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

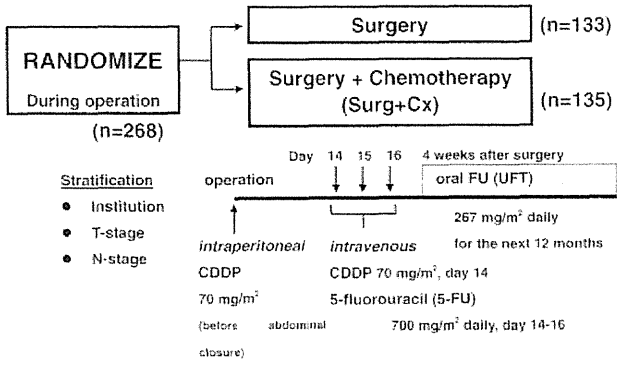

Fig. 1 Study scheme. All patients underwent gastrectomy with D2 or greater lymph node dissection. CDDP, *cis*-diamminedichloro-platinum (cisplatin)

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

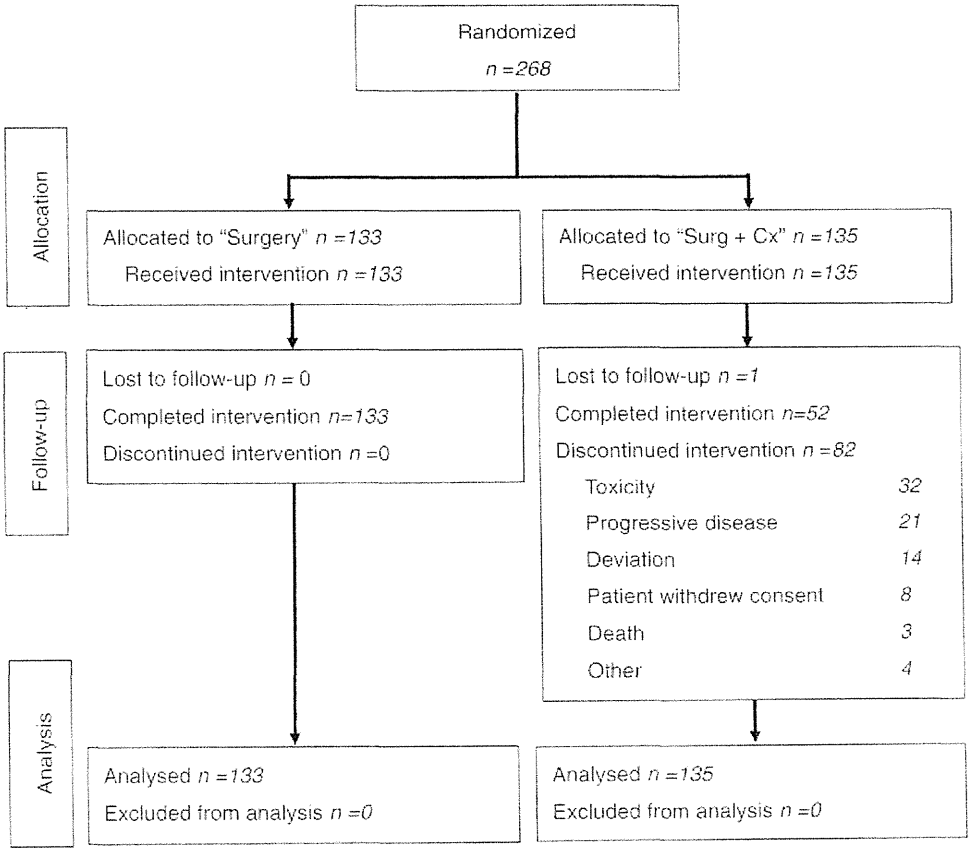

Fig. 2 CONSORT diagram

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

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.

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]

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

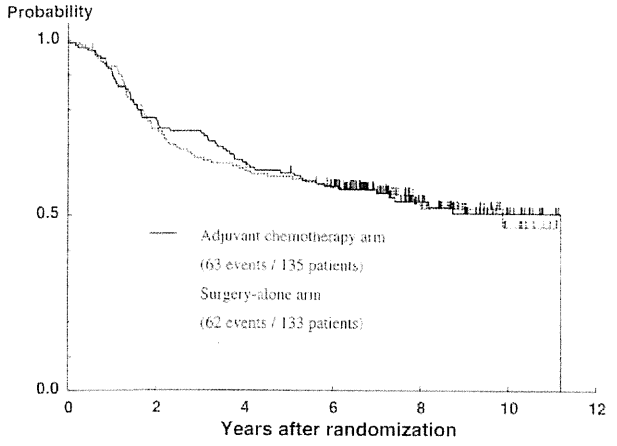

Fig. 3 Overall survival. There was no significant difference in overall survival: 5-year survival rate 62.0% (95% confidence interval 53.7–70.2) in the adjuvant chemotherapy group versus 60.9% (52.6–69.2) in the surgery-alone group, $P = 0.482$

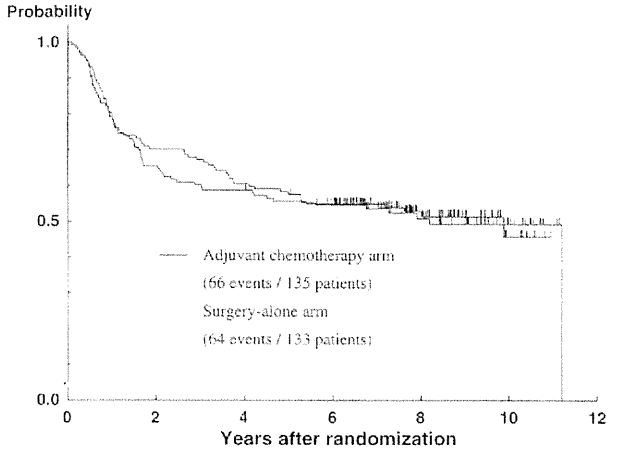

Fig. 4 Relapse-free survival. There was no significant difference in relapse-free survival: 5-year relapse-free survival rate 57.5% (95% confidence interval 49.1–65.9) in the adjuvant chemotherapy group versus 55.6% (47.2–64.1) in the surgery-alone group, $P = 0.512$

(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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References

1. Pisani P, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer. 1999;83(1):18–29.
2. Wanebo HJ, Kennedy BJ, Chmiel J, Steele G Jr, Winchester D, Osteen R. Cancer of the stomach. A patient care study by the American College of Surgeons. Ann Surg. 1993;218(5):583–92.
3. Miyashiro I, Takachi K, Doki Y, Ishikawa O, Ohigashi H, Murata K, et al. When is curative gastrectomy justified for gastric cancer with positive peritoneal lavage cytology but negative macroscopic peritoneal implant? World J Surg. 2005;29(9):1131–4.
4. Burke EC, Jr, Karpeh MS, Conlon KC, Brennan MF. Peritoneal lavage cytology in gastric cancer: an independent predictor of outcome. Ann Surg Oncol. 1998;5(5):411–5.
5. Gunderson LL, Sosin H. Adenocarcinoma of the stomach: areas of failure in a re-operation series (second or symptomatic look) clinicopathologic correlation and implications for adjuvant therapy. Int J Radiat Oncol Biol Phys. 1982;8(1):1–11.
6. Hiratsuka M, Iwanaga T, Furukawa H, Yasuda T, Nakano H, Nakamori S, et al. Important prognostic factors in surgically treated gastric cancer patients. Gan To Kagaku Ryoho. 1995;22(5):703–8.
7. Landry J, Tepper JE, Wood WC, Moulton EO, Koerner F, Sullinger J. Patterns of failure following curative resection of gastric carcinoma. Int J Radiat Oncol Biol Phys. 1990;19(6):1357–62.
8. Japanese Research Society for Gastric Cancer. Japanese Classification of Gastric Carcinoma. 1st English ed. Tokyo: Kanehara; 1995.
9. Japanese Gastric Cancer Association. Japanese Classification of Gastric Carcinoma—2nd English edition. Gastric Cancer. 1998;1(1):10–24.
10. Sakuramoto S, Sasako M, Yamaguchi T, Kinoshita T, Fujii M, Nashimoto A, et al. Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med. 2007;357(18):1810–20.
11. Nakajima T, Nashimoto A, Kitamura M, Kito T, Iwanaga T, Okabayashi K, et al. Adjuvant mitomycin and fluorouracil followed by oral uracil plus tegafur in serosa-negative gastric cancer: a randomised trial. Gastric Cancer Surgical Study Group. Lancet. 1999;354(9175):273–7.
12. Nashimoto A, Nakajima T, Furukawa H, Kitamura M, Kinoshita T, Yamamura Y, et al. Randomized trial of adjuvant chemotherapy with mitomycin, fluorouracil, and cytosine arabinoside followed by oral fluorouracil in serosa-negative gastric cancer: Japan Clinical Oncology Group 9206-1. J Clin Oncol. 2003;21(12):2282–7.
13. Nakajima T. Gastric cancer treatment guidelines in Japan. Gastric Cancer. 2002;5(1):1–5.
14. Shimoyama M, Fukuda H, Saijo N, Yamaguchi N. Japan Clinical Oncology Group (JCOG). Jpn J Clin Oncol. 1998;28(3):158–62.
15. Tobinai K, Kohno A, Shimada Y, Watanabe T, Tamura T, Takeyama K, et al. Toxicity grading criteria of the Japan Clinical Oncology Group. The Clinical Trial Review Committee of the Japan Clinical Oncology Group. Jpn J Clin Oncol. 1993;23(4):250–7.
16. Sano T, Katai H, Sasako M, Maruyama K. One thousand consecutive gastrectomies without operative mortality. Br J Surg. 2002;89(1):123.
17. Kang Y, Chang H, Zang D, Lee J, Kim T, Yang D, et al. Postoperative adjuvant chemotherapy for grossly serosa-positive advanced gastric cancer: a randomized phase III trial of intraperitoneal cisplatin and early mitomycin-C plus long-term doxifluridine plus cisplatin (iceMFP) versus mitomycin-C plus short-term doxifluridine (Mf) (AMC0101) (NCT00296322). J Clin Oncol. 2008;26(15s; ASCO annual meeting abstract LBA4511).
18. Chang H, Kang Y, Min Y, Zang D, Kim G, Yang D, et al. A randomized phase III trial comparing mitomycin-C plus short-term doxifluridine (Mf) versus mitomycin-C plus long-term doxifluridine plus cisplatin (MFP) after curative resection of advanced gastric cancer (AMC 0201) (NCT00296335). J Clin Oncol. 2008;26(15s; ASCO annual meeting abstract 4531).
19. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med. 2006;355(1):11–20.
20. Msika S, Benhamiche AM, Tazi MA, Rat P, Faivre J. Improvement of operative mortality after curative resection for gastric cancer: population-based study. World J Surg. 2000;24(9):1137–42.
21. Sasako M, Sano T, Yamamoto S, Sairenji M, Arai K, Kinoshita T, et al. Left thoracoabdominal approach versus abdominal-transhiatal approach for gastric cancer of the cardia or subcardia: a randomised controlled trial. Lancet Oncol. 2006;7(8):644–51.

