[5%]), thrombocytopenia ($n = 1$ [3%]), and febrile neutropenia ($n = 1$ [3%]) were also similar to the original. Among nonhematological toxicities, grade 3/4 anorexia was remarkably reduced to 3 patients (8%) and nausea also decreased to 1 patient (3%). The incidences of grade 3/4 fatigue and diarrhea slightly increased to 2 (5%) and 1 (3%) patients, respectively. There was no grade 3/4 creatinine elevation seen. There were no treatment-related deaths occurring within 30 days after completion of "SP step" treatment.

Compliance

As mentioned previously, 4 and 1 patients were determined to be ineligible after enrollment in the original and amended protocols, respectively, and therefore 21 and 37 patients were analyzed for compliance, respectively. Under the original protocol, 57% (12/21; 95% CI 34–78%) achieved treatment completion with 3 cycles of S-1 plus cisplatin, and 76% (16/21; 95% CI 53–92%) achieved treatment completion with 2 cycles. The proportion of patients receiving treatment according to protocol was 57% (12/21; 95% CI 34–78%). Of note, 6/21 (29%) patients did not complete the first cycle of the "SP step". Reasons for not completing the first cycle included neutropenia on the day of cisplatin administration (day 8) in 3 patients, anorexia in 2 patients, and infection in 1. Dose reductions of S-1 and cisplatin were required once in 9 (43%) and 8 (38%) patients, respectively, and twice in 1 (5%) and 1 (5%) patients, respectively. There were 6 patients (29%) withdrawn from treatment as follows: 3 because of toxicity (neutropenia), 2 because of patient refusal of additional treatment because of toxicity, and 1 because of refusal of additional treatment for other reasons.

Under the amended protocol, 81% (30/37; 95% CI 65–92%; $P < 0.001$ under the null hypothesis) achieved treatment completion with 3 cycles of S-1 plus cisplatin, and 95% (35/37; 95% CI 82–99%) achieved treatment completion with 2 cycles. The proportion of patients receiving treatment according to protocol was 78% (29/37; 95% CI 62–90%). The number of patients not completing the first cycle of the "SP step" was remarkably decreased to only 1 (3%) patient. There were 10 (27%) patients requiring S-1 dose reduction after the first chemotherapy cycle of S-1 monotherapy. Dose reductions of S-1 and cisplatin were required once in 12 (32%) and 8 (22%) patients, respectively, and twice in 7 (19%) and 6 (16%) patients, respectively. Withdrawal of treatment occurred in 2 (5%) patients as follows: one because creatinine elevation did not recover and the other because of patient refusal of additional treatment because of toxicity.

The relative dose intensities (RDIs) of S-1 were 0.67 in the original and 0.78 in the amended protocol, and for cisplatin were 0.65 and 0.81, respectively.

Discussion

To the best of our knowledge, this is the first report on a safety analysis of S-1 plus cisplatin treatment for stage III GC patients who have undergone curative resection with D2 lymphadenectomy. The overall frequencies of major toxicities under the original protocol were almost similar to those of the SPIRITS trial [16] (neutropenia 40 vs. 40%; anemia 20 vs. 26%; and anorexia 28 vs. 30% in this study and the SPIRITS trial, respectively). However, the completion rate of 3 cycles of S-1 plus cisplatin as a primary endpoint (57%) and RDI of S-1 or cisplatin were unexpectedly low in this study. Among the 9 patients who could not complete the 3 cycles of S-1 plus cisplatin, 6 patients could not complete treatment even during the first cycle, mainly because of neutropenia on day 8 and anorexia. We found that toxicity of chemotherapy was more likely to occur during the first cycle.

Therefore, to improve the completion rate of the treatment, we decided to amend the protocol by establishing S-1 monotherapy as the first cycle of chemotherapy, followed by 3 cycles of S-1 plus cisplatin. Although it might be possible that efficacy is decreased by changing the first cycle to S-1 monotherapy, we prioritized complying with postoperative adjuvant chemotherapy, which might also be important in improving survival [19, 20].

In our amended protocol, not only was cisplatin administration omitted in the first cycle, but also the dose of S-1 in subsequent combination cycles was reduced if there were severe toxicities during the "first-cycle" administration of S-1 monotherapy. In addition, the neutropenia count for delaying cisplatin administration was also changed, from $<1{,}500/mm^3$ to $<1{,}200/mm^3$. As a result, 81% of patients achieved treatment completion with 3 cycles of S-1 plus cisplatin with improved RDIs of both S-1 (0.78 from 0.65) and cisplatin (0.81 from 0.65). The frequency of grade 3/4 anorexia and nausea also decreased, from 28 to 8% and 8 to 3%, respectively, although we do not use Substance P inhibitor in both protocol because it was not approved in Japan at that time.

The actual cause of the poor compliance during the early post-gastrectomy course in this study was not discovered. There are several reports about the effect of gastrectomy on S-1 pharmacokinetics [21–23], although this issue remains controversial. Kim et al. reported that total gastrectomy significantly increased the maximum concentration and the areas under the curves of plasma fluorouracil and 5-chloro-2,4-dihydroxypyridine (CDHP) after S-1 administration, which may be one explanation for the toxicity seen in this study [23]. Additionally, there may be a hidden cause, such as relatively poor nutritional status due to gastrectomy, although this study included patients with sufficient oral intake and adequate organ function.

Although this was not a randomized study, in comparison with the original protocol, the amended protocol was more feasible, with a higher completion rate and higher RDIs. Relapse-free survival and overall survival were not reached in this study; therefore, it is difficult to speculate that the addition of 3 cycles of cisplatin might improve the prognosis compared with S-1 alone. Now in Japan, another feasibility study of S-1 plus docetaxel as postoperative adjuvant chemotherapy is ongoing [24]. The addition of any other agent to S-1 as an adjuvant chemotherapy needs to be validated in a randomized phase III trial with S-1 as the control arm.

In conclusion, the postoperative adjuvant S-1 plus cisplatin regimen of the amended protocol is more feasible than the original protocol, because of (1) early dose reduction of S-1 prior to cisplatin addition (2) greater recovery time from surgery prior to cisplatin. It should be regarded as a feasible experimental arm for the next adjuvant phase III trial comparing this S-1 plus cisplatin regimen and S-1 alone as adjuvant chemotherapy for stage III GC patients who have undergone curative resection with D2 lymphadenectomy.

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Conflicts of interest T. Sano has received lecture fees from Taiho Pharmaceutial (Tokyo, Japan). All other authors declared no conflicts of interest.

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ORIGINAL ARTICLE

Randomized clinical trial of adjuvant chemotherapy with intraperitoneal and intravenous cisplatin followed by oral fluorouracil (UFT) in serosa-positive gastric cancer versus curative resection alone: final results of the Japan Clinical Oncology Group trial JCOG9206-2

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Abstract
Purpose To evaluate the survival benefit of adjuvant chemotherapy after curative resection in serosa-positive gastric cancer, a multicenter phase III clinical trial was conducted in Japan.
Patients and methods From January 1993 to March 1998, 268 patients were randomized to adjuvant chemotherapy (135 patients) or surgery alone (133 patients). All patients underwent gastrectomy with D2 or greater lymph node dissection. The chemotherapy regimen consisted of intraperitoneal cisplatin soon after abdominal closure, postoperative intravenous cisplatin (day 14) and 5-fluorouracil (day 14–16), and daily oral FU (UFT) starting 4 weeks after surgery for 12 months. The primary endpoint was overall survival. Relapse-free survival and site of recurrence were secondary endpoints.
Results Fifty-two patients (38.5%) in the adjuvant chemotherapy arm completed the chemotherapy regimen. There were 4 (1.49%) treatment-related deaths, 1 in the surgery-alone and 3 in the adjuvant chemotherapy arm (2 did not receive chemotherapy). Grade 4 toxicity was observed in 3 patients in the surgery-alone and 2 patients in the adjuvant chemotherapy arm. There was no significant difference in 5-year overall survival (62.0% adjuvant chemotherapy vs. 60.9% surgery-alone, $P = 0.482$) and 5-year relapse-free survival rates (57.5% adjuvant chemotherapy vs. 55.6% surgery-alone; $P = 0.512$).
Conclusion There was no benefit in overall and relapse-free survival with this adjuvant chemotherapy regimen for

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patients with macroscopically serosa-positive gastric cancer after curative resection.

Keywords Randomized clinical trial · Adjuvant chemotherapy · Serosa-positive gastric cancer · Intraperitoneal chemotherapy

Introduction

A large number of gastric cancers are still diagnosed in advanced stages worldwide [1, 2]. Once the primary tumor invades the subserosal or serosal layers of the gastric wall, cancer cells are more likely to spread into the abdominal cavity and implant on peritoneal surfaces, resulting in peritoneal dissemination [3, 4]. The most frequent cause of recurrence and subsequent cancer death in serosa-positive gastric cancer is peritoneal metastasis even after curative resection [5–7]. The main goal of adjuvant chemotherapy for resected gastric cancer is to prevent such a distant recurrence and increase the potential of cure.

