

Fig. 2. Overall survival curves in patients with Siewert type 1 (a) and Siewert type 2 (b) tumors, by treatment groups. THO = Transthoracic esophagectomy; TTO = transhiatal esophagectomy.

via right thoracotomy with transhiatal one. The overall survival in the entire study population did not show statistically significant differences between the 2 groups. However, the actual difference in the survival curves was impressive and the overall 5-year survival rate was 29% for the transhiatal approach and 39% for the transthoracic one ($p = 0.38$; fig. 1). In the subgroup analysis according to the Siewert classification, the difference in overall 5-year survival was as large as 17% (95% CI -3 to 37%) for Siewert type 1 ($n = 90$), while it was only 1% for Siewert type 2 ($n = 115$; fig. 2) [22]. Due to the small sam-

ple size, this study was not able to show any statistically significant difference, but the results strongly suggest that thorough mediastinal dissection via right thoracotomy is needed for Siewert type 1 but not for type 2. With higher morbidity after transthoracic dissection, the transhiatal approach might be better treatment for Siewert type 2.

Sasako et al. [23] reported the results of a phase III trial for Siewert type 2 and 3 tumors, comparing a left thoraco-abdominal approach versus a transhiatal one. All these tumors were diagnosed to have esophageal in-

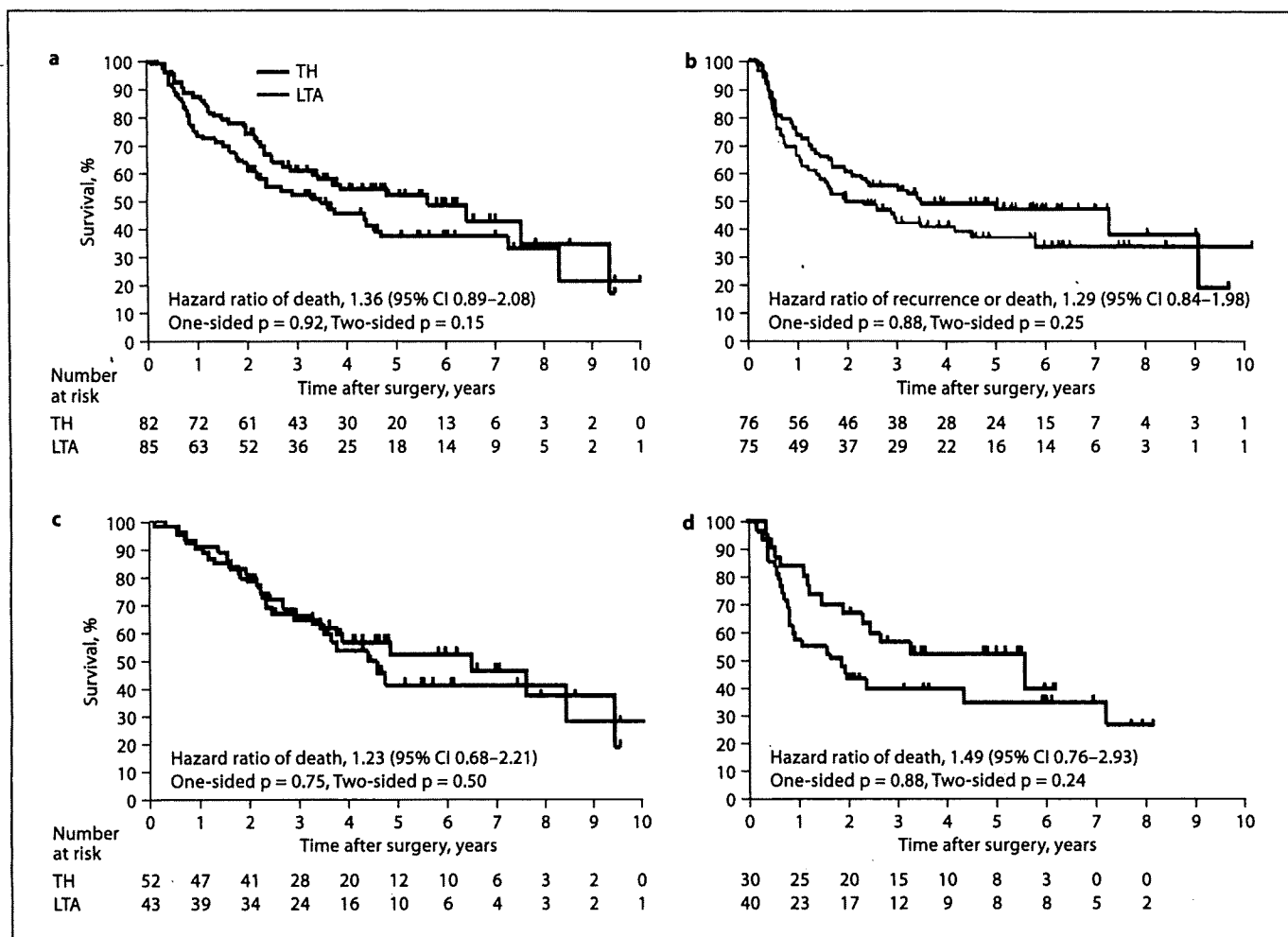


Fig. 3. Overall survival (a) and disease-free survival (b) of the entire patient population and overall survival in patients with Siewert type 2 (c) and type 3 (d) tumors by treatment groups. TH = Transhiatal; LTA = left thoraco-abdominal. Reprinted with permission from *The Lancet Oncology* [23].

Table 3. Comparison between the INT0116 study and JCOG9206-2 study

	INT0116/SWOG9008	JCOG9206-2
Surgery (D0/1/2), %	54/36/10	4/67/33
Adjuvant	Rad (45 Gy)+CX (5FU+LV)	CDDP+5FU+UFT (50%), none (50%)
Number of patients	281 (tested arm)	268 (control = 133, tested = 135)
Tumor location	A (53%), Corp (24%), cardia (21%), multifocal (2%)	L (31%), M (32%), U (28%), wide (9%)
pT (T1/T2/T3/T4)	14/74/175/18	5/87/165/11
Proportion of T3/4, %	69	66
Node positive, %	85	72
TRD	3 (1.1%)	4 (1.5%)
Overall survival (5 years), %	42	control 61, tested 62

Rad = Radiation; CX = chemotherapy; LV = leucovorin; 5FU = 5-fluorouracil; CDDP = cis-diamminedichloroplatinum; UFT = uracil-ftegafur; A = antrum; Corp = gastric body; L = distal one third; M = middle one third; U = upper one third; wide = wide spread; TRD = treatment-related death.

vasion of 3 cm or less. They clearly demonstrated that there was no survival benefit from the left thoraco-abdominal approach which was accompanied by a much higher morbidity and more remarkable deterioration of pulmonary function than the transhiatal approach. The subgroup analysis showed no survival benefit for both Siewert type 2 and 3. Especially for Siewert type 3, the

transhiatal approach showed much better survival than the left thoracotomy approach (fig. 3).