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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.

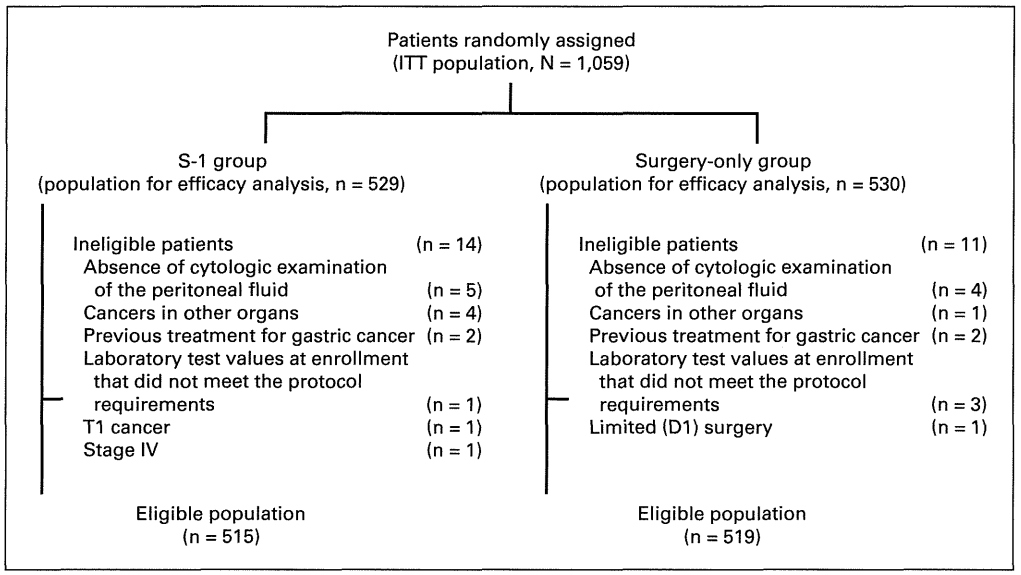

Fig 1. CONSORT diagram. D1 gastrectomy; ITT, intent-to-treat.

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

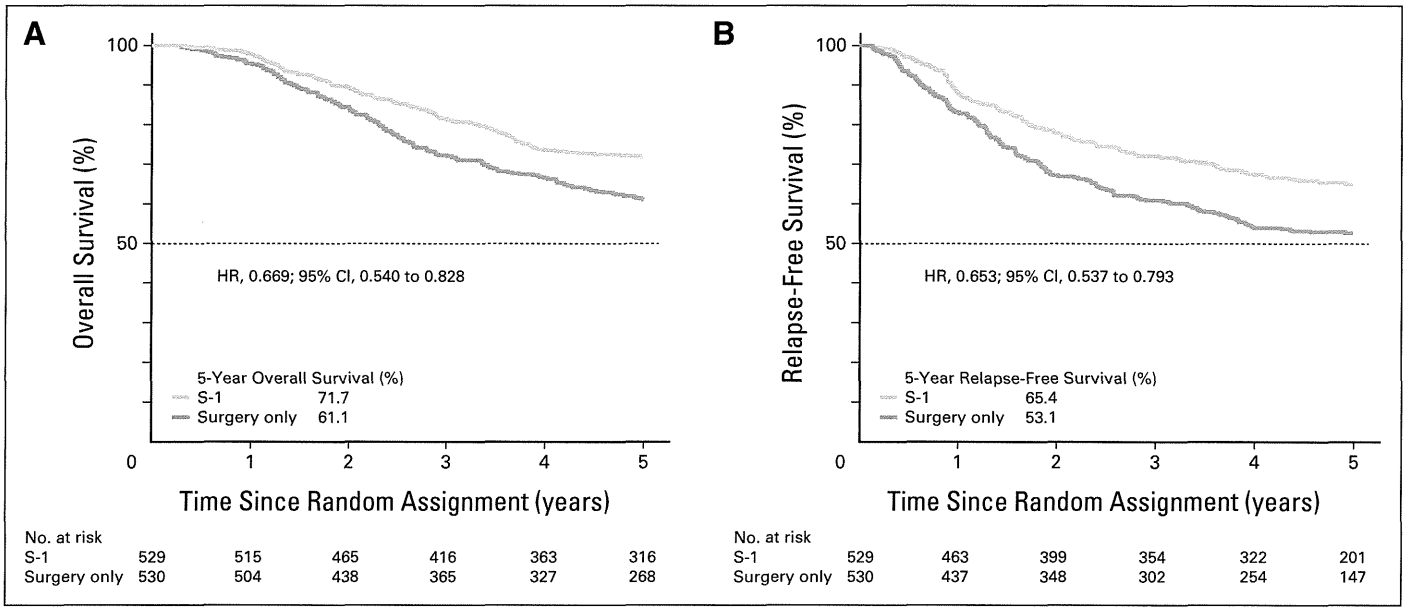

Fig 2. Kaplan-Meier estimates of (A) overall survival and (B) relapse-free survival for all randomly assigned patients. HR, hazard ratio.

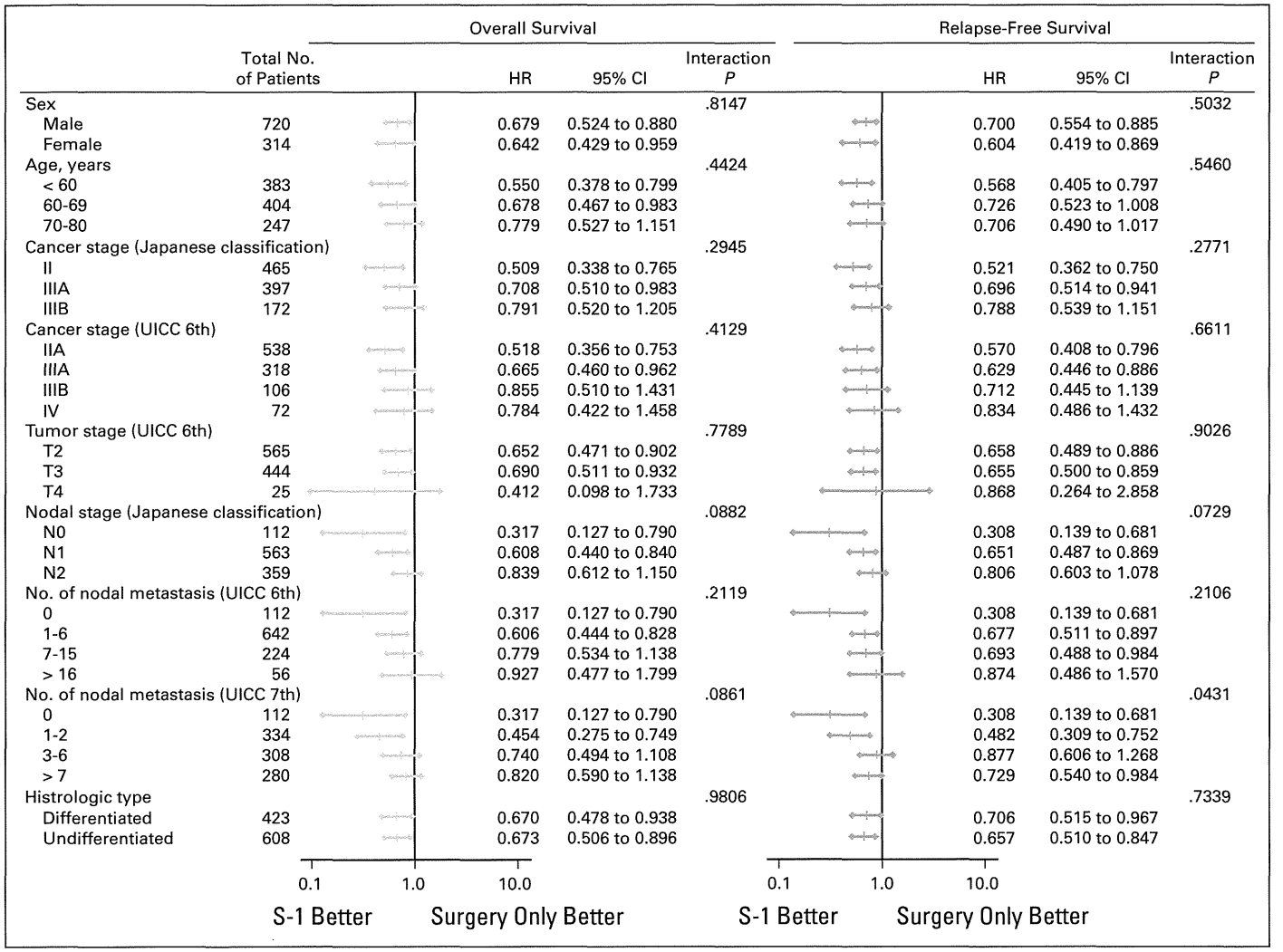

Fig 3. Subgroup analysis: overall survival and relapse-free survival for eligible population. In the surgery-only group, cancers in three patients could not be classified as differentiated or undifferentiated. HR, hazard ratio; UICC, International Union Against Cancer (UICC) TNM Classification of Malignant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

Statistical Analysis

The sample size was calculated as follows. Given that the 5-year survival rate would be 70% in the surgery-only group, with an HR of 0.70, $\alpha = .05$ (two-sided), and a statistical power of 80%, we estimated that 1,000 patients would be required. OS and RFS were estimated on the basis of all randomly assigned patients. The results in eligible patients were analyzed according to disease stage. OS was defined as the interval from the date of random assignment to the date of death from any cause. RFS was defined as the interval from the date of random assignment to the date of confirming recurrence or death from any cause, whichever came first. Data for up to 5 years from the date of random assignment were analyzed. Data obtained after 5 years were not included in this analysis. The survival rate was estimated by using the Kaplan-Meier method. The Cox proportional hazards model was used to calculate HRs. All statistical analyses were done with SAS, version 9.1 (SAS Institute, Cary, NC).