In Japan today, adjuvant chemotherapy with single agent S-1 is considered the standard of care for patients with pathological stage II/III (Japanese Classification of Gastric Carcinoma 2nd English Edition [8, 9]) gastric cancer after potentially curative D2 dissection, based on the results of the ACTS-GC clinical trial [10–13]. However, subgroup analysis of the ACTS-GC data suggest that S-1 may be less effective for patients with more advanced gastric cancer such as serosa-positive cancer.

The present Japan Clinical Oncology Group (JCOG) trial, JCOG9206-2, is a randomized controlled phase III clinical trial of adjuvant chemotherapy with intraperitoneal and intravenous cisplatin followed by oral fluorouracil (FU). Thirteen institutions in Japan participated in this trial as members of the Gastric Cancer Surgical Study Group (GCSSG), a subgroup of JCOG [14]. We report here the final results with 6 years of follow-up.

Patients and methods

Patients

Patients had to fulfill the following eligibility criteria: macroscopically complete operation; histologically proven gastric adenocarcinoma, macroscopically serosa-positive (T3–4), with no metastases to level 3–4 lymph node stations (N0–2) [8, 9]; age 75 years or younger; no previous treatment for gastric cancer; negative peritoneal lavage cytology; adequate organ function as assessed by laboratory studies: leukocyte count of at least 4000/mm^3; hemoglobin of at least 11.0 g/dl; platelet count of at least 100000/mm^3; AST, ALT, total bilirubin, blood urea nitrogen and creatinine no higher than 1.25 times the upper limit of normal; creatinine clearance no lower than 70 ml/min. All patients provided written informed consent. Patients who had undergone any chemotherapy or radiotherapy, or those with synchronous or metachronous cancer of other organs were excluded.

Treatment assignment and evaluation

The patients were randomized using the minimization method to balance the adjuvant chemotherapy and surgery-alone arms according to institution and the combination of the macroscopic depth of tumor invasion (T-category) and lymph node metastasis (N-category) according to the Japanese Classification of Gastric Carcinoma 2nd English Edition [9]. After the surgeon confirmed the above eligibility criteria, patients were randomly assigned to either arm by means of an intraoperative telephone call to the JCOG Data Center (Fig. 1).

The chemotherapy comprised intraperitoneal cisplatin (70 mg/m^2) soon after abdominal closure; intravenous cisplatin (70 mg/m^2) on postoperative day 14; intravenous 5-fluorouracil (5-FU) (700 mg/m^2) daily on postoperative days 14–16; and UFT (267 mg/m^2) daily, starting 4 weeks after surgery for 12 months. Intraperitoneal cisplatin (70 mg/m^2 with saline in total volume 1000 ml) was administered via drainage tubes that were clamped for following 2 h. Creatinine clearance was evaluated twice weekly before and after the administration of cisplatin. A full blood count was performed every week to assess for hematological toxicity during hospital stay. During UFT treatment, each patient was asked to visit the hospital every month for physical examinations and laboratory testing in both arms. Patients underwent upper gastrointestinal series, gastric endoscopy, ultrasonography, computed tomography or other investigations either as required or every 6 months

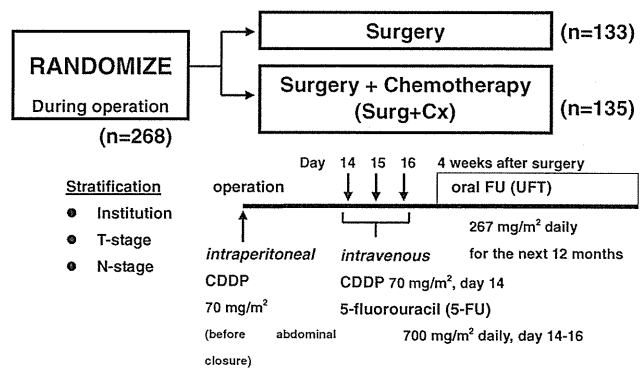

Fig. 1 Study scheme. All patients underwent gastrectomy with D2 or greater lymph node dissection. CDDP, *cis*-diamminedichloro-platinum (cisplatin)

to evaluate for recurrence. Adverse events were recorded according to the JCOG toxicity criteria [15]. Since some adverse events may occur after surgery even without chemotherapy, all potential adverse drug effects were compared with the adverse effects experienced by patients in the surgery-alone arm. Data on adverse events in the surgery-alone patients, except for postoperative morbidity and mortality, were collected retrospectively. Original case report forms were designed to collect adverse events during chemotherapy (only in the adjuvant chemotherapy arm). However, some adverse events happened even in the surgery-alone group. To keep the comparability between arms, we re-collected the data of adverse events from both arms at the final analysis. The surgery-alone arm received no additional treatment after surgery unless there was recurrence. The main prognostic factors, including age, gender, the depth of tumor invasion and nodal spread, operative procedures, and pathological findings, were described according to the general guidelines issued by the Japanese Research Society for Gastric Cancer Study [8, 9].

Study design and statistical analyses

This study was designed as a multicenter prospective randomized controlled phase III clinical trial. The study protocol was approved by the JCOG Clinical Trial Review Committee and the institutional review boards of all participating institutions. The primary endpoint was OS. Relapse-free survival and the site of recurrence were secondary endpoints. The original planned duration of accrual was 4 years with 5 years of follow-up. The planned sample size was 280 patients, with 140 patients in each arm to power the study at 80% to detect a 15% difference in 5-year OS rates between the surgery-alone arm (40%) and the chemotherapy arm (55%) with a two-sided significance level of 5%. The study design was amended to one projecting 5-year OS rates of 55% in the surgery-alone arm and 67% in the chemotherapy arm, with a 5-year accrual period and 6 years follow-up, because combined survival was better, and accrual poorer, than expected.

OS was measured from the date of random treatment assignment to the date of death or censored at the date of the last follow-up. Relapse-free survival was measured from the date of random treatment assignment to the date of the first observation of relapse or the date of death from any cause. If no progression was reported and if the patient remained alive, data on relapse-free survival were censored as of the date on which the absence of relapse was confirmed. OS and relapse-free survival were estimated by the Kaplan–Meier method and compared by the stratified log-rank test with the combination of the depth of the tumor invasion and lymph node metastasis as strata on the

