potassium oxonate; T_{max}, time to maximum plasma concentration.

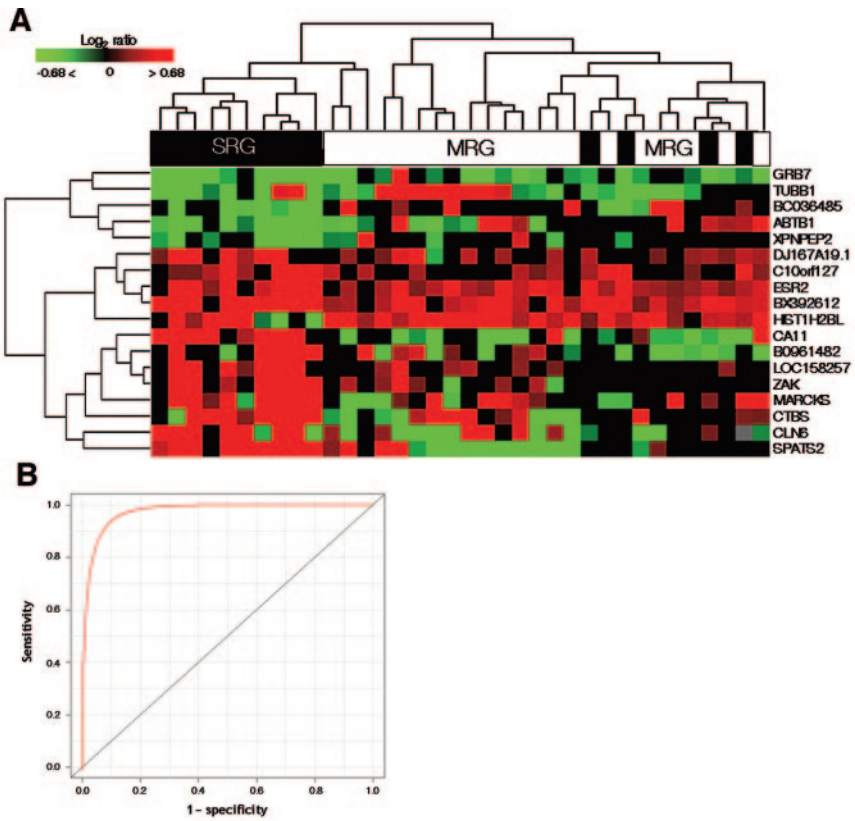

Figure 2. **(A):** Supervised hierarchical clustering of the selected 18 genes for which copy number changes were associated with hemoglobin reduction with S-1 treatment. The white block indicates the mild hemoglobin reduction group (MRG, $<$1.0 ΔHb/cycle) and the black block indicates the severe hemoglobin reduction group (SRG, $\geq$1.0 ΔHb/cycle). **(B):** Receiver operating characteristic (ROC) curve of prediction logistic regression model.

S-1 showed remarkably good tolerability in our patients at the initial dosage. All but two patients completed the planned schedule, which encouraged us to continue the study with an escalated dose of 40 mg/m^2 bid (80 mg/m^2 a day). Twenty-three patients (74%) in group 2 received S-1 at 140 mg or more. Even with this high dose level, the patients still showed favorable compliance. The actual dose intensity of our patients was 367 mg/m^2 per week, which is the highest ever obtained with S-1 monotherapy. These findings suggest that S-1 is a promising alternative to antimetabolites in gastric cancer with very good compliance in a Korean population.

This high compliance is a result of the favorable safety profile. It is well known that myelosuppression and diarrhea are the events that precluded dose escalation in Japan, whereas gastrointestinal toxicity and skin reaction were the limiting factors in western trials [11, 16]. On the other hand, the major adverse event in our patients was anemia. Thirteen percent of patients experienced grade 3 anemia at least once during their course of treatment, and 42% of the patients experienced grade 2–3 anemia in the first cycle. Although more grade 2–3 anemia was observed in group 2 patients as the cycle progressed, the overall incidences of anemia in the entire patient population did not change significantly throughout the cycles. We eliminated bleeding when counting anemia. There have been several reports of hemolytic anemia associated with 5-FU or uracil-tegafur (UFT) [29, 30]. Although no specific laboratory test was conducted to diagnose hemolytic anemia, there was sufficient evidence to exclude its possibility, because clinically no dark-colored urine, jaundice, or hepatosplenomegaly were observed. We had five cases of grade 2–3 hyperbilirubinemia, but they were not concordant with the cases of anemia. Moreover, most reports of 5-FU- or UFT-related hemolytic anemia were associated with long-term exposure or rechallenge of the drug, which is contrary to our observation of the early appearance of anemia. Another point of note is the hyperbilirubinemia that was exclusive to group 2 patients, which reminds us of the DLT in the U.S. phase I trial [18]. Its mechanism is still unclear, but it may be suggestive of the possibility of saturation of glucuronyltransferase enzyme activity or hepatobiliary transport of S-1 at this dose [31, 32].