RESULTS

Patients

From October 2001 through December 2004, a total of 1,059 patients were enrolled at 109 centers throughout Japan; 529 were assigned to the S-1 group and 530 to the surgery-only group (intention-to-treat population; Fig 1). In both groups combined, 474 patients (44.8%) had stage II disease, 409 (38.6%) had stage IIIA disease, and 175 (16.5%) had stage IIIB disease. The numbers of patients with each stage of disease were similar in the two treatment groups. The groups were also well balanced with respect to the type of gastrectomy performed, the combined resection of other organs, and other factors. Details of the patient demographics and baseline characteristics have been reported previously.[12]

Fourteen patients in the S-1 group and 11 in the surgery-only group were ineligible, as shown in Figure 1. In the S-1 group, 12 patients did not receive S-1. In the surgery-only group, four patients received adjuvant treatment at their strong request, violating the protocol.

Safety

Details of the safety analysis have been reported previously.[12] In brief, except for anorexia (incidence, 6%), grade 3 or 4 adverse events occurred in less than 5% of the patients in the S-1 group.

OS and RFS in All Randomly Assigned Patients

Among 1,059 patients, 145 and 199 died, 32 and 42 patients are alive with recurrence, and 352 and 289 patients are alive without recurrence in the S-1 and the surgery-only groups, respectively. Data on 131 patients lost to follow-up within 5 years from the date of random assignment were censored.

OS and RFS were analyzed in all 1,059 randomly assigned patients. The 5-year OS rate was 71.7% (95% CI, 67.8% to 75.7%) in the S-1 group and 61.1% (95% CI, 56.8% to 65.3%) in the surgery-only group. The HR for death in the S-1 group compared with the surgery-only group was 0.669 (95% CI, 0.540 to 0.828), indicating that S-1 reduced the risk of death by 33.1% (Fig 2A). The 5-year RFS rate was 65.4% (95% CI, 61.2% to 69.5%) in the S-1 group and 53.1% (95% CI, 48.7% to 57.4%) in the surgery-only group. The HR for relapse in the S-1 group compared with that in the surgery-only group was 0.653 (95% CI, 0.537 to 0.793). Treatment with S-1 thus reduced the risk of relapse by 34.7% (Fig 2B).

Subgroup Analysis

OS and RFS in eligible patients were analyzed according to sex, age, disease stage (Japanese Classification, 13th edition), and histologic type. There was no interaction between treatment and any of these factors (Fig 3). Kaplan-Meier estimates of OS and RFS are shown according to disease stage, which was used as a stratification factor when patients were randomly assigned (Figs 4, 5, and 6).

The 5-year OS rates of the patients with stage II disease were 84.2% (95% CI, 79.5% to 89.0%) in the S-1group and 71.3% (95% CI, 65.3% to 77.2%) in the surgery-only group, with an HR of 0.509 (95% CI, 0.338 to 0.765; Fig 4A). Their 5-year RFS rates were 79.2% (95% CI, 73.8% to 84.6%) in the S-1 group and 64.4% (95% CI, 58.1% to 70.7%) in the surgery-only group, with an HR of 0.521 (95% CI, 0.362 to 0.750; Fig 4B). The 5-year OS rates of stage IIIA patients were 67.1% (95% CI, 60.4% to 73.8%) in the S-1 group and 57.3% (95% CI, 50.3% to 64.2%) in the surgery-alone group, with an HR of 0.708 (95% CI, 0.510 to 0.983; Fig 5A). Their 5-year RFS rates were 61.4% (95% CI, 54.5% to 68.4%) in the S-1 group and 50.0% (95% CI, 42.9% to 57.0%) in the surgery-alone group, with an HR of 0.696 (95% CI,

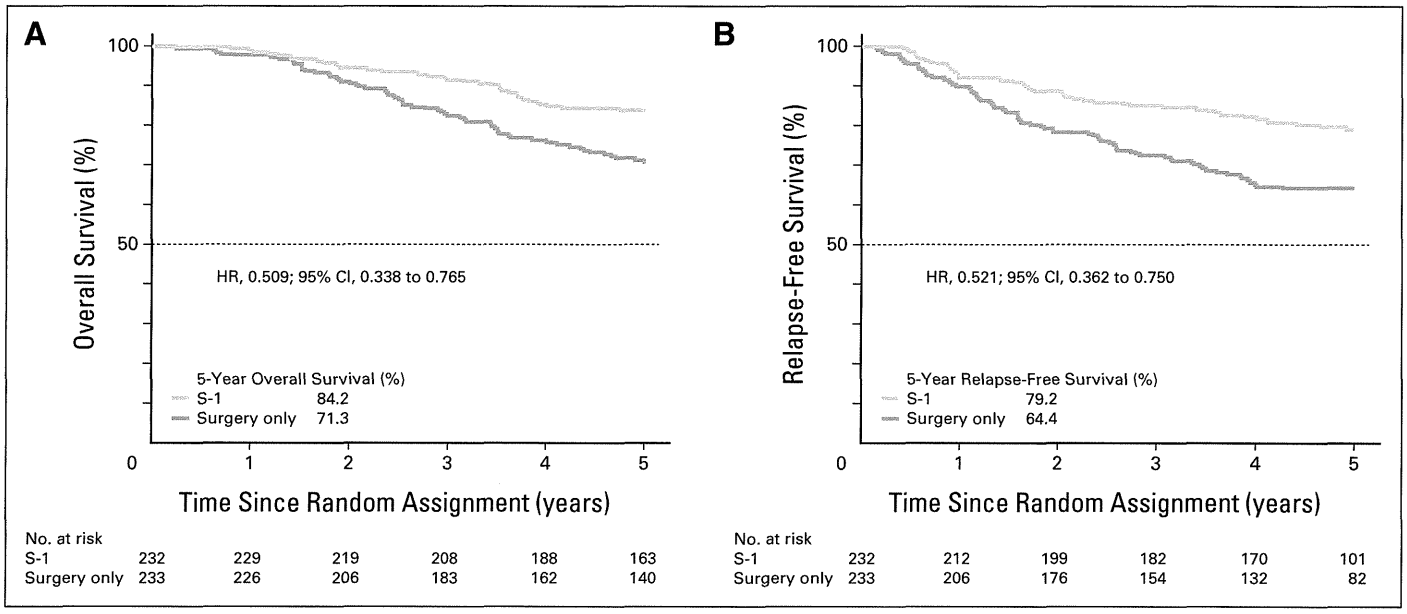

Fig 4. Kaplan-Meier estimates of (A) overall survival and (B) relapse-free survival for eligible patients with stage II gastric cancer. HR, hazard ratio.

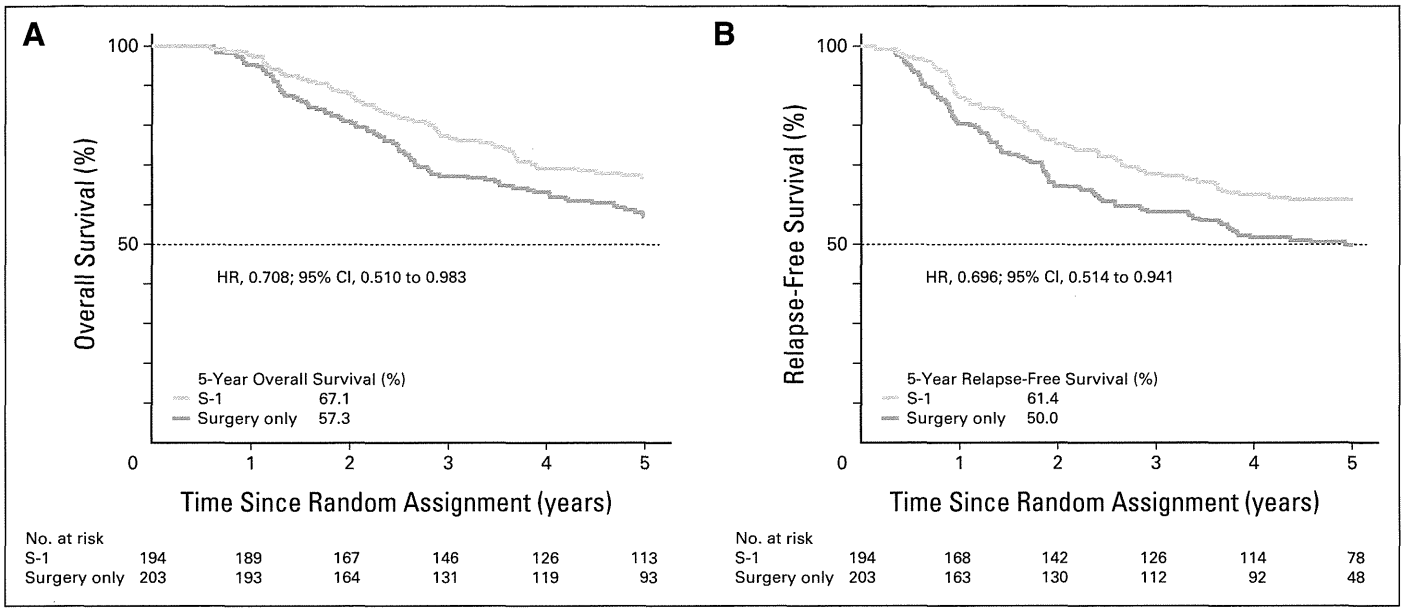

Fig 5. Kaplan-Meier estimates of (A) overall survival and (B) relapse-free survival for eligible patients with stage IIIA gastric cancer. HR, hazard ratio.