Fig. 2 CONSORT diagram

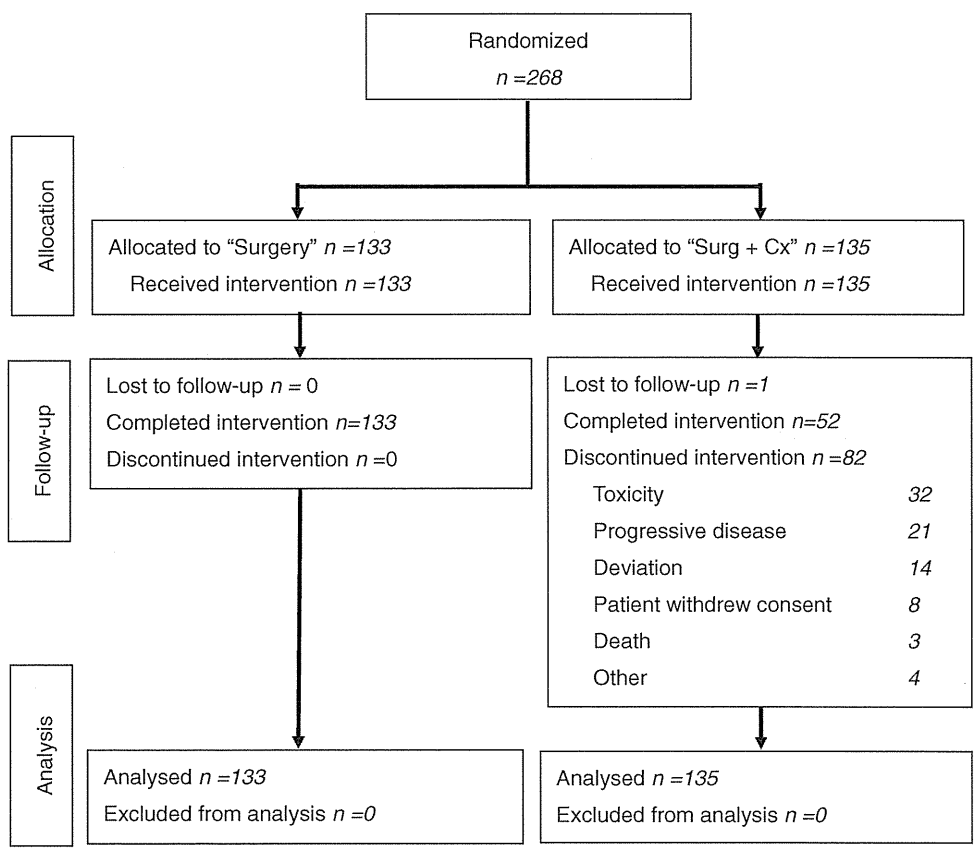

Table 1 Distribution of the main prognostic factors in the two treatment arms

No. of patients	Surgery ($n = 133$)	Surg + Cx ($n = 135$)	P*
Gender			0.601
Male	88	94	
Female	45	41	
Age (years)			0.043
Median	57	59	
Range	23–73	33–75	
Tumor diameter (cm)			0.324
Median	5.5	6.0	
Range	2.4–19.0	1.5–18.0	
Macroscopic type			0.603
0	0	0	
1	5	1	
2	31	32	
3	76	78	
4	14	15	
5	7	9	
Liver metastasis			–
Absent (H0)	133	135	
Present (H1)	0	0	
Peritoneal metastasis (macroscopic)			0.245
Absent (P0)	131	135	
Present (P1)	2	0	
Type of gastrectomy			1.000
Total	76	76	
Proximal	0	1	
Distal	57	58	
Combined resection			0.787
No	39	37	
Yes	94	98	
Spleen	73	74	
Pancreas	26	26	
Adrenal gland	7	5	
Colon	6	6	
Other	50	50	
Pathological depth of tumor invasion (T)			0.613
T1			
m, sm	2	3	
T2			
mp	5	7	
T2			
ss	34	41	
T3			
se	88	77	
T4			
si	4	7	
Pathological extent of lymph node metastasis (N)[a]			0.794
N0	32	41	
N1	51	49	
N2	38	35	
N3	5	4	
N4	7	5	
Involvement of the resection margin			
Proximal			1.000
Negative	133	134	
Positive	0	1	
Distal			0.498
Negative	133	133	
Positive	0	2	
Tumor histology			0.991
Common types			
Papillary	1	2	
Well differentiated	10	12	
Moderately differentiated	33	35	
Poorly differentiated	69	67	
Mucinous	6	5	
Signet ring cell	13	13	
Other types			
Carcinoid	1	0	
Unknown	0	1	

Surg + Cx Surgery plus adjuvant chemotherapy, *m* mucosa including muscularis mucosae, *sm* submucosa, *mp* muscularis propria, *ss* subserosal, *s* serosa, *si* serosa-infiltrating

* A *t*-test was used for continuous variables. Fisher's exact test was used for discrete variables

[a] Pathological extent of lymph node metastasis was classified based on the Japanese Classification of Gastric Carcinoma 1st English edition. Data are missing for one patient in the Surg + Cx arm

intention-to-treat basis. Analyses for toxicity were conducted for all of the randomly assigned patients. All statistical analyses were conducted with SAS software (version 8.1, SAS Institute, Cary, NC).

Results

From January 1993 to March 1998, 268 patients were enrolled in this phase III study. Of the 268 eligible patients enrolled, 133 patients were assigned to the surgery-alone arm and 135 patients to the adjuvant chemotherapy arm (Fig. 2). Distribution of the main prognostic factors across the two arms was well balanced (Table 1). There were no significant differences between the two groups in the

Table 2 Frequency of postoperative morbidity and mortality

	Surgery ($n = 133$)	Surg + Cx ($n = 135$)	P
Surgical morbidity			
Leakage	3	9	0.137
Pancreatic fistula	20	14	0.275
Peritoneal abscess	8	13	0.364
Pneumonia	3	1	0.369
Other infections	9	6	0.439
Stomal stenosis	2	1	0.621
Ileus	0	4	0.122
Miscellaneous	4	16	0.009
Non-surgical morbidity			
Creatinine ≥2.0	3	21	<0.001
AST, ALT ≥100	44	43	0.896
Hospital death	1	4	0.370

Surg + Cx Surgery plus adjuvant chemotherapy

Table 3 Adverse events

Arm	Grade[a]					% Grade 4	Total
	0	1	2	3	4		
Surgery							
Leukopenia	90	28	9	0	0	0	127
Anemia	58	31	34	4	–	–	127
Thrombocytopenia	125	1	1	0	0	0	127
Increase in bilirubin	83	–	32	11	1	0.8	127
Increase in AST	22	62	27	14	2	1.6	127
Increase in ALT	26	55	29	16	1	0.8	127
Increase in creatinine	109	13	4	1	0	0	127
Nausea or vomiting	110	16	4	0	0	0	130
Diarrhea	122	6	2	0	0	0	130
Stomatitis	129	1	0	0	0	0	130
Neuropathy (sensory)	130	0	0	0	0	0	130
Skin-other (pigmentation)	130	0	0	0	0	0	130
Surg + Cx							
Leukopenia	76	30	19	3	1	0.8	129
Anemia	31	24	56	18	–	–	129
Thrombocytopenia	123	4	1	0	1	0.8	129
Increase in bilirubin	67	–	43	15	1	0.8	126
Increase in AST	25	61	27	16	0	0	129
Increase in ALT	29	70	21	9	0	0	129
Increase in creatinine	70	38	16	5	0	0	129
Nausea or vomiting	80	31	19	1	0	0	131
Diarrhea	113	17	1	0	0	0	131
Stomatitis	126	4	1	0	0	0	131
Neuropathy (sensory)	128	3	0	0	0	0	131
Skin-other (pigmentation)	130	1	0	0	0	0	131

Surg + Cx Surgery plus adjuvant chemotherapy

[a] Toxicity graded according to JCOG criteria [13]

institution and the combination of macroscopic T-category and N-category as stratification factors. There were also no significant differences in gender, but the surgery-alone group was younger than the adjuvant chemotherapy group ($P = 0.0426$). All patients underwent gastrectomy with D2 or greater lymph node dissection. The operative procedures were similar in the two groups. Seventy-six of 133 patients (57.1%) in the surgery-alone arm and 76 of 135 (56.3%) in the adjuvant chemotherapy arm underwent total gastrectomy, and all the other patients except one underwent distal gastrectomy. Similar numbers of patients in each group underwent combined resections involving the spleen, pancreas, adrenal gland, colon or other organs. There were no significant differences between the two groups in tumor diameter, macroscopic type, presence of liver or macroscopic peritoneal metastasis, depth of tumor invasion, extent of lymph node metastasis, involvement of the resection margins and histological type.

Of the 135 patients of the adjuvant chemotherapy arm, 82 patients discontinued chemotherapy as is shown in Fig. 2. Thirty-two patients discontinued chemotherapy because of toxicity, among whom 19 patients could not start intravenous chemotherapy. Therefore, only the remaining 13 patients terminated chemotherapy during intravenous CDDP/5-FU or oral UFT.

The perioperative mortality was low. There were 4 treatment-related deaths. One of 133 patients in the surgery-alone arm died because of postoperative complications, and 3 of 135 in the adjuvant chemotherapy arm died because of postoperative complications or chemotherapy toxicity (2 of 3 did not receive chemotherapy). There were no significant differences in the frequency of surgical morbidity except for miscellaneous events such as wound infection (Table 2). As for postoperative non-surgical morbidity, renal dysfunction (JCOG grade 3–4) within 3 months after surgery was observed only in 5 patients in the adjuvant chemotherapy group. Adverse events were generally mild. The frequencies of adverse events according to JCOG criteria are listed in Table 3. Grade 4 toxicity was observed in 3 patients in the surgery-alone and 2 patients in adjuvant chemotherapy arm.

In 6 years of planned follow-up, there was no significant differences in OS (Fig. 3) and relapse-free survival (Fig. 4). The 5-year overall survival rate in the adjuvant chemotherapy arm was 62.0% (95% confidence interval 53.7–70.2) versus 60.9% (52.6–69.2) in the surgery-alone arm ($P = 0.482$, one-sided stratified log-rank test). The 5-year relapse-free survival rate was 57.5% (49.1–65.9) in the adjuvant chemotherapy group versus 55.6% (47.2–64.1) in the surgery-alone group ($P = 0.512$) one-sided stratified log-rank test). Sixty-six of 135 patients (48.9%) in the adjuvant chemotherapy arm and 64 of 133 patients

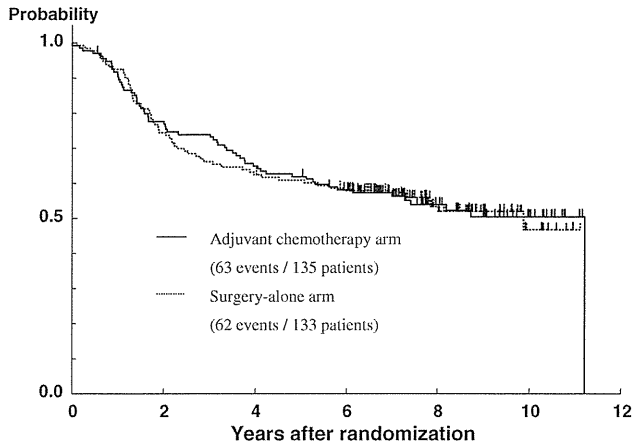

Fig. 3 Overall survival. There was no significant difference in overall survival: 5-year survival rate 62.0% (95% confidence interval 53.7–70.2) in the adjuvant chemotherapy group versus 60.9% (52.6–69.2) in the surgery-alone group, $P = 0.482$

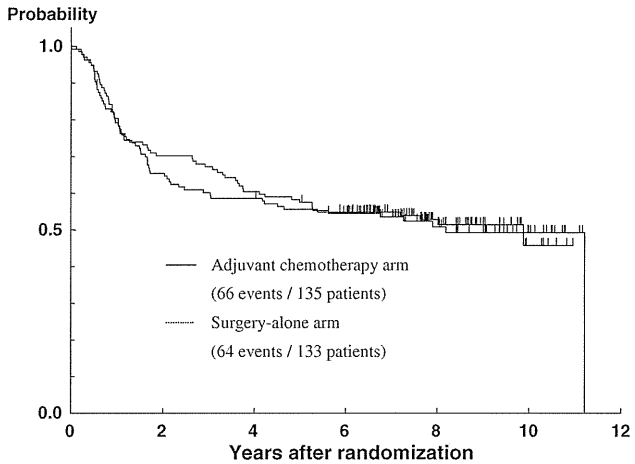

Fig. 4 Relapse-free survival. There was no significant difference in relapse-free survival: 5-year relapse-free survival rate 57.5% (95% confidence interval 49.1–65.9) in the adjuvant chemotherapy group versus 55.6% (47.2–64.1) in the surgery-alone group, $P = 0.512$

(48.1%) in the surgery-alone arm experienced cancer recurrence or death. The results for survival were not substantially changed after adjustment for age by Cox proportional hazards regression. Sites of recurrence, including peritoneal dissemination as the most common site, did not differ significantly between the two arms (Table 4).