From these two trials, the transhiatal approach is regarded as the standard treatment for Siewert type 2 and 3 tumors, while the transthoracic approach via right thoracotomy is recommended for Siewert type 1 tumors.

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Reprinted From

The NEW ENGLAND JOURNAL of MEDICINE

VOL. 357 NO. 18

ESTABLISHED IN 1812

NOVEMBER 1, 2007

WWW.NEJM.ORG



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ORIGINAL ARTICLE

Adjuvant Chemotherapy for Gastric Cancer with S-1, an Oral Fluoropyrimidine

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ABSTRACT

BACKGROUND

Advanced gastric cancer can respond to S-1, an oral fluoropyrimidine. We tested S-1 as adjuvant chemotherapy in patients with curatively resected gastric cancer.

METHODS

Patients in Japan with stage II or III gastric cancer who underwent gastrectomy with extended (D2) lymph-node dissection were randomly assigned to undergo surgery followed by adjuvant therapy with S-1 or to undergo surgery only. In the S-1 group, administration of S-1 was started within 6 weeks after surgery and continued for 1 year. The treatment regimen consisted of 6-week cycles in which, in principle, 80 mg of oral S-1 per square meter of body-surface area per day was given for 4 weeks and no chemotherapy was given for the following 2 weeks. The primary end point was overall survival.

RESULTS

We randomly assigned 529 patients to the S-1 group and 530 patients to the surgery-only group between October 2001 and December 2004. The trial was stopped on the recommendation of the independent data and safety monitoring committee, because the first interim analysis, performed 1 year after enrollment was completed, showed that the S-1 group had a higher rate of overall survival than the surgery-only group ($P=0.002$). Analysis of follow-up data showed that the 3-year overall survival rate was 80.1% in the S-1 group and 70.1% in the surgery-only group. The hazard ratio for death in the S-1 group, as compared with the surgery-only group, was 0.68 (95% confidence interval, 0.52 to 0.87; $P=0.003$). Adverse events of grade 3 or grade 4 (defined according to the Common Toxicity Criteria of the National Cancer Institute) that were relatively common in the S-1 group were anorexia (6.0%), nausea (3.7%), and diarrhea (3.1%).

CONCLUSIONS

S-1 is an effective adjuvant treatment for East Asian patients who have undergone a D2 dissection for locally advanced gastric cancer. (ClinicalTrials.gov number, NCT00152217.)

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N Engl J Med 2007;357:1810-20.
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META-ANALYSES HAVE SHOWN THAT ADJUVANT chemotherapy is effective in treating gastric cancer.¹⁻⁶ However, the effectiveness of specific regimens has not been verified in large clinical trials. In 2001, the Intergroup-0116 (INT-0116) study investigators reported that postoperative chemoradiotherapy was effective in treating adenocarcinoma of the stomach or gastroesophageal junction.⁷ Subsequently, the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial⁸ showed the efficacy of perioperative chemotherapy. Both studies assessed the benefits of adjuvant therapy after only limited surgery, but the type of surgical procedure for gastric cancer can influence the results of postoperative chemotherapy.^{9,10} In Japan, gastrectomy with extended (D2) lymph-node dissection alone is considered standard treatment.¹¹

S-1 (TS-1, Taiho Pharmaceutical) is an orally active combination of tegafur (a prodrug that is converted by cells to fluorouracil), gimeracil (an inhibitor of dihydropyrimidine dehydrogenase, which degrades fluorouracil), and oteracil (which inhibits the phosphorylation of fluorouracil in the gastrointestinal tract, thereby reducing the gastrointestinal toxic effects of fluorouracil) in a molar ratio of 1:0.4:1.^{12,13} The rate of response to treatment with S-1 alone exceeded 40% in two late phase 2 trials involving patients with advanced or recurrent gastric cancer.^{14,15} The pharmacokinetics of the fluorouracil that is derived from S-1 is not influenced by gastrectomy,¹⁶ and for this reason, S-1 is suitable for the postoperative adjuvant setting. In a pilot study,¹⁷ we examined the feasibility of using S-1 postoperatively in patients with gastric cancer. We report the results of a large-scale trial — the Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer (ACTS-GC) — involving patients with stage II or III gastric cancer who underwent D2 surgery.

METHODS

The trial was conducted in accordance with the World Medical Association Declaration of Helsinki and Japanese Good Clinical Practice guidelines. The protocol was approved by the institutional review board of each participating hospital. Written informed consent was obtained from all patients.

All members of the steering committee and the sponsor jointly designed the trial and collected the

data, which were held by the independent ACTS-GC Data Center. The data were analyzed by the independent data and safety monitoring committee. All academic members of the steering committee vouch for the validity and completeness of the data and the analysis. All of the authors reviewed and approved the final version of the manuscript before submission.

ELIGIBILITY CRITERIA

The criteria for eligibility were histologically proven gastric cancer of stage II (excluding T1 cases), IIIA, or IIIB; D2 or more extensive lymph-node dissection with R0 surgery (with the result of no residual tumor¹⁸); no hepatic, peritoneal, or distant metastasis; no tumor cells in peritoneal fluid on cytologic analysis; an age of 20 to 80 years; no previous treatment for cancer except for the initial gastric resection for the primary lesion; and adequate organ function (a leukocyte count of at least 4000 per cubic millimeter or the lower limit of the normal range; a platelet count of at least 100,000 per cubic millimeter; a total bilirubin level of no more than 1.5 mg per deciliter [25.7 μ mol per liter], aspartate aminotransferase and alanine aminotransferase levels no more than 2.5 times the upper limit of the normal range; and a serum creatinine level no greater than the upper limit of the normal range). Stage classification and the evaluation of resected specimens were performed in accordance with the guidelines of the Japanese Gastric Cancer Association.¹⁸

STUDY DESIGN AND TREATMENT

The primary end point was overall survival; secondary end points were relapse-free survival and the degree of safety of S-1. Patients were enrolled, within 6 weeks after surgery, over the telephone or by fax by staff at the ACTS-GC data center. Patients were randomly assigned to either the S-1 group or the surgery-only group, with the assignments made at the ACTS-GC data center by means of the minimization method and according to the cancer stage (II, IIIA, or IIIB). Zelen's adjustment¹⁹ was applied to balance the numbers of patients between each group at each participating hospital.