0.514 to 0.941; Fig 5B). As for stage IIIB disease, we enrolled 90 patients in the S-1 group and 85 in the surgery-only group; the 5-year OS rates were 50.2% (95% CI, 39.5% to 61.0%) in the S-1 group and 44.1% (95% CI, 33.1% to 55.0%) in the surgery-alone group, with an HR of 0.791 (95% CI, 0.520 to 1.205; Fig. 6A). Their 5-year RFS rates were 37.6% (95% CI, 27.0% to 48.2%) in the S-1 group and 34.4% (95% CI, 24.1% to 44.7%) in the surgery-alone group, with an HR of 0.788 (95% CI, 0.539 to 1.151; Fig 6B).

Site of First Relapse

Common sites of first relapse were the peritoneum, hematogenous sites, and lymph nodes (Table 1). Rates of metastasis and relapse were consistently lower in the S-1 group than in the surgery-only group for all sites. In particular, the rates of recurrence in lymph nodes and of peritoneal relapse were markedly lower in the S-1 group.

DISCUSSION

To the best of our knowledge, the ACTS-GC study is the first large clinical trial of adjuvant chemotherapy enrolling more than 1,000 patients who underwent D2 gastrectomy for gastric cancer. The results of this follow-up study showed that 1-year treatment with S-1 improved OS and RFS at 5 years compared with surgery alone, thus reconfirming the conclusions reached on early publication of the study results after a median follow-up of 3 years.

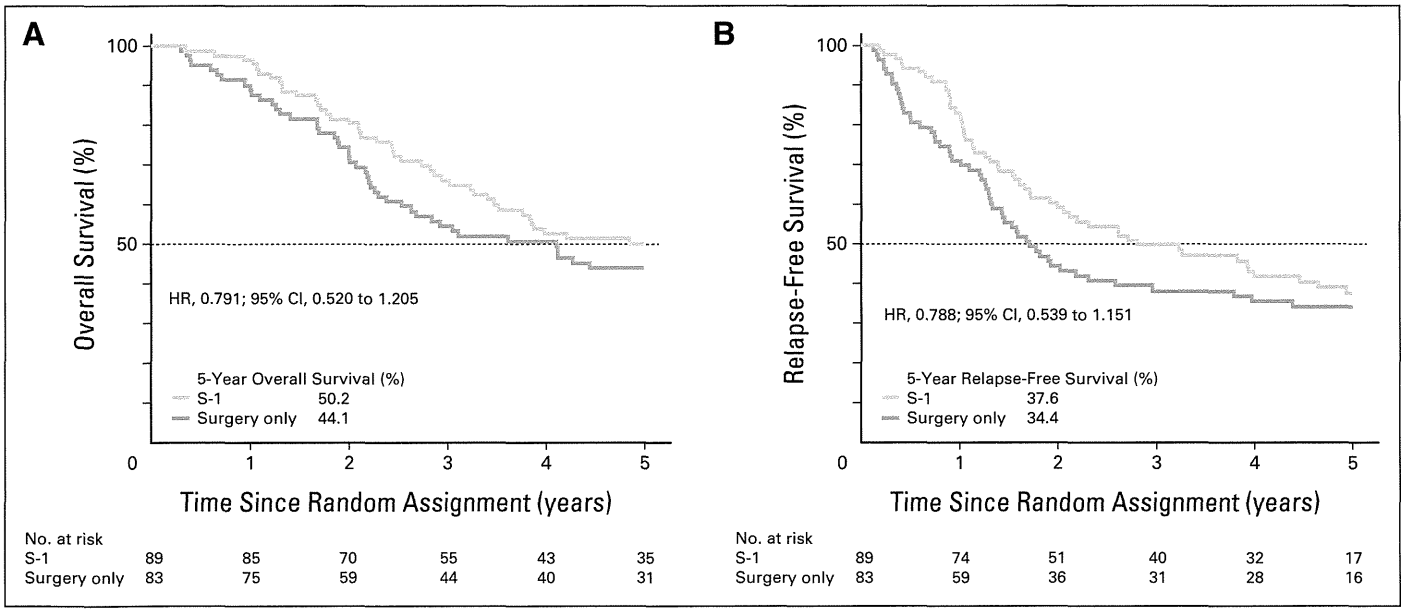

Fig 6. Kaplan-Meier estimates of (A) overall survival and (B) relapse-free survival for eligible patients with stage IIIB gastric cancer. HR, hazard ratio.

Table 1. Site of First Relapse (all randomly assigned patients)*

Site	S-1 (n = 529)		Surgery Only (n = 530)		HR	95%CI
	No.	%	No.	%		
Total No. of relapses	162	30.6	221	41.7	—	—
Local	11	2.1	17	3.2	0.572	0.268 to 1.221
Lymph nodes	30	5.7	54	10.2	0.505	0.323 to 0.789
Peritoneum	77	14.6	100	18.9	0.687	0.511 to 0.925
Hematogenous	61	11.5	71	13.4	0.784	0.557 to 1.105

Abbreviation: HR, hazard ratio.
*Some patients had a first relapse at more than one site.

Our present results confirmed that postoperative adjuvant chemotherapy with S-1 alone reduced the risk of death by 33.1%, thereby demonstrating that effectiveness was maintained since the previous analysis. This reduction in the risk of mortality is comparable with that obtained with combined regimens for adjuvant chemotherapy in the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial[14] and the Intergroup 0116 (INT-0116) trial.[15]

Whether the results of this study can be extrapolated to countries outside East Asia remains uncertain because of possible differences in pharmacokinetics of S-1 between whites and East Asians. If S-1 is used as adjuvant chemotherapy in whites, the dose should be carefully adjusted. A second reason is that all patients in this study underwent D2 gastrectomy although more limited surgery (D0/1) is commonly performed in the United States and some parts of Europe. In the surgery-only group, OS at 5 years was 61.1%, which was much better than that of patients undergoing D2 gastrectomy in Europe (33%) in a Dutch trial.[16] One of the reasons for this large difference may be the high level and widespread use of diagnostic technology in Japan, potentially leading to stage migration between Japan and Western countries.[17] Another important reason might be the high quality of D2 gastrectomy in Japan, whereas D0 or D1 gastrectomy remains the standard procedure in the United States and was the standard in Europe until recently. Although a Dutch trial comparing D1 with D2 gastrectomy reported negative results,[16,18] a 15-year follow-up study showed that the rate of mortality from gastric cancer was significantly lower in the D2 gastrectomy group.[19] Thus, the most recent European Society for Medical Oncology (ESMO) clinical practice guidelines recommend D2 gastrectomy as the standard procedure for curable advanced gastric cancer.[20]

The primary end point of this study was 5-year OS, although that of an ongoing adjuvant chemotherapy study in Korea and China is 3-year disease-free survival. The latter is designed to evaluate the efficacy of postoperative adjuvant chemotherapy with capecitabine and oxaliplatin compared with surgery alone. To justify the use of RFS or disease-free survival as the primary end point for adjuvant chemotherapy after curative resection of gastric cancer, more evidence is needed, but the results of this study may strongly suggest that RFS can be used as the primary end point of such studies. (In this follow-up analysis, the 3-year RFS rates were 72.4% and 61.1%, and the 5-year OS rates were 71.7% and 61.1% in the S-1 group and surgery-only group, respectively.)

To compare our results with those of other foreign studies, we also report the stage-specific 3- and 5-year OS and RFS according to the International Union Against Cancer (UICC) TNM Classification of Malignant Tumours, Sixth Edition. Three-year OS rates according to UICC staging in the S-1 and surgery-only groups were 91.1% and 80.9% (stage II), 77.8% and 68.3% (stage IIIA), 66.6% and 56.8% (stage IIIB), and 59.1% and 45.7% (stage IV). Three-year RFS rates were 84.3% and 73.5% (stage II), 69.1% and 56.7% (stage IIIA), 44.8% and 28.9% (stage IIIB), and 46.0% and 37.1% (stage IV). Five-year OS rates were 83.4% and 70.8% (stage II), 68.9% and 56.2% (stage IIIA), 43.7% and 40.1% (stage IIIB), and 45.1% and 42.7% (stage IV). Five-year RFS rates were 77.9% and 65.4% (stage II), 64.3% and 48.7% (stage IIIA), 35.9% and 28.9% (stage IIIB), and 26.8% and 25.0% (stage IV).

The approach for adjuvant chemotherapy differs among East Asian countries, including Japan, in which D2 gastrectomy has long been the standard procedure, and Western countries, in which D0 or D1 gastrectomy used to be or currently is standard. As Cunningham and Chua[21] stated, "surgery alone" is no longer standard treatment anywhere in the world for advanced gastric cancer. Some type of adjuvant chemotherapy, including the use of radiotherapy after D0/1 resection, can thus be considered standard treatment at present.

A meta-analysis by the Global Advanced/Adjuvant Stomach Tumor Research International Collaboration (GASTRIC) group[7] showed that some form of postoperative chemotherapy is associated with a higher survival rate than surgery alone; moreover, the use of monotherapy for postoperative adjuvant treatment resulted in good outcomes. The ACTS-GC trial demonstrated that S-1 monotherapy improved OS and RFS. In patients with early-stage (II and IIIA) tumors, the benefits of treatment with S-1 were considerable. However, the 5-year OS rate in patients with stage IIIB disease was 50.2% in the S-1 group and 44.1% in the surgery-only group, suggesting that there remains some room for improvement. Future studies should evaluate the effectiveness of intensive preoperative and/or postoperative chemotherapy with multiple agents in patients at high risk for relapse.

The results of the S-1 plus cisplatin versus S-1 in randomized controlled trial in the treatment for stomach cancer (SPIRITS) trial,[22] demonstrating that S-1 plus cisplatin is superior to S-1 alone with respect to survival in patients with unresectable or recurrent gastric cancer, and the V325 study [a randomized, multinational phase II/III trial of patients with untreated advanced gastric cancer],[23,24] showing that the addition of docetaxel to cisplatin plus fluorouracil prolongs survival, indicated that S-1 plus cisplatin and S-1 plus docetaxel are candidate regimens for postoperative adjuvant chemotherapy. These regimens were confirmed to be feasible in a postoperative setting,[25,26] and further studies should be performed to examine whether such regimens are superior to S-1 alone.

The Japan Clinical Oncology Group (JCOG) is now performing the JCOG 0501 study to compare S-1 plus cisplatin as neoadjuvant chemotherapy with surgery followed by S-1 monotherapy in patients with clinically resectable Borrmann type 4 (linitis plastica) and large type 3 gastric cancer. This trial is expected to be a landmark study, determining the future direction for preoperative chemotherapy in Japan.