Table 4 Site of cancer recurrence

Site of recurrence	Surgery ($n = 133$)	Surg + Cx ($n = 135$)	Total
Peritoneal dissemination	23	19	42
Liver metastases	9	16	25
Metastases to other organs	5	7	12
Local (remnant stomach)	0	2	2
Local (other sites)	4	2	6
Distant lymph nodes	10	6	16
Other	5	0	5
Death before recurrence	8	14	22
Total	64	66	130

Surg + Cx Surgery plus adjuvant chemotherapy

Discussion

The present study is a prospective randomized controlled phase III clinical trial of adjuvant chemotherapy with cisplatin followed by UFT conducted by the GCSSG subgroup of JCOG to clarify the efficacy of adjuvant chemotherapy after curative resection with extended (D2 or greater) lymphadenectomy for macroscopically serosa-positive gastric cancer. There was no benefit in overall and relapse-free survival with this regimen, and there was no difference between the arms in the site of recurrence. The frequency of postoperative morbidity was similar in the two groups, suggesting that administration of intraperitoneal cisplatin does not affect postoperative morbidity [16].

The recent AMC 0101 trial demonstrated that adjuvant chemotherapy with intraperitoneal cisplatin and early mitomycin-C plus long-term doxifluridine plus cisplatin (iceMFP) improved survival of patients with grossly serosa-positive advanced gastric cancer when compared with mitomycin-C plus short-term doxifluridine (Mf) [17]. Another Korean randomized trial, AMC 0201, showed there was no benefit in survival with adjuvant mitomycin-C plus long-term doxifluridine plus cisplatin when compared with Mf [18]. Taken together, these two studies suggest that improved OS might have been due to intraperitoneal cisplatin and/or mitomycin-C when given early. However, the AMC 0101 trial could not definitively demonstrate whether intraperitoneal chemotherapy itself contributed to improved survival. The adjuvant chemotherapy in the present study might be insufficient because it consisted of just one single course of intravenous cisplatin/5-FU. However, in the AMC 0201 trial, repeated administration of doxifluridine plus cisplatin did not show any benefit over short-term mitomycin-C plus doxifluridine.

Only 39% in the adjuvant chemotherapy arm actually completed the chemotherapy regimen in the present study, although UFT toxicity was generally mild. Patients tend to suffer from gastrointestinal disturbances after gastrectomy even without postoperative chemotherapy. Compliance of highly toxic regimens significantly decreases if given in early postoperative period, as observed in the MAGIC trial, which demonstrated the superiority in overall survival of pre- and postoperative chemotherapy compared with

surgery-alone [19]. The MAGIC trial reported that 57% of patients in the chemotherapy group were able to receive postoperative chemotherapy and that only 43% of patients in the chemotherapy group actually completed the full 6 cycles, although 88% of patients completed the 3 cycles of neoadjuvant chemotherapy. Thus, special consideration should be given to compliance when choosing a regimen for postoperative adjuvant chemotherapy. Powerful regimens should be planned for neoadjuvant settings in more advanced disease and less toxic drugs for postoperative use for earlier stage disease, such as stage II [12, 20, 21].

In conclusion, there was no benefit in overall and relapse-free survival with adjuvant cisplatin followed by UFT for patients with macroscopically serosa-positive gastric cancer after curative resection. When recurrence occurs, there was no difference in the site between the two treatment groups. Therefore, we do not recommend adjuvant chemotherapy with this regimen for this patient population in clinical practice.

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ORIGINAL ARTICLE – TRANSLATIONAL RESEARCH AND BIOMARKERS

Clinical Significance of Molecular Detection of *Matrix metalloproteinase-1* in Bone Marrow and Peripheral Blood in Patients with Gastric Cancer

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ABSTRACT

Purpose. Matrix metalloproteinases are responsible for proteolytic degradation of basement membrane and extracellular matrix. In tumor tissues, elevated expression of matrix metalloproteinase-1 (MMP-1) has been associated with tumor invasion and metastasis. However, little is known about the expression of *MMP-1* in peripheral blood (PB) and bone marrow (BM) in gastric cancer patients. Thus, the aim of the present study is to determine *MMP-1* messenger RNA (mRNA) expression levels in BM and PB of patients with gastric cancer.

Methods. The study group consisted of 857 patients with gastric cancer (577 males and 280 females) ranging in age from 27 to 87 years (average 61.6 years). *MMP-1* mRNA expression levels in BM and PB were evaluated quantitatively by real-time reverse-transcription polymerase chain reaction (RT-PCR).

Results. Expression of *MMP-1* mRNA in BM and PB of patients with gastric cancer was significantly higher than in noncancer patients. High levels of *MMP-1* mRNA expression were significantly associated with differentiated histology, tumor size, tumor invasiveness, lymph node metastasis, liver metastasis, and clinical stage. Particularly importantly, *MMP-1* mRNA expression in PB was an independent factor of distant metastasis.

Conclusions. We disclosed that *MMP-1* mRNA expression in peripheral blood and bone marrow of gastric cancer patients was very high, precisely reflecting staging of gastric cancer. *MMP-1* mRNA expression in peripheral blood may be a useful marker for distant metastasis in gastric cancer.

In gastric cancer, treatment often requires surgical removal of the tumor. Nevertheless, relapse may occur within several years, and the subsequent clinical outcome is poor. Tumor–node–metastasis (TNM) classification of cancer patients uses surgically resected specimens, but at present, this approach cannot predict recurrence. Therefore, identification of better markers to predict recurrence and metastasis of disease should allow clinicians to make more accurate prognosis.

Over the last two decades, detection of isolated tumor cells (ITC) in peripheral blood (PB) and bone marrow (BM) has become a superior predictor of recurrence than established clinicopathologic variables. However, the clinical significance of ITC has not been uniformly defined for all types of cancer. For instance, in breast cancer, Braun et al. reported that assessing circulating tumor cells immunohistochemically with pan-cytokeratin antibodies predicted prognosis.[1–8] Our laboratory reported that the epithelial markers carcinoembryonic antigen (CEA)/cytokeratin (CK)20 were good prognostic markers in colorectal cancer.[9] On the other hand, we recently showed that, in 810 cases of gastric cancer, there was no clinicopathologic significance of ITC in BM or PB as defined by CEA, CK7 or CK19 assessed by quantitative real-time reverse-transcription

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polymerase chain reaction (RT-PCR).[10] Therefore, it is important to discover a reliable biomarker capable of predicting recurrence and metastasis of gastric cancer.

In a previous report on prognostic biomarkers capable of predicting recurrence and metastasis in gastric cancer, Heiss et al. used immunocytological techniques to assess urokinase-type plasminogen activator (u-PA) and the receptor for urokinase-type plasminogen activator (u-PAR).[11–13] Gastric cancer patients who were positive for u-PAR expression had significantly worse prognoses than those who were negative. Our current study focuses on a different family of proteases, the matrix metalloproteinases (MMPs), using quantitative real-time RT-PCR to avoid subjective factors frequently complicating immunohistochemical studies.[14–16] Among the currently known 28 matrix metalloproteinases, we focused on MMP-1 (also known as interstitial collagenase) and assessed the clinical magnitude of *MMP-1* gene expression in BM as well as PB in gastric cancer cases.

The MMPs constitute a large family of proteolytic enzymes which are used by invasive cancer cells to hydrolyze the structural proteins that compose the extracellular matrix, such as collagen, elastin, laminin, fibronectin, and fibrinogen.[17–19] In particular, MMP-1 degrades types 1, 2, and 3 collagens, and its presence is reportedly closely correlated with the invasive and metastatic potentials of various cancers, including gastric, esophageal, colorectal, pancreatic, breast, and ovarian, and malignant melanoma [20–27].