Patients assigned to the S-1 group received two oral doses of 40 mg of S-1 per square meter of body-surface area per day, for 4 weeks, followed by 2 weeks of no chemotherapy. Specifically, during the treatment weeks, patients with a body-surface

Table 1. Baseline Characteristics of the Patients.

Characteristic	S-1 (N=529)	Surgery Only (N=530)	P Value*
Sex — no. (%)			0.98
Male	367 (69.4)	369 (69.6)	
Female	162 (30.6)	161 (30.4)	
Age			0.86
<60 yr — no. (%)	199 (37.6)	195 (36.8)	
60–69 yr — no. (%)	193 (36.5)	215 (40.6)	
70–80 yr — no. (%)	137 (25.9)	120 (22.6)	
Median — yr	63	63	
Range — yr	27–80	33–80	
Tumor stage — no. (%)			0.81
T1	1 (0.2)	0	
T2	289 (54.6)	286 (54.0)	
T3	225 (42.5)	232 (43.8)	
T4	14 (2.6)	12 (2.3)	
Nodal stage, Japanese classification — no. (%)†			0.72
N0	51 (9.6)	64 (12.1)	
N1	296 (56.0)	281 (53.0)	
N2	182 (34.4)	185 (34.9)	
N3	0	0	
No. of lymph-node metastases — no. (%)			0.37
0	51 (9.6)	64 (12.1)	
1–6	331 (62.6)	325 (61.3)	
7–15	117 (22.1)	113 (21.3)	
≥16	30 (5.7)	28 (5.3)	

area of less than 1.25 m² received 80 mg daily; those with a body-surface area of 1.25 m² or more but less than 1.5 m² received 100 mg daily; and those with a body-surface area of 1.5 m² or more received 120 mg daily. This 6-week cycle was repeated during the first year after surgery. If patients had hematologic toxic effects of grade 3 or grade 4 (highest possible grade) or nonhematologic toxic effects of grade 2, grade 3, or grade 4, their daily dose was reduced, from 120 mg to 100 mg, 100 mg to 80 mg, or 80 mg to 50 mg. The surgery-only group received no anticancer treatment after surgery, unless there was a confirmed relapse.

Patients in both groups were to be followed up for 5 years postoperatively. Adverse events were assessed according to the Common Toxicity Criteria of the National Cancer Institute (version 2.0).

FOLLOW-UP

Patients in the S-1 group underwent hematologic tests and assessments of clinical symptoms every 2 weeks. Patients in the surgery-only group underwent similar examinations at least every 3 months. Evaluation for adverse events was performed every 3 months for 1 year after surgery.

The presence of a relapse was determined by means of imaging studies, including ultrasonography, computed tomography (CT), gastrointestinal radiography series, and endoscopy. Patients underwent at least one type of imaging study, usually CT, at 6-month intervals during the first 2 years after surgery and at 1-year intervals thereafter until year 5 after surgery. Case-report forms, which included the results of these tests and evaluations and the survival status of patients, were submitted 1 year, 1.5 years, 2 years, 3 years, 4 years,

Table 1. (Continued.)

Characteristic	S-1 (N = 529)	Surgery Only (N = 530)	P Value*
Cancer stage, Japanese classification — no. (%)‡			0.78
II	236 (44.6)	238 (44.9)	
IIIA	202 (38.2)	207 (39.1)	
IIIB	90 (17.0)	85 (16.0)	
IV	1 (0.2)	0	
Cancer stage, TNM classification — no. (%)			0.37
IB	1 (0.2)	0	
II	264 (49.9)	282 (53.2)	
IIIA	170 (32.1)	157 (29.6)	
IIIB	54 (10.2)	56 (10.6)	
IV	40 (7.6)	35 (6.6)	
Type of lymph-node dissection — no. (%)			0.69
D1	0	1 (0.2)	
D2	501 (94.7)	497 (93.8)	
D3	28 (5.3)	32 (6.0)	
Type of gastrectomy — no. (%)			0.26
Total	220 (41.6)	201 (37.9)	
Distal	301 (56.9)	316 (59.6)	
Proximal	4 (0.8)	11 (2.1)	
Other	4 (0.8)	2 (0.4)	

* P values for sex and type of gastrectomy were calculated with the use of the chi-square test. P values for age, tumor stage, nodal stage, number of lymph-node metastases, cancer stage (Japanese and tumor-node-metastasis [TNM] classifications), and type of lymph-node dissection were calculated with the use of the Wilcoxon test.

† Nodal stages according to the Japanese classification were defined as follows: N0, no evidence of lymph-node metastasis; N1, metastasis to group 1 lymph nodes; N2, metastasis to group 2 lymph nodes; and N3, metastasis to group 3 lymph nodes. Groups 1, 2, and 3 are regional lymph-node classifications defined according to the location of the primary tumor and based on the results of studies of lymphatic flow at various tumor sites and the observed survival associated with metastasis at each nodal station (i.e., position in relation to primary node).

‡ Cancer stages according to the Japanese classification were defined as follows: stage IA, T1N0; stage IB, T1N1 or T2N0; stage II, T1N2, T2N1, or T3N0; stage IIIA, T2N2, T3N1, or T4N0; stage IIIB, T3N2 or T4N1; and stage IV, T4N2, any T stage with N3, or distant metastasis.

and 5 years after surgery. Patients, their physicians, endoscopists, and radiologists were aware of the group assignment after surgery, and no placebo was used. However, relapses and other events were evaluated by members of the steering committee, who were unaware of the group assignments.

STATISTICAL ANALYSIS

The results of a previous study conducted in Japan²⁰ served as the basis for determining the required numbers of patients.²¹ The 5-year overall survival rate in the surgery-only group was assumed to be 70%. We calculated that a total enrollment of 1000 patients was needed for a hazard ratio for death of 0.70 in the S-1 group as compared

with the surgery-only group, with the use of the log-rank test, a two-sided alpha of 5%, and a statistical power of 80%, assuming 3 years of recruitment and an additional 5 years of follow-up.

Efficacy was to be evaluated in two interim analyses performed by an independent data and safety monitoring committee 1 year and 3 years after the completion of enrollment. Significance was evaluated with the use of the method of Lan and DeMets²² and the O'Brien-Fleming boundary. Person-years were used to estimate information fractions for use in interim analyses. When calculating information fractions, we assumed that patients who had not completed the study before the interim analysis were continuously observed until the final analysis.

Data for all randomly assigned patients, whether eligible or not, were included in efficacy analyses. Data for eligible patients were also analyzed to evaluate the robustness of the results. Overall survival was defined as the period between randomization and death. All deaths, including those from other diseases, were considered to be events. Relapse-free survival was defined as the period between randomization and the occurrence of an event — relapse or death — whichever came first. Data for patients who had not had an event were censored as of the date of the final observation.