The use of molecular targeted agents for gastric cancer has been studied extensively. In the Trastuzumab in Combination with Chemotherapy Versus Chemotherapy Alone for Treatment of HER2-Positive Advanced Gastric or Gastro-Esophageal Junction Cancer (ToGA) study, trastuzumab combined with cisplatin and either fluorouracil or capecitabine significantly prolonged OS in patients with HER2-positive gastric cancer.[27] The effectiveness of adjuvant chemotherapy with molecular targeted agents such as trastuzumab also needs to be assessed in patients with HER2-positive gastric cancer.

In conclusion, this 5-year follow-up study confirmed that adjuvant chemotherapy with S-1 given for 1 year after surgery improved

OS and RFS at 5 years in patients with stage II or III gastric cancer who underwent D2 gastrectomy. Postoperative chemotherapy with S-1 can be recommended for patients with stage II or III gastric cancer who undergo D2 gastrectomy, at least in Asian populations.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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REFERENCES

1. Ferlay J, Shin HR, Bray F, et al: Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10, 2010. http://www.iarc.fr/en/publications/eresources/cancerbases/index.php
2. Hermans J, Bonenkamp JJ, Boon MC, et al: Adjuvant therapy after curative resection for gastric cancer: Meta-analysis of randomized trials. J Clin Oncol 11:1441-1447, 1993
3. Piedbois P, Buyse M: Meta-analyses need time, collaboration, and funding. J Clin Oncol 12:878-880, 1994
4. Earle CC, Maroun JA: Adjuvant chemotherapy after curative resection for gastric cancer in non-Asian patients: Revisiting a meta-analysis of randomised trials. Eur J Cancer 35:1059-1064, 1999
5. Mari E, Floriani I, Tinazzi A, et al: Efficacy of adjuvant chemotherapy after curative resection for gastric cancer: A meta-analysis of published randomised trials—A study of the GISCAD (Gruppo Italiano per lo Studio dei Carcinomi dell'Apparato Digerente). Ann Oncol 11:837-843, 2000
6. Panzini I, Gianni L, Fattori PP, et al: Adjuvant chemotherapy in gastric cancer: A meta-analysis of randomized trials and a comparison with previous meta-analyses. Tumori 88:21-27, 2002
7. GASTRIC (Global Advanced/Adjuvant Stomach Tumor Research International Collaboration) Group, Paoletti X, Oba K, et al: Benefit of adjuvant chemotherapy for resectable gastric cancer: A meta-analysis. JAMA 303:1729-1737, 2010
8. Shirasaka T, Shimamato Y, Ohshimo H, et al: Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. Anticancer Drugs 7:548-557, 1996
9. Diasio RB: Clinical implications of dihydropyrimidine dehydrogenase inhibition. Oncology (Williston Park) 13:17-21, 1999
10. Sakata Y, Ohtsu A, Horikoshi N, et al: Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients. Eur J Cancer 34:1715-1720, 1998
11. Koizumi W, Kurihara M, Nakano S, et al: Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer: For the S-1 Cooperative Gastric Cancer Study Group. Oncology 58:191-197, 2000
12. Sakuramoto S, Sasako M, Yamaguchi T, et al: Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med 357:1810-1820, 2007
13. Japanese Gastric Cancer Association: Japanese Classification of Gastric Carcinoma - 2nd English Edition. Gastric Cancer 1:10-24, 1998
14. Cunningham D, Allum WH, Stenning SP, et al: Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med 355:11-20, 2006
15. Macdonald JS, Smalley SR, Benedetti J, et al: Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. N Engl J Med 345:725-730, 2001
16. Bonenkamp JJ, Hermans J, Sasako M, et al: Extended lymph-node dissection for gastric cancer. N Engl J Med 340:908-914, 1999
17. Bunt AM, Hermans J, Smit VT, et al: Surgical/pathologic-stage migration confounds comparisons of gastric cancer survival rates between Japan and Western countries. J Clin Oncol 13:19-25, 1995
18. Bonenkamp JJ, Songun I, Hermans J, et al: Randomised comparison of morbidity after D1 and D2 dissection for gastric cancer in 996 Dutch patients. Lancet 345:745-748, 1995
19. Songun I, Putter H, Kranenbarg EM, et al: Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial. Lancet Oncol 11:439-449, 2010
20. Okines A, Verheij M, Allum W, et al: Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 21:v50-v54, 2010
21. Cunningham D, Chua YJ: East meets West in the treatment of gastric cancer. N Engl J Med 357:1863-1865, 2007
22. Koizumi W, Narahara H, Hara T, et al: S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): A phase III trial. Lancet Oncol 9:215-221, 2008
23. Van Cutsem E, Moiseyenko VM, Tjulandin S, et al: Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: A report of the V325 Study Group. J Clin Oncol 24:4991-4997, 2006
24. Ajani JA, Moiseyenko VM, Tjulandin S, et al: Quality of life with docetaxel plus cisplatin and fluorouracil compared with cisplatin and fluorouracil from a phase III trial for advanced gastric or gastroesophageal adenocarcinoma: The V-325 Study Group. J Clin Oncol 25: 3210-3216, 2007
25. Fujitani K, Tamura S, Kimura Y, et al: Phase II feasibility study of adjuvant S-1 plus docetaxel for stage III gastric cancer patients after curative D2 gastrectomy (OGSG 0604). J Clin Oncol 27, 2009 (suppl; abstr e15567)
26. Takahari D, Hamaguchi T, Yoshimura K, et al: Feasibility study of adjuvant chemothe rapy with S-1 plus cisplatin for gastric cancer. Cancer Chemother Pharmacol 67:1423-1428, 2011
27. Bang YJ, Van Cutsem E, Feyereislova A, et al: Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal *junction cancer (ToGA): A phase 3, open-label, ran*domised controlled trial. Lancet 376:687-697, 2010

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REGIV as a Potential Biomarker for Peritoneal Dissemination in Gastric Adenocarcinoma

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Background: This study examined the clinical significance of regenerating islet-derived family member 4 (REGIV) in surgically resected gastric tumors. The potential of REGIV as a biomarker in gastric cancer was also assessed including its predictive value for prognosis and recurrence after surgery.

Methods: Immunohistochemistry was performed to assess the clinical significance of REGIV expression status in surgically resected specimens. The quantitative genetic diagnostic method, transcription-reverse transcription concerted reaction (TRC) that targeted REGIV mRNA was applied for prediction of peritoneal recurrence in gastric cancer.

Results: Positive immunostaining for REGIV was observed in 85 cases (52.5%), and correlated significantly with diffuse type histopathology ($P = 0.001$), advanced T stage ($P = 0.022$), and frequent peritoneal recurrence ($P = 0.009$). Multivariate analysis identified advanced T stage ($P < 0.001$) and REGIV expression ($P = 0.034$) as independent prognostic factors for peritoneal recurrence-free survival. Overexpression of REGIV protein was evident in the majority of peritoneal tumors (93.8%). REGIV mRNA assessed by TRC could be a predictive marker for peritoneal recurrence after curative operation.

Conclusions: REGIV overexpression is common in primary gastric tumors and a potentially suitable marker of diffuse type histopathology and peritoneal dissemination. Overexpression of REGIV mRNA, assessed by the TRC method, is a potentially suitable marker of peritoneal recurrence after curative resection.

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KEY WORDS: **gastric cancer; REGIV; peritoneal dissemination; TRC; molecular diagnosis**

INTRODUCTION

The incidence of gastric cancer has decreased worldwide and particularly so in Western countries. Despite this, it remains the fourth most common cancer and the second most common cause of cancer-related death [1,2]. The prognosis of patients with advanced gastric cancer, especially those with serosa-invading tumors, remains poor even after curative operation. In such cases, peritoneal dissemination due to seeding of free cancer cells from the primary gastric cancer is the most common type of spread [3–5]. The identification of suitable biomarkers to predict peritoneal recurrence and prognosis is therefore important to advance the treatment of patients with gastric cancer.

Regenerating islet-derived family member 4 (REGIV) belongs to a superfamily of calcium-dependent lectins [6]. REGIV is expressed in various normal tissues including the stomach, colon, small intestine, and pancreas [7,8], and is overexpressed in various tumors such as gastric, colorectal, pancreas, prostate, and gallbladder cancers [7–11]. Overexpression of REGIV was shown in colorectal adenomas with severe dysplasia and adenocarcinoma, indicating the involvement of REGIV in the early stages of colorectal carcinogenesis [12]. REGIV protein expression was also reported in goblet cells of intestinal metaplasia and goblet-like cell vesicles of gastric cancer, implicating REGIV in the differentiation of stomach cancer. A recent in vitro study further showed that the carbohydrate-recognition domain of REGIV protein is critical for colorectal cell migration and invasion [13]. Several studies have identified REGIV as a potent activator of epidermal growth factor receptor (EGFR)/Akt/activator protein-1 (AP-1). Furthermore, colon cancer cells treated with recombinant REGIV showed increased expression of Bcl-2, Bcl-xl, and survivin, suggesting a role in the inhibition of apoptosis [14–16]. Finally, REGIV expression also correlated significantly with resistance to combination chemotherapy with 5-fluorouracil (5-FU) and cisplatin [15]. Despite these data linking REGIV and human cancers, the precise biological function of REGIV overexpression in human cancer remains unclear.

In this study, we examined the expression of REGIV protein in gastric cancer tissues and assessed the correlations between REGIV expression and clinicopathological characteristics. The results showed that overexpression of REGIV protein correlated significantly with diffuse type histopathology and peritoneal recurrence after surgery. Furthermore, REGIV overexpression was observed in most peritoneal disseminated tumors obtained by surgery or staging laparoscopy. We introduce a novel, rapid, and quantitative genetic diagnostic technique that targets REGIV mRNA and called it the

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transcription-reverse transcription concerted reaction (TRC) to detect occult cancer cells in the peritoneal cavity of patients with gastric cancer. In another study, we assessed the clinical significance of the molecular diagnosis and examined the association between REGIV expression and chemoresistance to the combination chemotherapy of S-1 plus cisplatin, which is a standard regimen for gastric cancer in Japan [17].