This is the first report to analyze the expression level of *MMP-1* mRNA in BM and PB of gastric cancer patients. In the current study, we analyzed samples from 857 gastric cancer patients using real-time RT-PCR. The data were correlated with clinicopathologic features and used to establish prognostic significance.

PATIENTS AND METHODS

Patient Samples

Eight hundred fifty-seven gastric cancer patients who underwent surgical treatment in the National Cancer Center Hospital, Japan, from 2001 to 2004 were studied. There were 577 males and 280 females patients with average age of 61.6 years (range 27–87 years). Sixteen patients with no history of cancer who underwent abdominal surgery in our hospital from 2001 to 2004 were recruited as negative controls. After analysis of those 16 nonmalignant cases that consisted of cholecystolithiasis and inguinal hernia without infection, no case was affected by cancer. Clinical stages and pathological features of primary tumors were defined according to the classification of the International Union against Cancer.[28] None of these patients underwent endoscopic mucosal resection. Additionally, gastric cancer patients with stage IV at the time of operation had palliative therapies (gastrointestinal reconstruction and control of bleeding), to improve patient quality of life. Written informed consent was obtained from all patients.

BM and PB Sampling

Both PB and BM were collected immediately prior to surgery under general anesthesia. BM aspirate was obtained from the sternum using a BM aspiration needle (MDTECH, Gainesville, FL), and PB was obtained through a venous catheter. The first 1 ml of PB and BM was discarded to avoid contamination by epidermal cells. Each 1-ml sample of PB and BM was immediately mixed vigorously with 4 ml ISOGEN-LS (NIPPON GENE, Toyama, Japan) and immediately stored at −80°C until RNA extraction.

Total RNA Extraction and First-Strand cDNA Synthesis

Total RNA was extracted according to the ISOGEN-LS manufacturer's protocol. All the clinical samples obtained at the National Cancer Center Hospital were sent to our institute. The reverse-transcription reaction was performed as described previously.[29,30] First-strand complementary DNA (cDNA) was synthesized from 2.7 μg total RNA in a 30 μl reaction mixture containing 5 μl 5× RT reaction buffer (BRL, Gaithersburg, MD), 200 μM dNTP, 100 μM solution of a random hexadeoxynucleotide mixture, 50 units Rnasin (Promega, Madison, WI), 2 μl 0.1 M dithiothreithol, and 100 units Moloney leukemia virus RT (BRL). The mixture was incubated at 37°C for 60 min, heated to 95°C for 10 min, and then chilled on ice.

Quantitative RT-PCR

MMP-1 and *Glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) mRNAs were quantitated with the LightCycler™ system (Roche Applied Science, Indianapolis, IN) using fluorescein isothiocyanate (FITC)- and LcRed-labeled hybriprobes. The primers and hybriprobes were synthesized by Nihon Gene Research Laboratories, Inc., Sendai, Japan. The sequences used for *MMP-1* mRNA were as follows: sense primer, 5′-TGCCGACAGAGATG AAGT-3′, and antisense primer, 5′-TCCAGTGTTTTCC TCAGA-3′. Hybriprobes used for *MMP-1* were 5′-TGG GCTGTTCAGGGACAGAATGTGCTAC-3′-FITC, and 5′-LcRed640-CGGATACCCCAAGGACATCTACAGCTC C-3′-phosphorylated. *GAPDH* was used as an internal control. The sequences of *GAPDH* mRNA were as follows: sense primer, 5′-TGAACGGGAAGCTCACTGG-3′, and antisense primer, 5′-TCCACCACCCTGTTGCTGTA-3′.

Hybriprobes used for *GAPDH* were 5′-GAGTGGGTGTCG CTGTTGAAGTCA-3′-FITC/5′-LcRed640-AGGAGACCACC TGGTGCTCAGTGTA-3′-phosphorylated. For amplification of *MMP-1*, an initial denaturation at 95°C for 10 min was followed by 10 s at 95°C, 15 s at 62°C, and 8 s at 72°C. For *GAPDH* amplification, an initial denaturation was followed by 15 s at 95°C, 15 s at 60°C, and 13 s at 72°C.

Data Analysis

A standard curve was prepared with 200–20,000 copies of purified plasmids containing *MMP-1* and *GAPDH*. After proportional baseline adjustment, the fit point method was employed to determine the cycle in which the log-linear signal was first distinguishable from the baseline, and then that cycle number was used as the crossing-point value. The standard curve was produced by measuring the crossing points of standard values and plotting them against the logarithmic value of concentrations. Those concentrations were calculated by plotting their crossing points against the standard curve and were adjusted by *GAPDH* content. Taking into consideration the clinical application of the current study, the 95% confidence interval (CI) was used as the upper limit of a normal case cutoff value (BM, 1.7×10^{-6}; PB, 1.6×10^{-7}). The 95% value of a normal case according to the reference intervals of the Clinical and Laboratory Standards Institute was established, and the reference limit was regarded as the cutoff value.[31] Levels higher or lower than the cutoff value were considered "positive" and "negative," respectively. The sensitivity and specificity of the data were determined to evaluate the legitimacy of the cutoff value.

Statistics

For continuous variables, data are expressed as mean ± standard deviation. The relationships between *MMP-1* mRNA expression and the clinicopathological factors were analyzed using the chi-square test and the Kruskal–Wallis test. A logistic regression model was used to identify the independent predictors of distant metastasis. All tests were analyzed using JMP software (SAS Institute Inc., Cary, NC, USA). Statistical significance was determined from two-sided tests as $p < 0.05$.

RESULTS

Patient Characteristics

BM and PB samples from 16 normal control cases and 857 gastric cancer patients were analyzed for *MMP-1* and *GAPDH* gene expression values by quantitative real-time RT-PCR. The expression levels of *MMP-1* mRNA were corrected for those of *GAPDH* mRNA. An overview of patient characteristics is given in Table 1.

Expression of MMP-1 mRNA in BM and PB of Gastric Cancer Patients with Surgery

In BM, the mean level of *MMP-1* expression in gastric cancer patients ($7.6 \times 10^{-5} \pm 2.3 \times 10^{-4}$) was significantly higher ($p < 0.0001$) than that of control cases ($1.1 \times 10^{-5} \pm 4.0 \times 10^{-5}$), as shown in Fig. 1a. In PB, the mean value of *MMP-1* expression for gastric cancer patients ($9.8 \times 10^{-4} \pm 5.4 \times 10^{-3}$) was also significantly

TABLE 1 Clinicopathologic characteristics of the patients

Factor	*n*	%
Patients enrolled	857	100.0
Age (mean ± standard deviation), years	61.6 ± 11.5	
Sex		
Male	577	67.3
Female	280	32.7
Histology		
Differentiated	192	22.4
Undifferentiated	665	77.6
Tumor size (mean ± standard deviation), mm	56.8 ± 42.2	
Tumor stage		
T1	454	53.0
T2	210	24.5
T3	180	21.0
T4	13	1.5
Lymph node metastasis		
N0	501	58.5
N1	200	23.3
N2	74	8.6
N3	82	9.6
Lymphatic involvement		
Negative	489	57.1
Positive	368	42.9
Vascular involvement		
Negative	709	82.7
Positive	148	17.3
Distant metastasis		
M0	754	88.0
M1	103	12.0
Stage		
I	533	62.2
II	109	12.7
III	82	9.6
IV	133	15.5

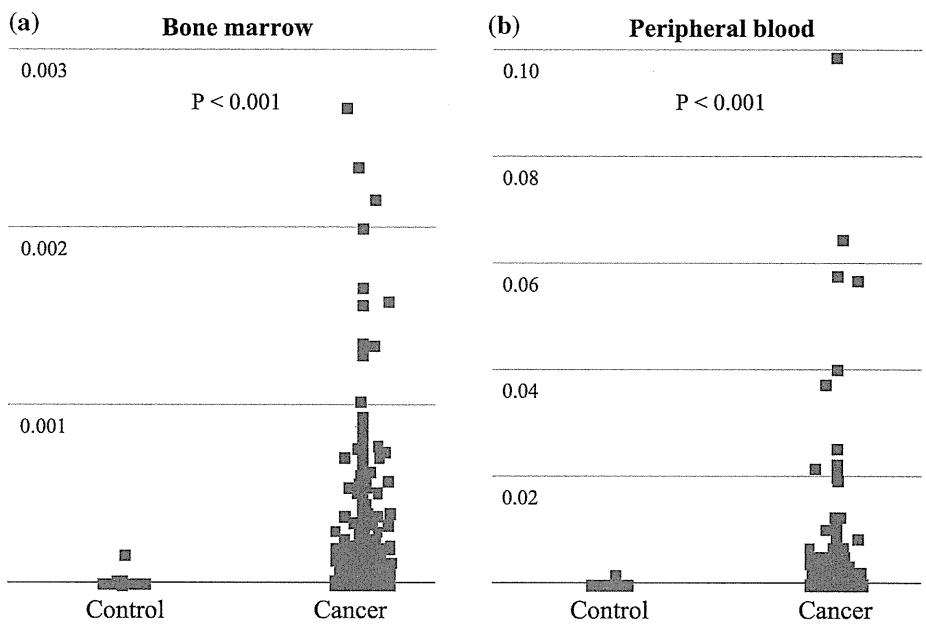

FIG. 1 Expression of MMP-1 mRNA is significantly elevated in bone marrow and peripheral blood in 857 cases of gastric cancer in comparison with that in 16 normal control cases

higher ($p < 0.0001$) than that of control cases ($1.2 \times 10^{-4} \pm 4.7 \times 10^{-4}$), as shown in Fig. 1b.