The median time from surgery to randomization was 28 days (range, 7 to 42) in the S-1 group and 28 days (range, 6 to 42) in the surgery-only group. Because the number of days from surgery to randomization varied among patients, we also calculated the overall survival from the date of surgery. In the first interim analysis, overall survival was also measured from the date of surgery. The Kaplan–Meier method was used to estimate the cumulative survival. The primary confirmatory analysis was performed with the use of the stratified log-rank test, with the cancer stage — which was used in the random assignment of patients at enrollment — as a stratification factor. The Cox proportional-hazards model was used to calculate the hazard ratios. All P values calculated in the subgroup analysis were two-sided and were not adjusted for multiple testing. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

CHARACTERISTICS OF PATIENTS

We enrolled and randomly assigned 1059 patients — 529 to the S-1 group and 530 to the surgery-only group — at 109 centers between October 2001 and December 2004. After randomization, 25 patients (14 in the S-1 group and 11 in the surgery-only group) were found to be ineligible. The reasons for ineligibility were as follows: the absence of cytologic examination of the peritoneal fluid (nine patients), cancers other than gastric cancer (five), previous treatment for gastric cancer (four), laboratory test values at enrollment that did not meet the protocol requirements (four), limited (D1) surgery (one), stage IV cancer (one), and T1 cancer (one). The main analyses were based on data from all randomly assigned patients, including those who were ineligible. The two groups were well

balanced with regard to baseline clinical characteristics, surgical procedures, and pathological findings (Table 1).

INTERIM ANALYSIS

The first interim analysis was based on data derived from case-report forms submitted by December 2005, 1 year after enrollment of the last patient. This analysis (median follow-up, 2.0 years) was conducted by the independent data and safety monitoring committee in June 2006. In this interim analysis, both overall survival and relapse-free survival differed between the two groups, both for all randomly assigned patients (overall survival, $P=0.002$; relapse-free survival, $P<0.001$) and for all eligible patients (overall survival, $P<0.001$; relapse-free survival, $P<0.001$). The significance level of the differences was close to the predetermined threshold for the interim analysis, $P=0.001$. Given these results, the data and safety monitoring committee recommended discontinuation of the trial and publication of the results based on updated data (from follow-up surveys as of June 30, 2006).

ADVERSE EVENTS AND TREATMENT COMPLIANCE

Data on 517 patients in the S-1 group and 526 in the surgery-only group were analyzed for adverse events. Data from the remaining 12 patients in the S-1 group, who did not receive S-1, and from the remaining 4 patients in the surgery-only group, who requested that their treatment assignment be changed after randomization, were not included in the safety analysis. Adverse events of grade 1, 2, 3, or 4 (defined according to the Common Toxicity Criteria of the National Cancer Institute, version 2.0) — including leukopenia, anemia, thrombocytopenia, elevated total serum bilirubin levels, and nonhematologic toxic effects — were more frequent in the S-1 group than in the surgery-only group. The adverse events of grade 3 or 4 that were more frequent in the S-1 group were anorexia, nausea, diarrhea, leukopenia, anemia, elevated total serum bilirubin level, stomatitis, and rash (Table 2).

Among the 517 patients in the safety population who received S-1, treatment was continued for at least 3 months in 452 patients (87.4%), at least 6 months in 403 patients (77.9%), at least 9 months in 366 patients (70.8%), and 12 months in 340 patients (65.8%). The reasons for withdrawal of treatment included refusal of the patient to continue treatment because of adverse events

Table 2. Adverse Events, According to Treatment Group.*

Event	S-1 (N=517)					Surgery Only (N=526)				
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 3 or 4	Grade 1	Grade 2	Grade 3	Grade 4	Grade 3 or 4
	no. of patients				%	no. of patients				%
Leukopenia	157	144	6	0	1.2	93	32	2	0	0.4
Anemia	293	167	6	0	1.2	311	64	3	1	0.8
Thrombocytopenia	123	10	1	0	0.2	32	2	2	0	0.4
Elevated AST level	193	30	9	0	1.7	177	30	17	1	3.4
Elevated ALT level	192	26	6	0	1.2	182	27	16	1	3.2
Elevated total serum bilirubin level	155	75	7	1	1.5	40	13	5	1	1.1
Elevated creatinine level	25	2	0	0	0.0	24	2	1	1	0.4
Stomatitis	139	26	1	0	0.2	16	2	0	0	0.0
Anorexia	213	72	30	1	6.0	63	9	8	3	2.1
Nausea	146	37	19	—	3.7	40	7	6	—	1.1
Vomiting	88	23	6	0	1.2	42	6	7	3	1.9
Diarrhea	227	66	16	0	3.1	85	11	1	0	0.2
Rash	111	52	5	0	1.0	6	4	2	0	0.4
Pigmentation	204	37	—	—	—	2	0	—	—	—
Fatigue	242	60	3	0	0.6	88	4	3	0	0.6

* Grades of adverse events were defined according to the Common Toxicity Criteria of the National Cancer Institute (version 2.0). AST denotes aspartate aminotransferase, and ALT alanine aminotransferase; dashes indicate not available.

or other factors (71 patients), the decision of the investigators to terminate treatment because of adverse events or complications (72), the detection of metastasis or relapse (25), the presence of cancers other than gastric cancer (2), post-enrollment ineligibility (5), and transfer to another hospital (2). The dose of S-1 was decreased in 219 of the 517 patients (42.4%) who received S-1. Of the 340 patients who received treatment for 12 months, the dose was decreased in 158 patients (46.5%).