MATERIALS AND METHODS

Patients and Specimens

We obtained gastric cancer tissues from 162 patients who underwent gastrectomy at the Department of Gastroenterological Surgery, Osaka University Hospital between 2000 and 2008. All tumors were confirmed as gastric adenocarcinoma by histopathological examination. The patients comprised 115 males and 47 females, aged 34–92 years (median, 66 years). Table I lists the characteristics of patients registered in this study. The pathological features were classified based on the 13th edition of the Japanese Classification of Gastric Cancer [18]. Sixteen peritoneal disseminated tumors were obtained from patients by surgery or staging laparoscopy and the corresponding 15 primary tumor specimens were also obtained from patients by surgery or upper gastrointestinal endoscopy. Twenty specimens biopsied during upper gastrointestinal endoscopy and three surgically resected tumor specimens were also obtained from patients treated with the combination chemotherapy of S-1, 5-FU derivative, and cisplatin [17]. The expression of REGIV mRNA by TRC in peritoneal lavage specimens of 95 patients was examined to test for correlation between TRC and cytology. Of those sampled, 50 patients who received no neoadjuvant chemotherapy and whose peritoneal lavage cytology was diagnosed as negative were assessed for further survival analyses.

Evaluation of Clinical Response to Chemotherapy

Before and after chemotherapy with S-1 plus cisplatin, conventional examinations including multidetector row computed tomography and gastric endoscopy were performed to assess the clinical response. The tumor response of measurable metastatic lesions was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) [19]. A complete response (CR) was defined as the disappearance of all evidence of cancer for more than 4 weeks. A partial response (PR) was defined as more than 50% reduction in the sum of the products of the perpendicular diameters of all lesions without any evidence of new regions or progression on any lesions. Stable disease (SD) was defined as less than a 50% reduction or less than a 25% increase in the sum of the products of the perpendicular diameters of all lesions, without any evidence of new lesions. Progressive disease (PD) was defined as a more than 25% increase in more than one region or the appearance of new regions.

Immunohistochemical Analysis

REGIV protein expression was evaluated by immunohistochemical (IHC) analysis of 4-μm thick sections from 10% formalin-fixed and paraffin-embedded blocks. For IHC staining, tissue slides were deparaffinized in xylene, and then rehydrated through a graded ethanol series. For antigen retrieval, slides were autoclaved in 10 mM citrate buffer (pH 6.0) at 121°C for 10 min. Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxide in methanol for 20 min, and then nonspecific binding was blocked in 10% normal serum for 20 min. The sections were then incubated overnight at 4°C in a moist chamber with anti-REGIV antibody (dilution 1:50; R&D Systems, Minneapolis, MN). The sites of antibody binding were visualized with the ABC peroxidase detection system (Vector Laboratories, Burlingame, CA). Finally, the sections were incubated in 3,3′-diaminobenzidine tetrahydrochloride with 0.05% H_2O_2 for 3 min and counterstained with 0.1% hematoxylin. The percentage of cancer cells stained with the antibody was evaluated. The presence of REGIV protein was judged as positive if more than 10% of the total observed cancer cells were positively stained; any less was judged as negative.

RNA Extraction

Total cellular RNA was extracted from cell pellets of peritoneal lavage fluid samples and cancer cell lines using TRIZOL reagent according to the manufacturer's protocol. In brief, the cell source mixture was minced using disposable homogenizers (IEDA^{TM}, Tokyo, Japan), mixed with 0.2 ml chloroform, and then centrifuged at 12,000*g* for 15 min. The supernatant was transferred to a fresh tube and mixed with 0.5 ml 100% isopropyl alcohol. After incubation for 10 min at room temperature, RNA was precipitated by centrifugation, washed with 75% ethanol, and then diluted with diethyl pyrocarbonate (DEPC)-treated water.

TABLE I. Relationship Between REGIV Expression and Various Clinicopathological Characteristics in Patients With Gastric Cancer (n = 162)

		REGIV		
	n	Negative	Positive	*P*-value
Age <70/≥70	99/63	45/32	54/31	0.507
Gender (M/F)	115/47	55/22	60/25	0.906
Histological type				
Differentiated	77	47	30	0.001
Undifferentiated	85	30	55	
pT T1/T2/T3/T4	27/82/48/5	19/34/20/4	8/48/28/1	0.022
pN N0/N1/N2/N3	72/55/33/2	37/26/12/2	35/29/21/0	0.232
pStage I/II/III/IV	61/41/51/9	34/18/19/6	27/23/32/3	0.148
Cytology (negative/positive)	157/5	75/2	82/3	0.497
Lymph node recurrence (negative/positive)	152/10	73/4	79/6	0.623
Liver recurrence (negative/positive)	146/16	64/13	79/6	0.052
Peritoneal recurrence (negative/positive)	144/18	74/3	71/14	0.009

pStageI includes pStageIA and pStageIB.
pStageIII includes pStageIIIA and pStageIIIB according to the 13th edition of the Japanese Classification of Gastric Cancer.

Sequences of Primers and Probes for TRC

Synthetic oligonucleotide sequences of a pair of primers, a scissors probe for TRC amplification, and an intercalation-activating fluorescence (INAF) probe for detection of REGIV mRNA are listed in Table II. Numbers in parentheses indicate the corresponding position of the target genome sequences (Gene Bank Accession NM_032044.2). Sequences of the promoter primers indicated in italics are the T7 RNA polymerase-binding sequences. The primers, a scissors probe, and the INAF probe were designed to bind to the secondary-structure-free sites of REGIV mRNA. The INAF probe is a DNA oligonucleotide linked with an intercalating fluorescence dye, oxazole yellow. The 3′-OH end of the scissors probe and INAF probe was capped with an amino group and glycolic acid, respectively, to avoid undesired enzymatic elongation by the Avian Myeloblastosis Virus (AMV) reverse transcriptase reaction. Synthetic oligonucleotides of primers and the scissors probe were provided by Sawady Technology (Tokyo, Japan). Synthesis of the INAF probe for REGIV amplicons was performed as described previously [20].

TRC Reaction

The TRC reaction was conducted as described previously [20]. In brief, 20 μl of the TRC buffer was added to 5 μl of the RNA extract in a thin-wall PCR tube, followed by the addition of 5 μl of enzyme mix. The tube containing the mixture was closed and set in a dedicated instrument, the "TRC monitor," to measure the fluorescence intensity of the reaction mixture incubated at 44°C (excitation wavelength 470 nm, emission wavelength 520 nm).

Real-Time Monitoring of TRC Reaction

The "TRC monitor" was constructed on a round incubator block and rotating fluorescence scanning unit [20]. The temperature of the incubator block was controlled at optimal TRC conditions (44°C) and 32 thin-wall PCR tubes were installed and set in a circle. These were assembled into 1 U to enable synchronous scanning of the fluorescence while irradiating the tube. The LED turns like a beacon to irradiate the excitation light of 470 nm into a tube from outside. The fluorescence (520 nm) is then transferred from the bottom of the tube to a photomultiplier through a light guide.

TABLE II. Synthetic Oligonucleotide Sequences of a Pair of Primers, a Scissors Probe for Amplification, and an INAF Probe for Detection of REGIV mRNA in the TRC Reaction

Scissors probe (68–93)
26 base antisense
5-TATATCTTCTTGCCTCAGGAATTAAT-3
Forward primer (83–106)
45 base sense
5-*CTAATACGACTCACTATAGGG*AAGAAGATATAAAAGCTCCAGAAA-3
Reverse primer (168–194)
27 base antisense
5-GGGTTCTCCTTGATCTGCAAATCTGTT-3
INAF probe (147–166)
20 base antisense
5-GGCAACCAAGACTCTAAGGG-3

INAF, intercalation activating fluorescence; TRC, transcription-reverse transcription concerted reaction.
Numbers in parentheses indicate the corresponding position of the target genome sequences. The sequence indicated by the italicized letters of the promoter primers is the T7 RNA polymerase-binding sequence.

Statistical Analysis

Statistical analysis was performed with JMP® software (JMP version 8.0.2, SAS Institute, Cary, NC). The associations of REGIV expression with the patients' clinicopathological features were assessed by the chi-squared test. Disease-free survival (DFS) and overall survival (OS) were assessed using the Kaplan–Meier method and compared by the log-rank test. Multivariate survival analysis was performed on all parameters that were found to be significant by univariate analysis using the Cox proportional hazard model. P-values <0.05 were considered significant.

RESULTS

REGIV Protein Expression in Gastric Cancer Tissues

The expression of REGIV was investigated in 162 cases of gastric adenocarcinoma by IHC. Of these, 85 cases (52.5%) were considered positive for REGIV, which was detected mainly in the cytoplasm of tumor cells (Fig. 1A). The remaining 77 cases (47.5%) showed negative staining (Fig. 1B). The positive cells for REGIV were detected in various areas of the formed tumor including the surface, central, and deepest areas of the gastric wall.

Correlations Between REGIV Expression and Clinicopathological Parameters

Table II shows the correlations between REGIV overexpression detected by IHC and various clinicopathological parameters for the 162 patients with gastric cancer. The proportion of REGIV-positive cases was significantly higher with diffuse type histology, advanced pathological T stage, and frequent peritoneal recurrence, and REGIV-positive cases tended to harbor infrequent liver metastasis ($P = 0.052$). Other parameters listed in Table II (age, gender, pathological N stage, pathological S stage, and lymph node metastasis) showed no significant correlation with REGIV expression. However, REGIV overexpression did not correlate with recurrence-free survival, but was significantly associated with poorer peritoneal recurrence-free survival and tended to be associated with better recurrence-free survival at sites other than the peritoneum (Fig. 2A–C).

Prognostic Significance of REGIV Expression for Peritoneal Recurrence

Univariate analysis by Cox's proportional hazard model identified several clinicopathological parameters as significant predictors of prognosis (Table III), namely pathological T stage, pathological N stage, and REGIV expression (HR = 8.773, HR = 4.440, and HR = 4.113, respectively; Table III). However histological type was not a significant prognostic factor (HR = 2.253). Multivariate analysis that included all the above significant parameters identified pathological T stage and REGIV expression as significant independent prognostic predictors (HR = 6.359 and HR = 3.362, respectively; Table III).