MMP-1 Expression and Clinicopathological Factors Associated with Surgically Treated Gastric Cancer Patients

Table 2 summarizes the correlations between *MMP-1* mRNA levels and clinicopathological factors. Using the respective cutoff values, sensitivities were 42.1% in BM and 49.1% in PB, and specificities were 75.0% in BM and 81.2 % in PB.

In BM, a significantly higher number of *MMP-1* mRNA-positive cases belonged to the following clinical subgroups: differentiated histological grade ($p < 0.05$), large tumor size ($p < 0.01$), invasion deeper than the muscularis propria ($p < 0.01$), lymph node metastasis ($p < 0.01$), lymphatic involvement ($p < 0.05$), vascular involvement ($p < 0.01$), perioperative overt distant metastasis (including liver metastasis and/or distant lymph node metastasis, $p < 0.01$), and clinical stage ($p < 0.01$). In contrast, in PB, there was a significant difference observed depending upon histological grade ($p < 0.01$), large tumor size ($p < 0.05$), invasion deeper than the muscularis propria ($p < 0.05$), lymph node metastasis ($p < 0.05$), perioperative overt distant metastasis ($p < 0.01$), and clinical stage ($p < 0.05$).

Relationship between MMP-1 mRNA Expression and Distant Metastasis

Levels of *MMP-1* mRNA expression in both BM and PB were significantly associated with liver metastasis. *MMP-1* mRNA expression in BM was significantly associated with distant lymph node metastasis (pN3). There was no significant difference between *MMP-1* mRNA expression in PB and pN3, but it tended towards association ($p = 0.07$). No significant differences were observed between *MMP-1* mRNA expression level and peritoneal dissemination (Table 3).

Multivariate Analysis for Distant Metastasis

Univariate and multivariate logistic regression analyses were performed on distant metastasis (Table 4). Univariate regression analysis showed that the following factors were significantly related to distant metastasis: histological grade, tumor size, lymph node metastasis, lymphatic invasion, venous invasion, and *MMP-1* mRNA expressions levels in BM and PB ($p < 0.01$, in each case). Multivariate regression analysis indicated that the PB *MMP-1* mRNA high-expression group was an independent predictor for distant metastasis [relative risk (RR), 1.82; 95 % CI, 1.03–3.11, $p < 0.05$] along with lymph node metastasis (RR, 7.14; 95 % CI, 2.95–15.8, $p < 0.0001$), tumor size (RR, 4.45; 95 % CI, 2.51–7.62, $p < 0.0001$), and lymphatic invasion (RR, 3.44; 95 % CI, 1.66–6.80, $p < 0.001$).

DISCUSSION

We found that *MMP-1* mRNA levels in BM and PB from 857 cancer patients were elevated in comparison with those from 16 normal controls. It is disclosed that, in gastric cancer, MMP-1 protein is derived from interstitial tissues and cancer cells. For the following reasons, it is likely that the expression of *MMP-1* detected in BM and PB originated from host monocytes, macrophages,

TABLE 2 Correlation of clinicopathologic characteristics and MMP-1 mRNA expression

	MMP-1 mRNA in bone marrow					*MMP-1* mRNA in peripheral blood				
	Negative		Positive			Negative		Positive		
	(n = 496)		(n = 361)			(n = 436)		(n = 421)		
Factor	n	%	n	%	p Value	n	%	n	%	p Value
Age (years) (mean ± standard deviation)	61.3 ± 11.7		62.0 ± 11.2		N.S.	61.5 ± 11.6		61.7 ± 11.5		N.S.
Gender										
Male	336	67.7	241	66.8	N.S.	296	67.9	281	66.7	N.S.
Female	160	32.3	120	33.2		140	32.1	140	33.3	
Histology										
Differentiated	97	19.6	95	26.3	<0.05	80	18.3	112	26.6	<0.01
Undifferentiated	399	80.4	266	73.7		356	81.7	309	73.4	
Tumor size										
Tumor maximal diameter (mm) (mean ± standard deviation)	53.6 ± 41.1		61.2 ± 43.3		<0.01	53.3 ± 38.7		60.5 ± 45.2		<0.05
Tumor stage										
pT1	283	57.1	171	47.4	<0.01	246	56.4	208	49.4	<0.05
pT2–pT4	213	42.9	190	52.6		190	43.6	213	50.6	
Lymph node metastasis										
Negative	309	62.3	192	53.2	<0.01	269	61.7	231	54.9	<0.05
Positive	187	37.7	169	46.8		167	38.3	190	45.1	
Lymphatic involvement										
Negative	296	59.7	191	52.9	<0.05	250	57.3	239	56.8	N.S.
Positive	200	40.3	170	47.1		186	42.7	182	43.2	
Vascular involvement										
Negative	425	85.7	284	78.7	<0.01	367	84.2	342	81.2	N.S.
Positive	71	14.3	77	21.3		69	15.8	79	18.8	
Distant metastasis										
M0	449	90.5	305	84.5	<0.01	398	91.3	356	84.6	<0.01
M1	47	9.5	56	15.5		38	8.7	65	15.4	
Stage										
I	328	66.1	205	56.8	<0.01	288	66.1	245	58.2	<0.05
II	51	10.3	58	16.1		44	10.1	65	15.4	
III	51	10.3	31	8.6		45	10.3	37	8.8	
IV	66	13.3	67	18.6		59	13.5	74	17.6	

N.S. not significant

endothelial cells, and BM stromal cells. First, monocyte and/or macrophage activated by interleukin (IL)-1 alpha and/or transforming growth factor (TGF)-alpha produce MMP-1. Those cytokines are reportedly abundant in PB of advanced gastric cancer cases.[32] Second, *MMP-1* may also be derived from the increasing number of circulating endothelial cells (CEC) or endothelial progenitor cell (EPC) found during tumor progression. The number of CEC in PB was reported to be much higher in malignant cases in comparison with nonmalignant cases.[33,34] CEC are derived from mature peripheral vessels, neovessels in tumors, and differentiated EPC in BM. [35–37] Asahara et al. initially described the concept of EPC.[38] The origin of EPC was neovessels in tumors and/or from BM.[39] Third, Barille et al. reported that stromal cells in BM secrete interleukin-6 (IL-6) and the soluble agonist receptor (sIL-6R) to activate MMP-1 and MMP-2, provoking progression of myeloma.[40]

It is, however, possible that some of the *MMP-1* signal might be derived from isolated tumor cells themselves. Inoue et al. performed immunohistochemical studies of gastric cancer cases, and detected MMP-1 expression in interstitial tissues, as well as cancer cells.[26] However, the main component of the basal cell layer of vascular vessels consists of type IV collagen, which is not the major

TABLE 3 Correlation of liver metastasis, distant lymph node metastasis, and peritoneal dissemination with MMP-1 mRNA expression

	MMP-1 mRNA in bone marrow					*MMP-1* mRNA in peripheral blood				
	negative ($n = 496$)		positive ($n = 361$)			negative ($n = 436$)		positive ($n = 421$)		
Factor	*n*	%	*n*	%	*p* Value	*n*	%	*n*	%	*p* Value
Liver metastasis										
Negative	496	100.0	356	98.6	<0.01	436	100.0	416	98.8	<0.01
Positive	0	0.0	5	1.4		0	0.0	5	1.2	
Distant lymph node metastasis (pN3)										
Negative	482	97.2	339	93.9	<0.05	423	97.0	398	94.5	N.S.
Positive	14	2.8	22	6.1		13	3.0	23	5.5	
Peritoneal dissemination										
Negative	482	97.2	348	96.4	N.S.	424	97.2	406	96.4	N.S.
Positive	14	2.8	13	3.6		12	2.8	15	3.6	

N.S. not significant

TABLE 4 Univariate and multivariate analysis for distant metastasis (logistic regression model)

Factor	Univariate analysis			Multivariate analysis		
	RR	95% CI	*p* Value	RR	95% CI	*p* Value
Histological grade (diff/undiff)	2.38	1.32–4.66	<0.01	1.76	0.88–3.78	N.S.
Tumor size (<60 mm/>61 mm)	9.9	6.00–17.1	<0.0001	4.45	2.51–7.62	<0.0001
Lymph node metastasis (negative/positive)	26.1	12.8–62.6	<0.0001	7.14	2.95–15.8	<0.0001
Lymphatic involvement (negative/positive)	13.1	7.30–25.5	<0.0001	3.44	1.66–6.80	<0.001
Vascular involvement (negative/positive)	4.66	2.99–7.25	<0.0001	1.30	0.84–2.35	N.S.
MMP1 mRNA expression in BM (negative/positive)	1.75	1.16–2.66	<0.01	–	–	–
MMP1 mRNA expression in PB (negative/positive)	1.91	1.26–2.95	<0.01	1.82	1.03–3.11	<0.05

RR relative risk, *CI* confidence interval, *BM* bone marrow, *PB* peripheral blood, *N.S.* not significant

substrate for MMP-1. Thus, the concept of *MMP-1* expression by cancer cells at the invading front of the vessel wall as well as those circulating in PB is rather doubtful.[41] In addition, the number of circulating cancer cells would be quite low. Considering the host condition exposing cancer cells and microenvironment, all candidate cells might produce *MMP-1*. Thus, we have to isolate responsible cells by using flow cytometry with cellular surface markers.