OVERALL SURVIVAL AND RELAPSE-FREE SURVIVAL

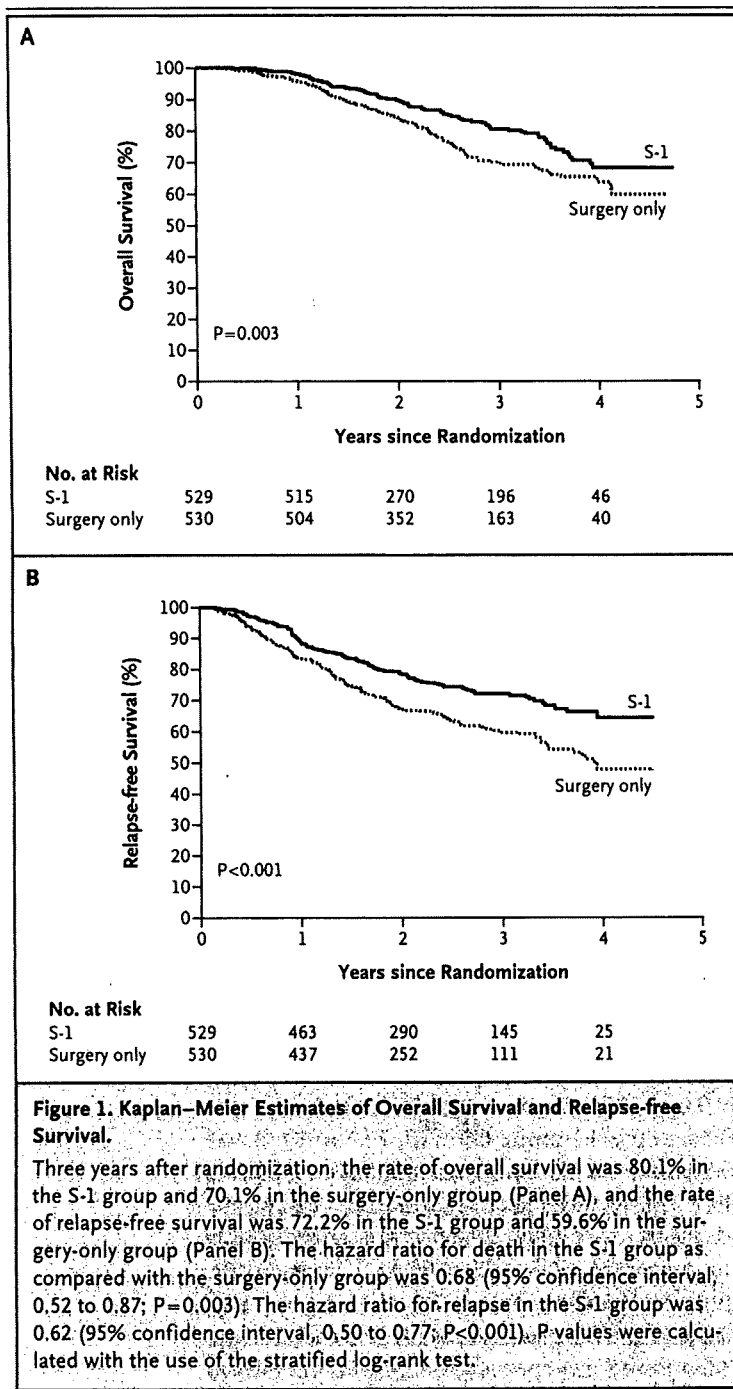
On the basis of follow-up data updated on June 30, 2006, the median time from randomization to follow-up was 2.9 years in both the S-1 group and the surgery-only group. Seven patients in the S-1 group and six patients in the surgery-only group were lost to follow-up. A total of 102 patients died in the S-1 group, and 140 patients died in the surgery-only group. The causes of death in the S-1 and surgery-only groups were as follows: relapse (in 96 and 124 patients, respectively), other cancer (1 and 2), a cause other than cancer (4 and 7), and unknown causes (1 and 7). The number of patients who had

recurrent metastasis was 133 in the S-1 group and 188 in the surgery-only group.

The hazard ratio for death in the S-1 group, as compared with the surgery-only group, was 0.68 (95% confidence interval [CI], 0.52 to 0.87; $P=0.003$). The 3-year overall survival rate was 80.1% in the S-1 group (95% CI, 76.1 to 84.0) and 70.1% in the surgery-only group (95% CI, 65.5 to 74.6) (Fig. 1A). The hazard ratio for relapse in the S-1 group, as compared with the surgery-only group, was 0.62 (95% CI, 0.50 to 0.77; $P<0.001$). The rate of relapse-free survival at 3 years was 72.2% in the S-1 group (95% CI, 67.9 to 76.4) and 59.6% in the surgery-only group (95% CI, 54.9 to 64.3) (Fig. 1B). Among eligible patients, the hazard ratio for death in the S-1 group, as compared with the surgery-only group, was 0.66 (95% CI, 0.51 to 0.86; $P=0.002$). The results for all randomly assigned patients were similar.

SITE OF FIRST RELAPSE

Common sites of first relapse were the peritoneum, hematogenous sites, and lymph nodes (Table 3). Local relapse occurred in 7 patients in the



group, 59 patients (11.2%) had peritoneal relapse, and 27 (5.1%) had lymph-node relapse.

SUBGROUP ANALYSIS

The overall survival of eligible patients was analyzed according to sex, age, cancer stage (Japanese classification and tumor–node–metastasis [TNM] classification), tumor stage, nodal stage (Japanese classification¹⁸ and TNM classification), and histologic type (Fig. 2). A total of 25 ineligible patients (14 in the S-1 group and 11 in the surgery-only group), including those who had stage IV disease, were excluded. There was no significant interaction between the treatment group and any of the variables studied.

DISCUSSION

Few large-scale trials (those with >500 patients) have compared postoperative adjuvant therapy with surgery alone among patients with gastric cancer. Such large trials have been performed by Nakajima et al. (the JCOG [Japan Clinical Oncology Group] 8801 study),²⁰ Macdonald et al. (the INT-0116 study),⁷ and Cunningham et al. (the MAGIC trial).⁸ The JCOG 8801 study in Japan failed to demonstrate therapeutic benefits of adjuvant chemotherapy. The authors suggested that surgery probably resulted in a cure only in patients with T1 tumors, who accounted for nearly one third of all patients in the study, possibly masking differences in overall survival. The INT-0116 study, performed in the United States, showed that adjuvant chemoradiotherapy prolonged overall survival and relapse-free survival. Most patients in the INT-0116 study underwent either D0 or D1 surgery, with only 10% undergoing D2 surgery. The characteristics of patients in the INT-0116 study differed from those in the JCOG 8801 study and those in our trial. An analysis of benefit according to the type of lymph-node dissection showed no effect of adjuvant chemoradiotherapy among patients who underwent D2 surgery. In the MAGIC trial, conducted mainly in the United Kingdom, perioperative and postoperative chemotherapy with epirubicin, cisplatin, and infused fluorouracil significantly prolonged overall survival and relapse-free survival. In that study, D2 surgery was not performed as a standard procedure.