Expression of REGIV in Peritoneal Metastatic Tumors

Subsequent IHC analysis of REGIV expression in 16 peritoneal tumors metastasized from gastric cancer revealed 15 (93.8%) with overexpressed REGIV (Fig. 1C). Furthermore, 14 out of 15 corresponding primary tumors that overexpressed REGIV protein in peritoneal metastasis showed overexpression of REGIV (Fig. 1D).

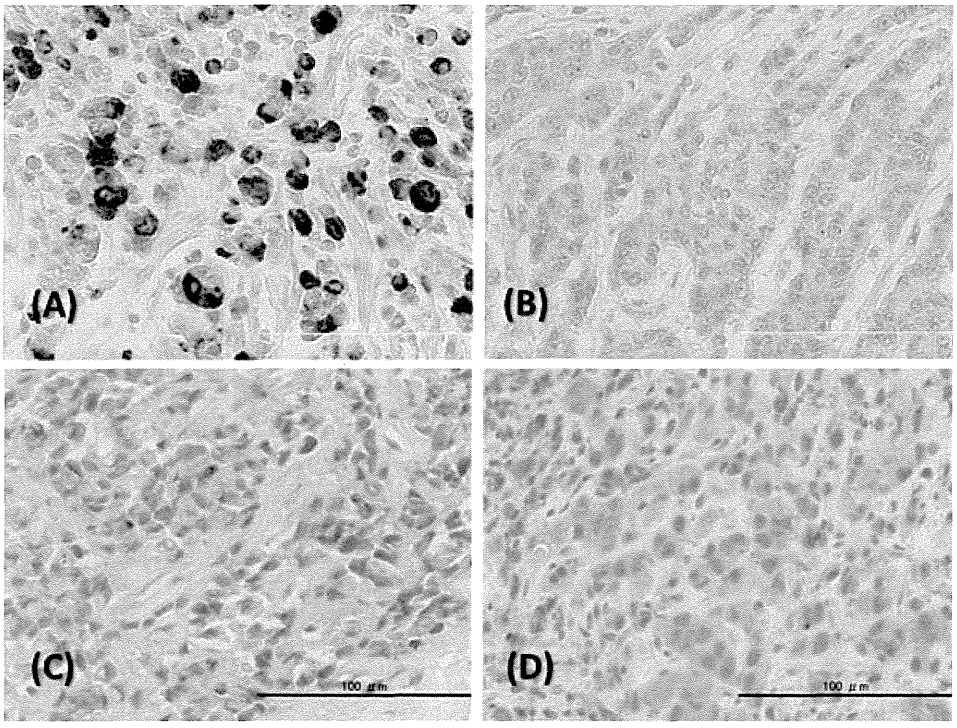

Fig. 1. Immunohistochemistry for REGIV protein in gastric cancer tissues. **A**: Representative positive staining for REGIV in primary tumor. **B**: Representative negative staining for REGIV in primary tumor. **C**: Representative positive staining in endoscopically biopsied specimen from primary tumor. **D**: Representative positive staining in peritoneal metastatic tumor.

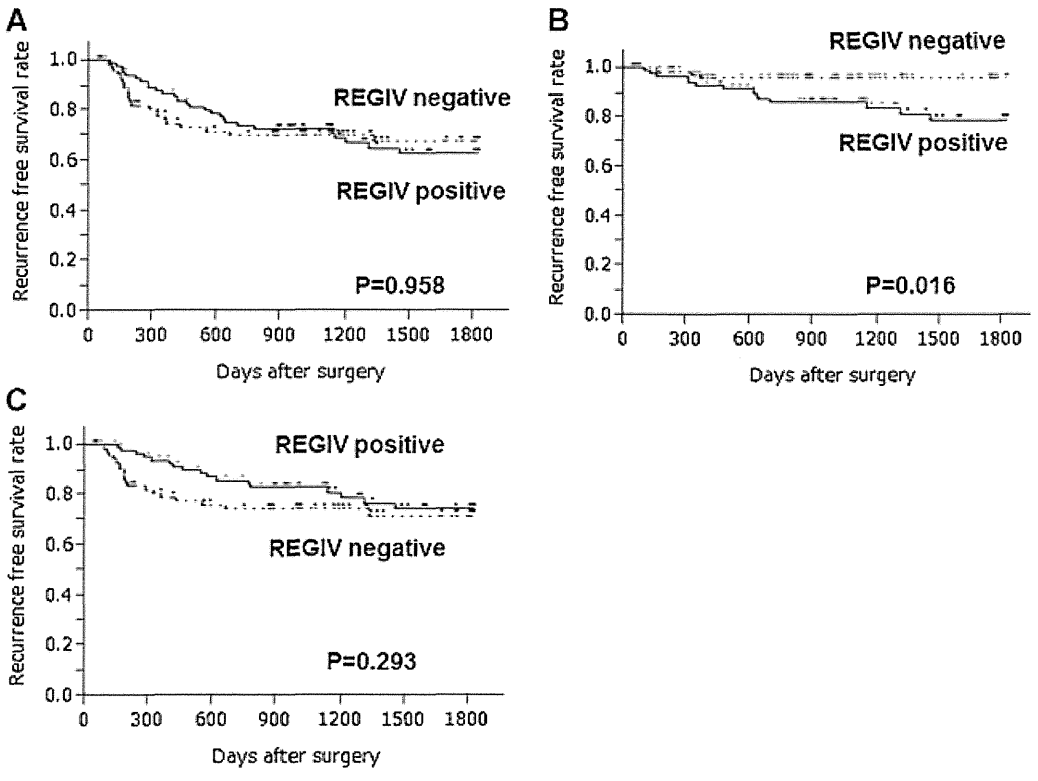

Fig. 2. Survival analysis according to REGIV expression in primary gastric cancer. **A**: Overall survival. **B**: Peritoneal recurrence-free survival. **C**: Recurrence-free survival outside of peritoneal sites.

TABLE III. Result of Univariate and Multivariate Survival Analyses of Peritoneal Recurrence-Free Survival by Cox's Proportional Hazard Model (n = 162)

	n	HR	95% CI	*P*-value
Univariate survival analysis				
Age (≥70/<70)	63/99	0.613	0.172–1.741	0.378
Gender (female/male)	47/115	0.675	0.190–1.909	0.477
Histological type (undifferentiated/differentiated)	85/77	2.253	0.836–7.082	0.111
pT(3–4/1–2)	53/109	8.773	3.096–31.205	<0.001
pN(1–3/0)	90/72	4.440	1.445–19.286	0.008
Cytology (positive/negative)	5/157	3.478	0.191–17.509	0.303
RegIV expression (positive/negative)	85/77	4.113	1.342–17.842	0.011
Multivariate survival analysis				
pT(3–4/1–2)	53/109	6.359	2.157–23.404	<0.001
N(1–3/0)	90/72	2.226	0.687–10.012	0.195
RegIV expression (positive/negative)	85/77	3.362	1.089–14.641	0.034

HR, hazard ratio; 95% CI, 95% confidence interval.

Correlations Between REGIV Protein Expression and Efficacy of Chemotherapy With S-1 Plus Cisplatin

Twenty preoperative specimens were biopsied by upper gastrointestinal endoscopy and 3 were surgically resected from patients subjected to combination chemotherapy of S-1 plus cisplatin [17]. There was no significant correlation between REGIV expression in these specimens and the effect of chemotherapy (CR + PR vs. SD + PD) in these cases.

TRC Analysis of Peritoneal Lavage Samples for REGIV mRNA

Finally, we examined the expression of REGIV mRNA by TRC in peritoneal lavage specimens of 95 patients to test for correlation between TRC and cytology. Of those sampled, 50 patients who received no neoadjuvant chemotherapy and whose peritoneal lavage cytology was diagnosed as negative were assessed for survival analyses. Table IV shows the correlative results, with 24 (96.0%) out of 25 cytology-positive specimens and 12 (17.1%) out of 70 cytology-negative specimens showing a positive TRC diagnosis. Figure 3 shows the comparative OS statistics for patients with gastric cancer after curative resections according to the TRC diagnosis for REGIV from peritoneal lavage specimens. Peritoneal recurrence-free survival in patients with positive TRC was significantly worse than in patients with negative TRC, although OS was not significantly different between the groups.

DISCUSSION

The present study indicated overexpression of REGIV protein in 52.5% of gastric cancers examined and identified an association between this expression and diffuse-type histopathology, tumor progression (advanced pT status), and frequent peritoneal recurrence. Furthermore, the REGIV overexpresssion was significantly associated with poorer peritoneal recurrence-free survival, although with no other type of recurrence-free survival in gastric cancer patients. The clinical significance of REGIV overexpression in gastric cancer is controversial. Oue et al. [7] reported REGIV overexpression in about 30% of gastric adenocarcinomas, in a significant association with poorly differentiated gastric cancer, although they found no associations with T status, N status, or pathological stage. In another study of 63 gastric cancer tumors, Yamagishi et al. [21] observed REGIV overexpression in 49% of cases, but found no relationship with any clinicopathological features including histology, lymph node metastasis, and clinical stage. In the study overexpression of REGI alpha, one of REG family, but not REGIV was an independent prognostic factor.

TABLE IV. Relationship Between TRC and Cytology for Peritoneal Lavage Specimens in Patients With Gastric Cancer (n = 95)

	TRC		
	Negative	Positive	Total
Cytology			
Negative	58	12 (17.1%)	70
Positive	1	24 (96.0%)	25
Total	59	36	95

Mitani et al. [15] reported that REGIV expression correlated significantly with resistance to combination chemotherapy with 5-FU and cisplatin. However, in our study, there was no significant correlation between REGIV expression and the effect of combination chemotherapy with a 5-FU derivative, S-1, and cisplatin.

The present study showed for the first time that REGIV overexpression was common in peritoneal metastatic tumors obtained during surgery or through staging laparoscopy (15/16, 94%), although REGIV protein was expressed in only 52.5% of primary tumors. These results suggested that REGIV overexpression could provide a biomarker for peritoneal dissemination in gastric cancer. Kuniyasu et al. [16] demonstrated that REGIV-transfected gastric cancer cell lines showed increased levels of BCL-2, BCL-XL, survivin, phosphorylated AKT, and phosphorylated EGFR, while peritoneal dissemination mouse models inoculated with REGIV-transfected gastric cancer cells showed increased number and size of peritoneal tumors and lower survival rates compared to untransfected controls. These authors also examined REGIV protein in peritoneal lavage samples obtained from gastric cancer surgery by immunoblot assay and showed that a REGIV-positive peritoneal lavage might be a good marker for peritoneal dissemination. In addition, REGIV mRNA expression assessed by quantitative RT-PCR was shown to be a sensitive predictive marker for peritoneal dissemination in gastric cancer [22]. However, RT-PCR procedures are complicated and time-consuming, thus further refinements are required for the clinical application of molecular diagnostic techniques for REGIV expression.