Even though we found a high compliance rate, our trial

showed a lower tumor response rate of 19%, compared with previous Japanese trials [12–14, 16]. The first point to consider with this discrepancy is whether the gastric mass was included for response evaluation or not. Most Japanese trials of S-1 have traditionally considered the gastric mass as measurable disease, but their criteria depend on gastrographic and/or endoscopic findings, which are subjective and have a risk of bias [12, 14]. The second point is our high rate of unconfirmed PR (19%). Most of these resulted from a short response duration, which was not long enough to be confirmed in next cycle. Moreover, one third of the patients showed progressive disease with new lesions. This may reflect the high tumor burden and biologic heterogeneity of gastric cancer, leading to the rapid appearance of metastatic clones even with responding pre-existing lesions [33, 34]. Nevertheless, the efficacy rate of S-1 is comparable with that of PCI 5-FU (18%–26%) and other oral fluoropyrimidines such as UFT (10%–28%) and capecitabine (19%–34%), which reaffirms the efficacy of S-1 in gastric cancer [35–37].

Pharmacokinetic evaluation can offer useful information regarding the ethnic influence on treatment outcome. At an equivalent dose level of 35 mg/m^2, the pharmacokinetic parameters of 5-FU, FT, and CDHP were similar to the Japanese findings, rather than those of the U.S. (Table 5) [28]. The dose increase to 40 mg/m^2 showed an upward tendency in the general pharmacokinetic parameters compared with 35 mg/m^2, but the AUC of 5-FU increased only marginally. This suggests that the capacity to convert FT to 5-FU (i.e., cytochrome P4502A6) is saturated at this dose level. The conversion level would also be similar to that of the Japanese population considering the interethnic profiles of cytochrome P4502A6 polymorphism of the two populations [38]. Nevertheless, this pharmacokinetic study cannot explain the discrepancy in the typical adverse events observed between our results and the Japanese results. This may imply that other pharmacodynamic or pharmacogenetic factors are involved.

Most anticancer drugs act directly upon hematopoietic progenitors or indirectly on the bone marrow microenvironment, hematopoiesis regulatory factors [39]. We assumed that the S-1–induced anemia was related to erythropoiesis rather than bleeding or hemolysis, based on the following: (a) the anemia occurred continuously after initial treatment; (b) there existed interpersonal variability in the velocity of mean Hb reduction per cycle after S-1 treatment; (c) 6 weeks per cycle is long enough to affect erythropoiesis by S-1; (d) there was gradual macrocytosis with S-1 treatment (from 91 to 106 fl of mean corpuscular volume), reflecting deranged DNA synthesis and mitosis; and (e) the anemia comprised a unique toxicity profile of the S-1–treated Korean population in colorectal cancer [40]. Based on these findings, we tried to find genetic changes that are related to S-1–induced anemia with pharmacogenomic evaluation.

A pharmacogenomic approach could help to predict the quantitative and qualitative differences in individual susceptibility and ethnic differences in treatment outcome. We performed a pharmacogenomic study with genomic DNA from peripheral blood mononuclear cells, not with tumoral DNA from cancer tissues. Even though there are still some controversies, genomic DNA is considered to have the genetic information of normal tissue rather than tumor DNA [41]. Thus, we thought that it was more rational to use genomic DNA to predict the influence of S-1 on normal tissue (toxicity) rather than on tumor tissue (response).

Our primary aim was to discriminate the patient at high risk for Hb reduction from among the patients receiving S-1. The point is that it is not an absolute risk estimation but a relative comparison of the risk between the patients who showed more rapid Hb reduction and those who did not. For this purpose, we focused on the patient's velocity of Hb reduction during treatment rather than the nadir value itself as a marker of personal susceptibility to S-1. We indicated the velocity as the average slope, which is the difference between the Hb levels ($Hb_{initial} - Hb_{nadir}$) divided by the number of treatment cycles when the initial anemia occurred. Thus, 18 genes with copy number changes were identified and they discriminated between the high and low Hb reduction groups. Among these, *Grb7* was recently reported to participate in the proliferation and maturation of colony-forming units–erythroid [42, 43]. Another gene, estrogen receptor β (*ESR2*), was also associated with differentiation of pluripotent hematopoietic cells [44]. However, the clinical importance of genetic classifiers is their potential role as predictive markers. The multifactorial nature of genetic heterogeneity in pharmacologic pathways makes it unlikely that a single marker will accurately detect treatment-related factors. A more promising and powerful approach is to use panels of genetic classifiers for predictive information. By combining clinical and genetic factors, we established a more robust prediction model with high accuracy (92%), sensitivity, and specificity (93% and 96%, respectively). This is the first example, to our knowledge, of using microarray-based CGH as a pharmacogenomic tool for the prediction of chemotherapy-related outcome. Of course, the role of a pharmacogenomic approach to explain interethnic variability remains speculative at this time. These selected genes could offer an insight for future functional studies concerning chemotherapy-induced anemia. They might offer subjects on population studies, such as single nucleotide polymorphisms, to clarify ethnic differences. We also believe that this predictive model can be clinically optimized through prospective validation.

In conclusion, this phase II trial obtained the highest dose intensity of S-1 ever reported in gastric cancer. Considering the efficacy of PCI 5-FU of around 20%, we believe that S-1 demonstrates acceptable efficacy and comparable survival with good tolerability. Considering only a modest improvement in efficacy and an increasing incidence of hyperbilirubinemia with dose elevation, we suggest that a dose of 35 mg/m^2 bid is optimal when designing future combination chemotherapy in gastric cancer, and that pharmacogenomics may represent a promising area of future research for individualized chemotherapy.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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