In addition to the JCOG 8801 study, the JCOG

S-1 group (1.3%) and in 15 patients in the surgery-only group (2.8%). Postoperative treatment with S-1 reduced the frequencies of peritoneal and lymph-node relapses. In the surgery-only group, 84 patients (15.8%) had peritoneal relapse, and 46 patients (8.7%) had lymph-node relapse. In the S-1

9206-1,²³ JCOG 9206-2,²⁴ and National Surgical Adjuvant Study Group for Gastric Cancer (N-SAS-GC)²⁵ studies have evaluated postoperative adjuvant chemotherapy after D2 surgery in Japan. These studies involved only about 200 patients each. Although the results of the N-SAS-GC study showed that adjuvant chemotherapy with uracil-tegafur (an oral fluoropyrimidine prodrug) was effective, confirmatory studies were needed. Because of the high incidence of gastric cancer in Japan, an effective regimen for adjuvant chemotherapy is particularly important there. Our decision to perform this phase 3 trial of S-1 in patients who underwent curative resection was based on the results of previous clinical trials showing that S-1 alone is effective for the treatment of advanced gastric cancer and may therefore be useful as adjuvant chemotherapy.

More than 100 centers located throughout Japan participated in our trial. To ensure a uniform level of surgical quality, the participating centers were selected from among hospitals performing at least 100 operations annually for gastric cancer. All of our patients, except one who received D1 surgery and was therefore ineligible, underwent surgery that was at least D2. The rate of local relapse in the surgery-only group was 2.8% (15 of 530 patients), indicating that surgery alone was satisfactory in terms of local control. In all, 29% of patients underwent splenectomy, whereas 4% underwent pancreatectomy.

After a median follow-up of 2.9 years, the rate of overall survival was higher in the S-1 group than in the surgery-only group. This outcome is consistent with the results of a previous trials conducted in Japan.^{3,25} Our results are also consistent with those of a meta-analysis showing that the hazard ratio for death among patients who received S-1, as compared with those who did not, ranged from 0.70 to 0.82.¹⁻⁶ In addition, the effectiveness of postoperative adjuvant chemotherapy with S-1 for gastric cancer is consistent with the high response rates among patients with advanced gastric cancer.^{14,15} Adverse events of grade 3 or grade 4 occurred in less than 5% of patients in the S-1 group, except for anorexia (which occurred in 6% of patients), and compliance with S-1 treatment was good. We therefore believe that S-1 is useful as adjuvant chemotherapy after curative surgery in patients with gastric cancer.

Table 3. Site of First Relapse, According to Treatment Group.*

Site	S-1 (N=529) <i>no. of patients (%)</i>	Surgery Only (N=530) <i>no. of patients (%)</i>	Hazard Ratio for Relapse in the S1 Group (95% CI)	P Value
Total no. of relapses	133 (25.1)	188 (35.5)		
Local	7 (1.3)	15 (2.8)	0.42 (0.16–1.00)	0.05
Lymph nodes	27 (5.1)	46 (8.7)	0.54 (0.33–0.87)	0.01
Peritoneum	59 (11.2)	84 (15.8)	0.64 (0.46–0.89)	0.009
Hematogenous	54 (10.2)	60 (11.3)	0.84 (0.58–1.21)	0.35

* Some patients had a first relapse at more than one site.

Our results were obtained at a median follow-up of 2.9 years after randomization (median follow-up after surgery, 3.0 years). The survival rate 3 years after surgery was 80.5% in the S-1 group and 70.1% in the surgery-only group. These results were similar to those obtained when survival rates were measured from the date of randomization, with a hazard ratio for death in the S-1 group, as compared with the surgery-only group, of 0.68 ($P=0.002$). The results may change marginally at the 5-year follow-up. However, the number of events in the surgery-only group has already reached nearly 80% of that initially predicted for 5 years. At the first interim analysis, the predicted probability that overall survival in the S-1 group would be significantly better than that in the surgery-only group at final analysis was estimated to be 99.3%.²⁶

Although it has sometimes been suggested that there may be differences in certain aspects of gastric cancer in Japan and the West, there have been no significant differences identified between Japan and the United Kingdom with regard to possible genetic influences or between Japan and European countries in the distribution of important prognostic factors.²⁷⁻²⁹ Moreover, Italian investigators have reported that pancreas-preserving D2 dissection performed at centers with experience in this procedure can provide a survival benefit.³⁰ If adequate D2 dissection were performed, we believe that treatment outcomes in Western countries would be similar to those in Japan. We acknowledge that the results of our trial may not be valid in countries where D2 surgery is not considered the standard operation.