We reported previously a novel method of quantitative genetic diagnosis using the TRC reaction system for detection of cancer micrometastasis and prediction of cancer recurrence in patients with gastric cancer [23]. The method amplifies and measures a cancer-specific mRNA in a single tube at constant temperature (no thermal cycling) and with only three steps: denaturing, annealing, and extension for PCR. The single temperature reaction is likely to be more

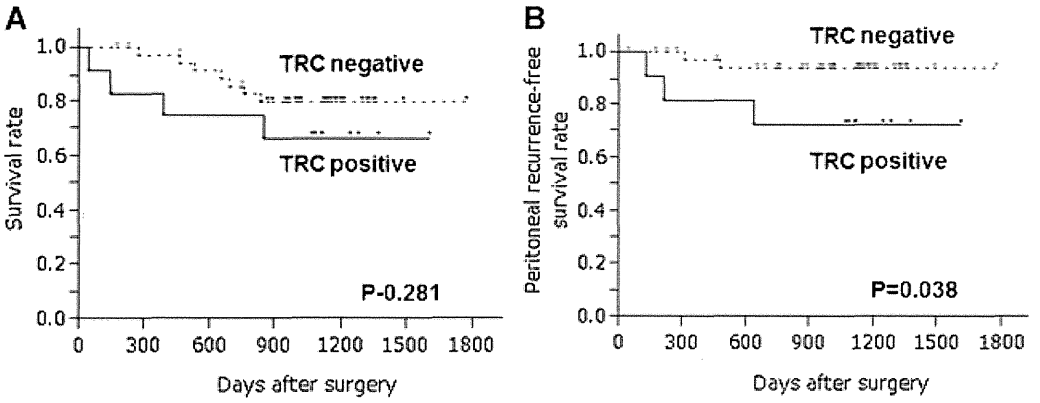

Fig. 3. Survival analysis according to the TRC diagnosis from peritoneal lavage specimens. **A**: Peritoneal recurrence-free survival. **B**: Overall survival.

stable and more accurate with respect to quantification. Another advantage is that this method amplifies RNA directly, avoiding the need for reverse transcription to convert RNA to cDNA prior to amplification. These advantages may allow the establishment of more reliable and practical genetic diagnosis of cancer micrometastasis. We reported previously on TRC using carcinoembryonic antigen (CEA) as a biomarker marker for the early detection of peritoneal recurrence after gastric cancer surgery [23]. However, CEA is not a cancer-specific marker and some regions in gastric tumors show no expression of CEA. Additional markers will therefore improve the sensitivity and specificity of our TRC method for predicting peritoneal recurrence following gastric cancer treatment. Our analyses in this study implicated TRC for REGIV as a potential molecular diagnostic method for predicting peritoneal dissemination in advanced gastric cancer in a simple and rapid manner.

In conclusion, we identified REGIV overexpression in peritoneal dissemination of advanced gastric cancer and that the detection of REGIV mRNA in peritoneal lavage fluid by TRC could be a predictor of peritoneal recurrence after curative gastrectomy. Overexpression of REGIV could become a predictor of peritoneal recurrence, although further studies will be needed in a larger population.

REFERENCES

1. Ferlay J, Shin HR, Bray F, et al.: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893–2917.
2. Parkin DM, Bray F, Ferlay J, et al.: Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
3. Dupont JB, Jr., Lee JR, Burton GR, et al.: Adenocarcinoma of the stomach: Review of 1,497 cases. Cancer 1978;41:941–947.
4. Yamada E, Miyaishi S, Nakazato H, et al.: The surgical treatment of cancer of the stomach. Int Surg 1980;65:387–399.
5. Yoo CH, Noh SH, Shin DW, et al.: Recurrence following curative resection for gastric carcinoma. Br J Surg 2000;87:236–242.
6. Hartupee JC, Zhang H, Bonaldo MF, et al.: Isolation and characterization of a cDNA encoding a novel member of the human regenerating protein family: Reg IV. Biochim Biophys Acta 2001;1518:287–293.
7. Oue N, Mitani Y, Aung PP, et al.: Expression and localization of Reg IV in human neoplastic and non-neoplastic tissues: Reg IV expression is associated with intestinal and neuroendocrine differentiation in gastric adenocarcinoma. J Pathol 2005;207: 185–198.
8. Gu Z, Rubin MA, Yang Y, et al.: Reg IV: A promising marker of hormone refractory metastatic prostate cancer. Clin Cancer Res 2005;11:2237–2243.
9. Violette S, Festor E, Pandrea-Vasile I, et al.: Reg IV, a new member of the regenerating gene family, is overexpressed in colorectal carcinomas. Int J Cancer 2003;103:185–193.
10. Takehara A, Eguchi H, Ohigashi H, et al.: Novel tumor marker REG4 detected in serum of patients with resectable pancreatic cancer and feasibility for antibody therapy targeting REG4. Cancer Sci 2006;97:1191–1197.
11. Tamura H, Ohtsuka M, Washiro M, et al.: Reg IV expression and clinicopathologic features of gallbladder carcinoma. Hum Pathol 2009;40:1686–1692.
12. Zhang Y, Lai M, Lv B, et al.: Overexpression of Reg IV in colorectal adenoma. Cancer Lett 2003;200:69–76.
13. Guo Y, Xu J, Li N, et al.: RegIV potentiates colorectal carcinoma cell migration and invasion via its CRD domain. Cancer Genet Cytogenet 2010;199:38–44.
14. Bishnupuri KS, Luo Q, Murmu N, et al.: Reg IV activates the epidermal growth factor receptor/Akt/AP-1 signaling pathway in colon adenocarcinomas. Gastroenterology 2006;130:137–149.
15. Mitani Y, Oue N, Matsumura S, et al.: Reg IV is a serum biomarker for gastric cancer patients and predicts response to 5-fluorouracil-based chemotherapy. Oncogene 2007;26:4383–4393.
16. Kuniyasu H, Oue N, Sasahira T, et al.: Reg IV enhances peritoneal metastasis in gastric carcinomas. Cell Prolif 2009; 42:110–121.
17. Koizumi W, Narahara H, Hara T, et al.: S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): A phase III trial. Lancet Oncol 2008;9:215–221.
18. Japanese Gastric Cancer A: Japanese classification of gastric carcinoma—2nd English edition. Gastric Cancer 1998;1:10–24.
19. Therasse P, Arbuck SG, Eisenhauer EA, et al.: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–216.
20. Ishiguro T, Saitoh J, Horie R, et al.: Intercalation activating fluorescence DNA probe and its application to homogeneous quantification of a target sequence by isothermal sequence amplification in a closed vessel. Anal Biochem 2003;314:77–86.
21. Yamagishi H, Fukui H, Sekikawa A, et al.: Expression profile of REG family proteins REG Ialpha and REG IV in advanced gastric cancer: Comparison with mucin phenotype and prognostic markers. Mod Pathol 2009;22:906–913.
22. Miyagawa K, Sakakura C, Nakashima S, et al.: Overexpression of RegIV in peritoneal dissemination of gastric cancer and its potential as A novel marker for the detection of peritoneal micrometastasis. Anticancer Res 2008;28:1169–1179.
23. Ishii T, Fujiwara Y, Ohnaka S, et al.: Rapid genetic diagnosis with the transcription-reverse transcription concerted reaction system for cancer micrometastasis. Ann Surg Oncol 2004;11: 778–785.

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Phase II trial of preoperative S-1 plus cisplatin followed by surgery for initially unresectable locally advanced gastric cancer

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Abstract

Background: The aim of this study was to evaluate the efficacy and feasibility of preoperative chemotherapy with S-1 plus cisplatin in patients with initially unresectable locally advanced gastric cancer.
Methods: We enrolled patients with initially unresectable locally advanced gastric cancer because of severe lymph node metastases or invasion of adjacent structures. Preoperative chemotherapy consisted of S-1 at 80 mg/m^2 divided in two daily doses for 21 days and cisplatin at 60 mg/m^2 intravenously on day 8, repeated every 35 days. If a tumor decreased in size, patients received 1 or 2 more courses. Surgery involved radical resection with D2 lymphadenectomy.
Results: Between December 2000 and December 2007, 27 patients were enrolled on the study. No CR was obtained, but PR was seen in 17 cases, and the response rate was 63.0%. Thirteen patients (48.1%) had R0 resections. There were no treatment related deaths. The median overall survival time (MST) and the 3-year overall survival (OS) of all patients were 31.4 months and 31.0%, respectively. Among the 13 patients who underwent curative resection, the median disease-free survival (DFS) and the 3-year DFS were 17.4 months and 23.1%, respectively. The MST and the 3-year OS were 50.1 months and 53.8%, respectively. The most common site of initial recurrence after the R0 resection was the para-aortic lymph nodes.
Conclusions: Preoperative S-1 plus cisplatin can be safely delivered to patients undergoing radical gastrectomy. This regimen is promising as neoadjuvant chemotherapy for resectable gastric cancer. For initially unresectable locally advanced gastric cancer, new trials using more effective regimens along with extended lymph node dissection are necessary.

Keywords: Neoadjuvant chemotherapy; Lymph node dissection; Bulky lymph node; TS-1; Cisplatin; Para-aortic lymph node

Introduction

Gastric cancer is still one of the most common cancers in the world; 876,000 new cases were anticipated worldwide in the year 2000.[1] In Japan, 110,323 new cases were anticipated in the year 2003 and the 5-year survival rate of gastric cancer diagnosed from 1993 to 1996 was 54.4%.[2,3]

Currently, surgery remains the mainstay of curative treatment. However, only an R0 resection is associated with significant cure rates. Patients having microscopic (R1) or macroscopic (R2) residual tumor have an extremely poor prognosis.[4]

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