Based on the clinicopathologic analysis presented here, gastric cancer cases with *MMP-1* expression in BM and/or in PB showed higher incidence of lymph node metastasis as well as liver metastasis. Multivariate analysis demonstrated that *MMP-1* could be an independent factor for distant metastasis. However, *MMP-1* expression in BM and PB was not associated with peritoneal dissemination, which is the main cause of death in gastric cancer patients. Those findings suggest that circulating cells expressing *MMP-1* contributed mainly to the establishment of hematogenous metastasis. Further study is required to elucidate the mechanism by which *MMP-1*-expressing BM cells and PB cells play a significant role in hematogenous metastasis of gastric cancer.

This study demonstrated an intimate relationship between expression of *MMP-1* mRNA and hematogenous metastasis by real-time RT-PCR. Moreover, from the standpoint of clinical application of our data, our finding of the importance of *MMP-1* in PB, not in BM, will be important for postoperative follow-up of patients. Quantitative evaluation by real-time RT-PCR assay should enable us to predict distant metastasis much more conveniently and objectively than in immunohistochemical studies.

Regarding recurrence and metastasis of gastric cancer, we recently reported the importance of the simultaneous presence of isolated tumor cells and *VEGFR-1*-expressing cells probably from monocytes and/or hematopoietic progenitor cells in PB.[10,42] Furthermore, *MMP-1* expression in PB or BM must have a valuable role to play in management of distant metastasis. Therefore, to clarify the mechanism of distant metastasis, we have to consider both cancer cells

and host-side factors. Further study is required to clarify the role of *MMP-1* in PB as well as in BM.

In conclusion, our study shows that expression of *MMP-1* mRNA in BM and PB of gastric cancer patients is significantly higher than in patients without cancer. Furthermore, high expression of *MMP-1* in PB correlates with the clinicopathological malignancy of gastric cancer, especially distant metastasis.

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ORIGINAL REPORT

Five-Year Outcomes of a Randomized Phase III Trial Comparing Adjuvant Chemotherapy With S-1 Versus Surgery Alone in Stage II or III Gastric Cancer

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See accompanying editorial on page 4348; listen to the podcast by Dr Mayer at www.jco.org/podcast

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ABSTRACT

Purpose
The first planned interim analysis (median follow-up, 3 years) of the Adjuvant Chemotherapy Trial of S-1 for Gastric Cancer confirmed that the oral fluoropyrimidine derivative S-1 significantly improved overall survival, the primary end point. The results were therefore opened at the recommendation of an independent data and safety monitoring committee. We report 5-year follow-up data on patients enrolled onto the ACTS-GC study.

Patients and Methods
Patients with histologically confirmed stage II or III gastric cancer who underwent gastrectomy with D2 lymphadenectomy were randomly assigned to receive S-1 after surgery or surgery only. S-1 (80 to 120 mg per day) was given for 4 weeks, followed by 2 weeks of rest. This 6-week cycle was repeated for 1 year. The primary end point was overall survival, and the secondary end points were relapse-free survival and safety.

Results
The overall survival rate at 5 years was 71.7% in the S-1 group and 61.1% in the surgery-only group (hazard ratio [HR], 0.669; 95% CI, 0.540 to 0.828). The relapse-free survival rate at 5 years was 65.4% in the S-1 group and 53.1% in the surgery-only group (HR, 0.653; 95% CI, 0.537 to 0.793). Subgroup analyses according to principal demographic factors such as sex, age, disease stage, and histologic type showed no interaction between treatment and any characteristic.

Conclusion
On the basis of 5-year follow-up data, postoperative adjuvant therapy with S-1 was confirmed to improve overall survival and relapse-free survival in patients with stage II or III gastric cancer who had undergone D2 gastrectomy.

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INTRODUCTION

In 2008, there were 737,000 deaths from gastric cancer worldwide. Gastric cancer is the second leading cause of cancer-related death, with the highest mortality rates in East Asia, including Japan, Korea, and China (28.1 per 100,000 in males; 13.0 per 100,000 in females).[1] Approximately 60% of gastric cancers in the world are diagnosed in this area. The mainstay of treatment for gastric cancer is surgery. However, in stages II (excluding T1 disease) and III (moderately advanced), an appreciable proportion of patients have recurrence, even after curative resection. Consequently, various regimens for adjuvant chemotherapy have been implemented to prevent postoperative recurrence.

Although the results of many randomized, controlled studies conducted to verify the effectiveness of postoperative adjuvant chemotherapy for gastric cancer were negative on an individual study basis, meta-analyses of these results have suggested that postoperative adjuvant chemotherapy is therapeutically useful in patients with gastric cancer.[2-7] However, no regimens have been clearly recommended for adjuvant chemotherapy after gastrectomy with D2 lymphadenectomy (D2 gastrectomy), established as the standard procedure for advanced gastric cancer in East Asia.

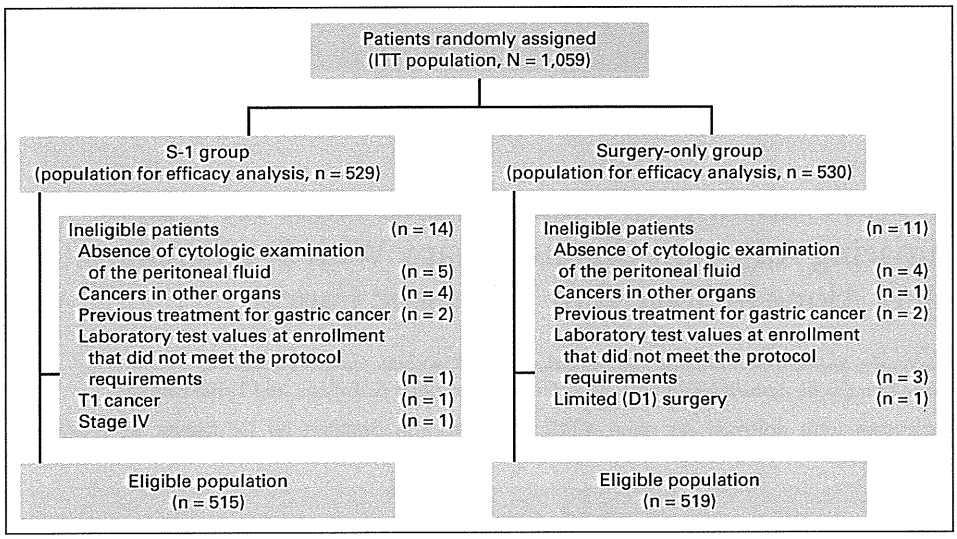

Fig 1. CONSORT diagram. D1 gastrectomy; ITT, intent-to-treat.

The Adjuvant Chemotherapy Trial of S-1 for Gastric Cancer (ACTS-GC) is a randomized phase III trial to confirm the effectiveness of 1-year postoperative treatment with S-1 compared with surgery alone in patients with stage II or III gastric cancer who underwent D2 gastrectomy. S-1 (TS-1; Taiho Pharmaceutical, Tokyo, Japan) is a dihydropyrimidine dehydrogenase inhibitory fluoropyrimidine preparation combining tegafur, gimeracil, and oteracil potassium in a molar ratio of 1:0.4:1.[8,9] Two phase II studies[10,11] in patients with advanced or recurrent gastric cancer obtained high response rates exceeding 40%. Postoperative adjuvant chemotherapy with S-1 was thus expected to be effective.