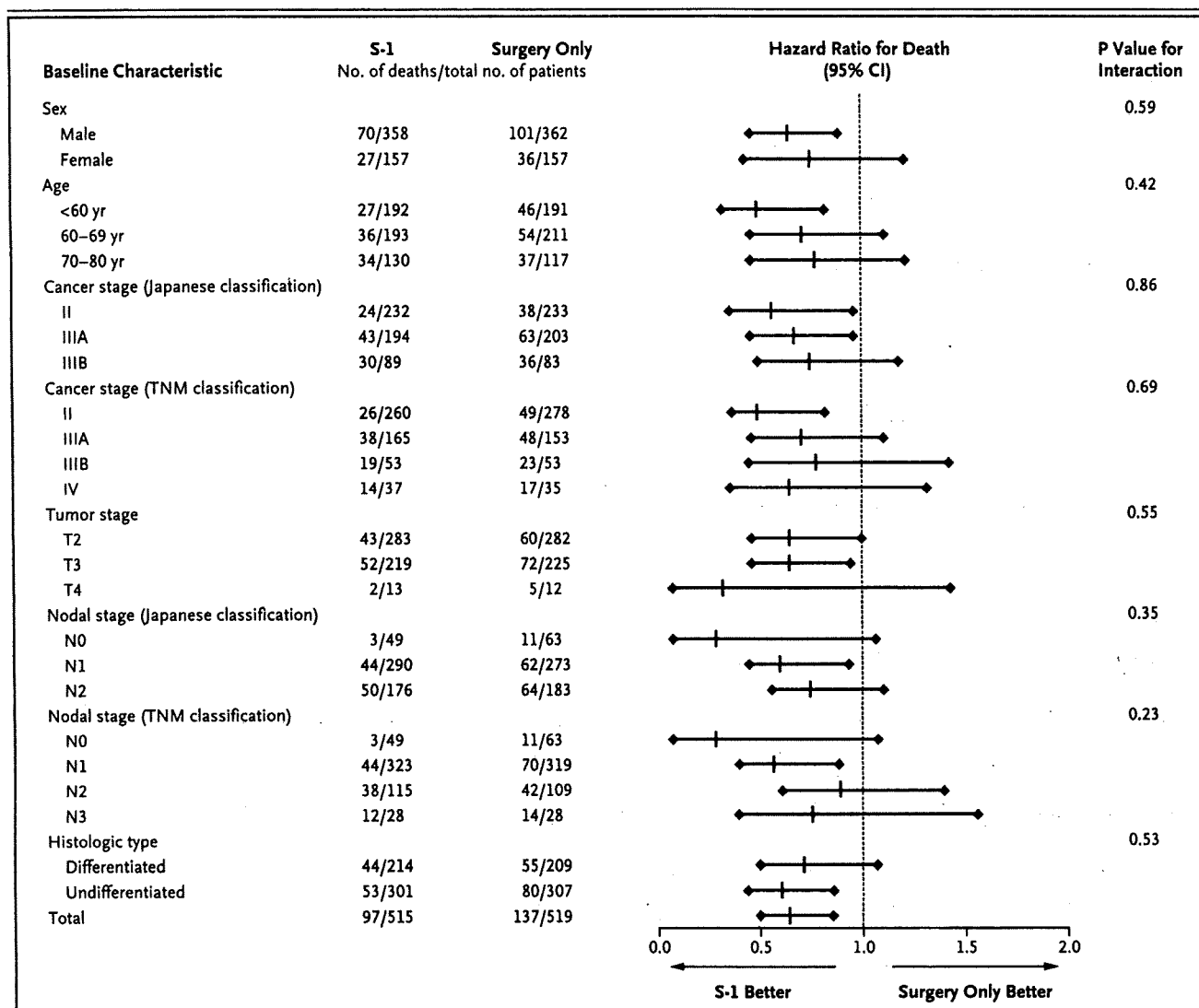


Figure 2. Hazard Ratios for Death and P Values for the Interaction of Treatment Group and Baseline Characteristic among Eligible Patients.

In the surgery-only group, the cancers in three patients could not be classified as differentiated or undifferentiated; one of these patients is still alive.

Supported by a grant from Taiho Pharmaceutical, Tokyo.
 Drs. Sakuramoto, Sasako, Yamaguchi, Kinoshita, Fujii, Nashimoto, Nakajima, Imamura, Yamamura, and Kurita report receiving lecture fees from Taiho Pharmaceutical; and Dr. Ohashi, consulting and lecture fees from Taiho Pharmaceutical. No

other potential conflict of interest relevant to this article was reported.

We thank Professor J. Patrick Barron of the International Medical Communications Center of Tokyo Medical University for his review of an earlier version of this manuscript.

APPENDIX

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Tsubo Hospital — N. Koyama, K. Nakayama, N. Sakurazawa; Saiseikai Niigata Daini Hospital — T. Aiba, Y. Sakai; Niigata Cancer Center Hospital — A. Nashimoto; Gunma University Hospital — E. Mochiki, R. Aihara; Gunma Prefectural Cancer Center — T. Fukuda, N. Kawano; Tsuchiura Kyodo General Hospital — S. Hiranuma; Tsukuba University Hospital — K. Ohta, N. Ohkohchi, S. Inagawa; Dokkyo Medical University Hospital — K. Sasaki; Tochigi Cancer Center — T. Inada; Saitama Cancer Center — Y. Tanaka; Dokkyo Medical University Koshigaya Hospital — S. Akao, M. Yamaguchi; Saitama Medical University Hospital — I. Koyama, R. Hirayama, W. Ichikawa; Chiba University Hospital — T. Suzuki, Y. Gunji, Y. Nabeya; National Cancer Center Hospital East — T. Kinoshita; Chiba Rousai Hospital — H. Nose, K. Kusashio; Chiba Cancer Center — N. Takiguchi; International Medical Center of Japan — T. Shimizu; Showa University Toyosu Hospital — K. Kumagai; National Cancer Center Hospital — M. 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The New England Journal of Medicine (ISSN 0028-4793) is published weekly in the English language from Editorial Offices at 10 Shattuck Street, Boston, MA 02115-6094 USA – Fax: (617) 734-4457. Business and Subscription Offices are at 860 Winter Street, Waltham, MA 02451-1412 USA – Fax: (781) 893-0413; Tel: (781) 893-3800 x5515; website: www.nejm.org. Those wishing to order subscriptions from outside The Americas may also contact European Magazine Distribution (EMD) – Fax: (49) 30 3132032 (Berlin, Germany).

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Detection of Minimal Gastric Cancer Cells in Peritoneal Washings by Focused Microarray Analysis with Multiple Markers: Clinical Implications

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Background: Peritoneal cytology is an important prognostic factor of gastric cancer. However, peritoneal cytology requires great skill, which may explain its low prevalence. A reverse transcriptase–polymerase chain reaction–based assay with multiple marker genes or immunocytochemistry was assessed as an alternative method of gathering the same kind of data as cytology.

Methods: Peritoneal washings from 179 patients with gastric cancer were analyzed by multiplex reverse transcriptase–polymerase chain reaction with 10 marker genes and subsequent hybridization to a customized oligo-nucleotide array. Results with this assay were either validated as a prognostic factor or confirmed by demonstrating the presence of cancer cells by immunocytochemical cytology.

Results: Only 1 (2.2%) of 44 disease-free cases was shown to be positive by the microarray assay, whereas 13 (93%) of 14 conventional cytology–positive cases were found to be positive. This assay further detected approximately one-third of cytology-negative patients either with peritoneal recurrence (7 of 20, 35%) or with non-peritoneal recurrence (6 of 22, 27%). A high concordance between the microarray assay and immunocytochemical cytology with five antibodies against CK20, FABP1, MUC2, TFF1, and MASPIN was confirmed. The clinical outcome of the microarray assay–positive cases was poor, as was that of the cytology-positive cases.

Conclusions: Our assay, though time-consuming and requiring special equipment, demonstrated a specificity and sensitivity equal to or better than cytology in our institutes. The minimal

Received August 17, 2006; accepted November 9, 2006; published online: February 9, 2007.

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free peritoneal cancer cells detected by the microarray assay may provide the same clinical information as larger amounts of cancer cells for patients with gastric cancer. An anti-MASPIN antibody may be helpful in peritoneal cytology of gastric cancer.

Key Words: Gastric cancer—Peritoneal cytology—RT-PCR—Microarray—MASPIN.

Gastric cancer is one of the most frequently encountered malignancies.¹ Although it can often be cured through surgical resection, leaving no evident residual disease, recurrences are experienced after a potentially curative surgical operation, with peritoneal metastasis as the most common form.² Therefore, peritoneal cytology as an assessment of the risk for peritoneal recurrence is often performed for advanced disease, and it is recognized as one of the most important prognostic factors for gastric cancer.³ Beginning in 1999, the Japanese Gastric Cancer Association began including a description of the intraoperative peritoneal wash cytology as "CY status" in its documentation system, and any surgical procedure labeled as CY1 (cytology positive) is considered noncurative.⁴ In addition, usage of laparoscopic disease evaluation and peritoneal cytology before surgical resection is being advocated for patients with advanced gastric cancer.⁵ Neoadjuvant therapies may be indicated as a potentially better treatment option for cases with T3 or T4 disease (tumor penetrates serosa or invades adjacent structures) or positive peritoneal cytology proven by a laparoscopic staging system.⁶ Thus, peritoneal cytology is establishing an increasing clinical implication in the management of advanced gastric cancer.