In this phase III trial, 1,059 patients with histologically confirmed stage II or III gastric cancer who underwent D2 gastrectomy were enrolled. A protocol-based interim analysis performed 1 year after the completion of enrollment (median follow-up, 3 years) confirmed that S-1 was effective. Because statistical analysis indicated that there was minimal probability that the results of this study would turn out to be negative after 5 years of follow-up, an independent data and safety monitoring committee recommended that the results should be disclosed at that time. An analysis of the results available at that time showed that the 3-year overall survival (OS) was 80.1% in the S-1 group compared with 70.1% in the surgery-only group. S-1 was demonstrated to reduce the risk of death by 32% (hazard ratio [HR], 0.68; 95% CI, 0.52 to 0.87; $P = .003$).[12] Although the study results were disclosed early because of these promising results, we considered it important to have 5-year follow-up data available. Such data would facilitate a comparison of our results for 5-year OS and other outcomes with those of previous trials. Moreover, this analysis may justify

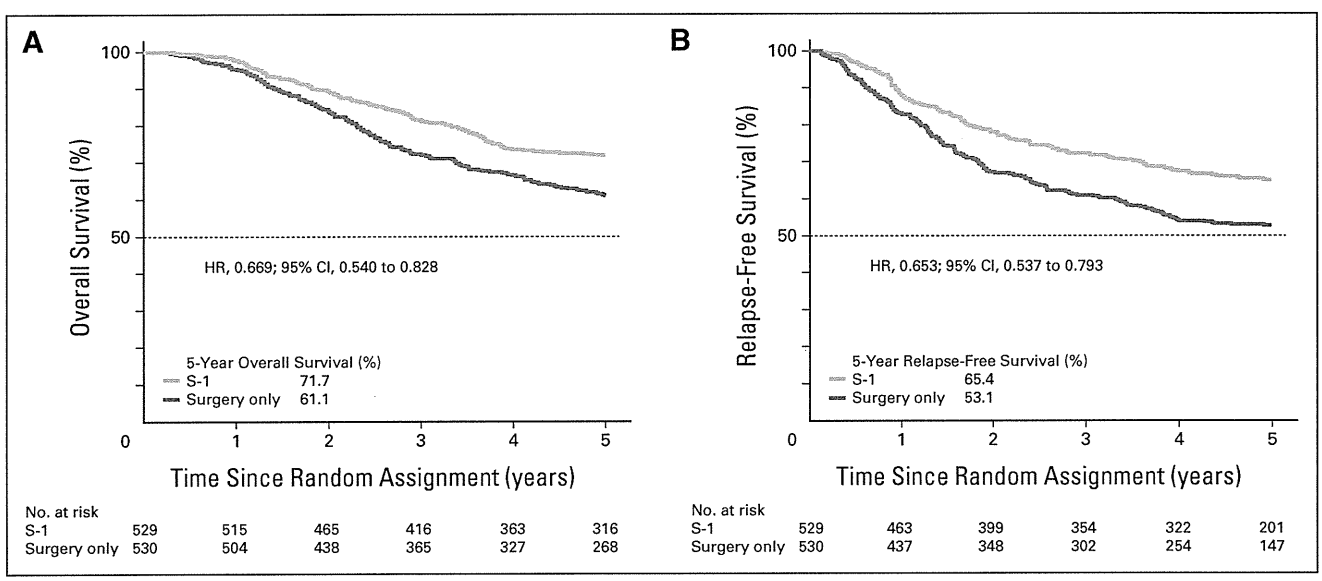

Fig 2. Kaplan-Meier estimates of (A) overall survival and (B) relapse-free survival for all randomly assigned patients. HR, hazard ratio.

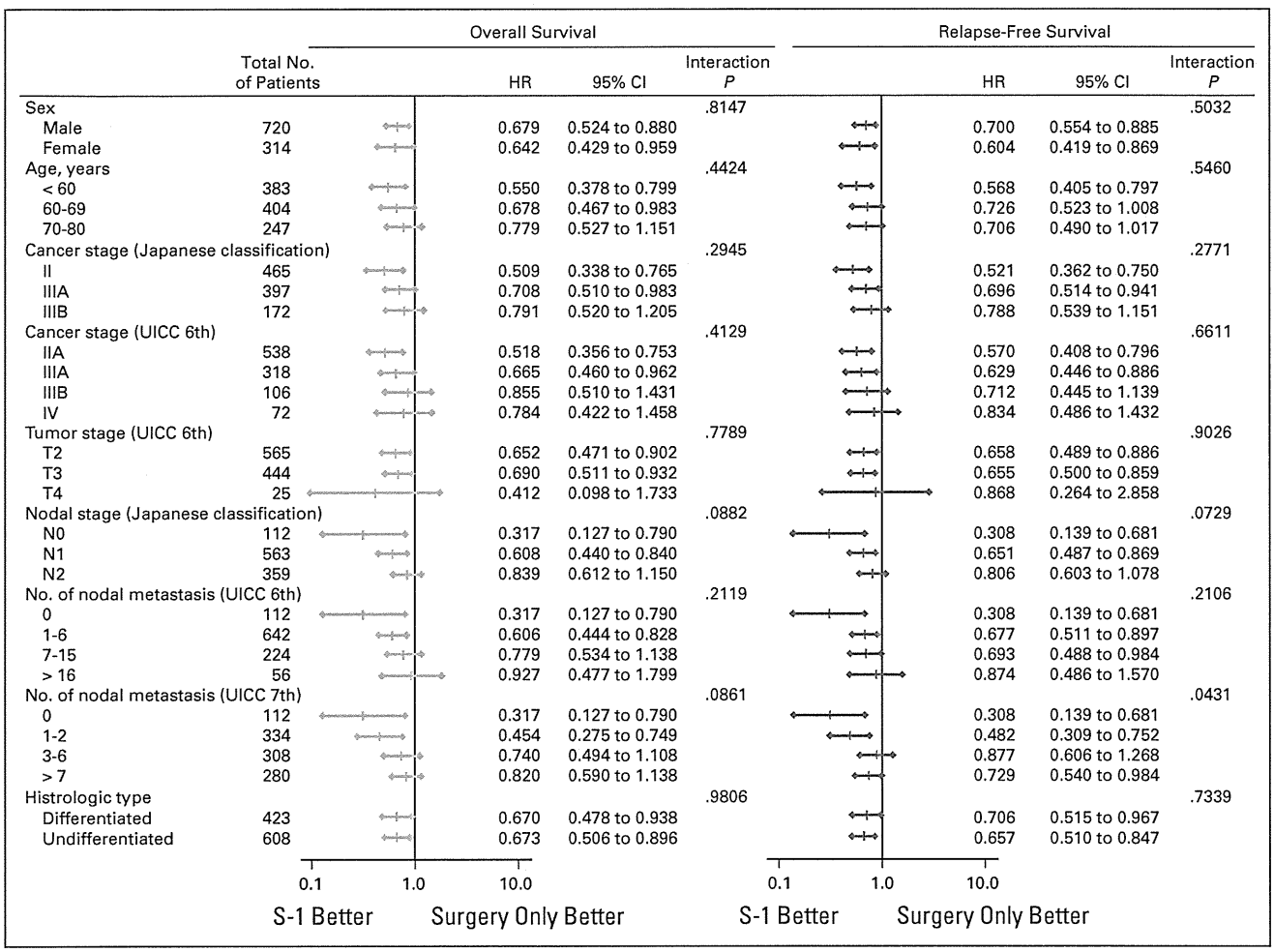

Fig 3. Subgroup analysis: overall survival and relapse-free survival for eligible population. In the surgery-only group, cancers in three patients could not be classified as differentiated or undifferentiated. HR, hazard ratio; UICC, International Union Against Cancer (UICC) TNM Classification of Malignant Tumours.

the present controversial use of 3-year relapse-free survival (RFS) as the primary end point in clinical trials of adjuvant chemotherapy for potentially curable gastric cancer.

PATIENTS AND METHODS

The trial was conducted in accordance with the World Medical Association Declaration of Helsinki and Japanese Good Clinical Practice guidelines. This protocol was approved by the institutional review board of each participating hospital (see Data Supplement). Written informed consent was obtained from all patients. Tumor stage classification and D classification were in accordance with the Japanese Classification of Gastric Carcinoma (Second English Edition).[13]

Patients and Treatment

Eligibility criteria were as follows: a histopathologically confirmed diagnosis of stage II (except for T1 disease), IIIA, or IIIB gastric cancer; R0 resection (with no tumor cells at the margin) with D2 or more extensive lymph node dissection; no evidence of hepatic, peritoneal, or distant metastasis; no tumor cells in peritoneal fluid on cytologic analysis; age 20 to 80 years; no previous treatment for cancer except for the initial gastric resection for the primary lesion; and adequate organ function. Patients were enrolled within 6 weeks after surgery over the telephone or by means of facsimile. Patients were randomly assigned to either the S-1 group or the surgery-only group. The assignments were made by the minimization method according to disease stage (II, IIIA, or IIIB) at the ACTS-GC data center.

Patients assigned to the S-1 group received S-1 in a daily dose of 80, 100, or 120 mg in two divided doses. The dose of S-1 was assigned on the basis of body surface area. S-1 was given for 4 weeks, followed by 2 weeks of rest. Treatment was continued for 1 year after surgery. Patients assigned to the surgery-only group received no anticancer treatment postoperatively until the confirmation of recurrence. The criteria for dose reduction and toxicity were described previously.[12]

Follow-Up

In the S-1 group, the results of blood tests and clinical findings were assessed at 2-week intervals during treatment with S-1. In the surgery-only group, patients came to the hospital for re-examination at least once every 3 months for the first year after surgery. From the second year onward, all patients were followed up in the same manner. Relapse was confirmed by imaging studies, including ultrasonography, computed tomography, and GI radiography, as well as endoscopy. Patients underwent at least one imaging study at 6-month intervals for the first 2 years after surgery and at 1-year intervals until 5 years after surgery. Individual patients were followed up for 5 years from the date of random assignment.