However, because conventional peritoneal cytology provides only a limited sensitivity, short-time peritoneal recurrences or even synchronous peritoneal metastases are not rare in cytology negative cases. Specimens containing few atypical cells or containing indeterminate cells mimicking reactive mesothelium are often defined as negative, perhaps because the job requires great skill by trained cytologists (or pathologists). With these problems, the peritoneal cytology in gastric cancer operation is not prevailing in spite of its importance. Trained cytologists are always used in large specialist hospitals, such as those of a university or cancer center, but are often unavailable at other general hospitals. Our goals were twofold: first, to discover a molecular biological method that could be used in a clinical laboratory agency as a substitute for skilled cytology; and second, to ensure that the procedure has uniform accuracy, regardless of the site where the procedure is performed.

To improve the sensitivity of peritoneal cytology, reverse transcriptase-polymerase chain reaction

(RT-PCR)-based methods or immunocytochemical cytology have been introduced.⁷⁻⁹ However, these challenges experienced problems resulting from the use of a single marker gene or only a few marker genes to detect cancer cells even though the cancer cells show expressional variations from cell to cell and from case to case. For example, *CEA* has been reported to be lacking in specificity and *CK20* in sensitivity.^{10,11} We previously identified 11 genes as potentially useful markers: *CK20*, *FABP1*, *MUC2*, *TFF1*, *TFF2*, *MASPIN*, *GW112*, *PRSS4*, *MDK*, *SOX9*, and *CDX1*. Among them, five markers, *CK20*, *FABP1*, *MUC2*, *TFF1*, and *TFF2*, showed highly specific results in nested RT-PCR for peritoneal cytology of gastric cancer.¹² Considering the advantage in the use of multiple markers,¹³ we here report a sensitive microarray assay that uses multiple marker genes for detecting minimal cancer cells in peritoneal washings of patients with gastric cancer. Concurrently, we also validated the usefulness of our previously identified five marker genes (*CK20*, *FABP1*, *MUC2*, *TFF1*, and *MASPIN*) in immunocytochemical peritoneal cytology.

METHODS

Clinical Materials

A total of 179 peritoneal wash samples from patients with gastric cancer who gave written informed consent were collected at the National Cancer Center (NCC, 98 cases), the Aichi Cancer Center (ACC, 47 cases), and the University of Tokyo Hospital (34 cases). The NCC samples were those used in our previous studies,¹² and the ACC samples were representative, and thus not consecutive, cases (18 stage Ia cases, 12 recurrence-free cases, and 17 relapsed cases) described in another previous study.⁷ Each set of samples derived from these three institutes was used for three analyses: the capability of predicting recurrence, the impact on clinical outcome, and concordance with findings of immunostaining of the assay. Our study design was approved in the institutional review board of each institute. The method for the RNA extraction is detailed in our previous reports.^{7,10}

TABLE 1. Sequences of primers and probes

Gene	Forward and reverse primers	Probe
<i>TFF1</i>	^a CCTTTGGAGCAGAGAGGAGGCAAT ^b TCAGAGCAGTCAATCTGTGTGTGAGC	TTCGACGACACCGTTCGTGGGGTCCCCTGGT GCTTCTATCCTAATACCATCGAC
<i>TFF2</i>	ATAACAGGACGAACTGCGGCTTCC AGCTGATAAGGCGAAGTTCTTCTTGG	TTGAAGTGCCTGGTGTCTTCCCCTGAACTCT GTGGAAGACTGCCATTAAGAGAGGC
<i>FABP1</i>	TCATGAAGGCAATCGGTCTG CAATGTCACCCAATGTCATGG	CATTCTGCACGATTTCCGACACCCCTTGATA TCCITCCCCTTCTGGATGAGCTCTCCG
<i>CK20</i>	ACACGGTGAACATATGGGAGCGATCT CTCCAGAAGGCGGCGGTAAGTAG	GTACGAMCCMCGCCCGAGGGCTGGTCGC GACTACAGTGCATATTACAGAC
<i>MUC2</i>	CCGGGGAGTGCTGTAAGAAG GCTCTCGATGTGGGTGTAGG	CTTGAAGTCCCCGGGCTTCAGGATGACGTGC TGGTTGTCGGGGCCGTTTGTATGATA
<i>CEA</i>	AACTTCTCCTGGTCTCTCAGCT GCAAATGCTTTAAGGAAGAAG	GGTTGGGGTTGCTCTGATATAGGAGCCCTGG TGTAGTTTCTTCAATTCAGGAAGACTGAC
<i>TACSTD1</i>	TGCTGGGGTCAAGAAGAACAG TTGAGTTCCTATGCATCTCA	GCAGGGTCTAAAAGCTGGTGTATTGCTGTTA TTGTGGTTGTGGTGATAGCAGTGTGTTGC
<i>MASPIN</i>	TCCGGGGTAGTTGGCAGAAATACAG TGCATGTCAAGGAAGAGATGGGAGA	GTAATTGTAAAGTTGGGTGAGTAAGCTATCC CTGTTGCCGGTTCATGGATTACTTCTCT
<i>PRSS4</i>	CTGGGCACAGTTGCTGTCCC GGCCACCAGAGTACGCTGG	GATGCTCCGGTGCTGACCCAGGCTGAGTGTA AAGCCTCCTACCTGGAAAGATTACCAAC
<i>GW112</i>	CAGAAGCCCCAGTAAGCTGTTTAGGA GCACCTTGTCACTGCCATCAGATTTT	AACCAGAGTTACTAACCAATTCACCCCCCAC CAACCCCTTCTACTGCCTACTTAAAAA
β -Actin	TCATCACCATTGGCAATGAG CACTGTGTTGGCGTACAGGT	CCTGTGGCATCCAGAACTACCTCAACTCC ATCATGAAGTGTGACGTGGACATCCGCA

^aForward primer.^bReverse primer.

Marker Gene Set

After identifying 11 potentially useful marker genes (*CK20*, *FABP1*, *MUC2*, *TFF1*, *TFF2*, *MASPIN*, *GW112*, *PRSS4*, *MDK*, *SOX9*, and *CDX1*) in our previous study,¹² we further screened additional thousands of genes for marker genes with a new version of microarray GeneChip U133A (Affimetrix, Santa Clara, CA) by use of the same gene screening procedures.¹² We first selected four marker candidates, *PRSS2*, *PRSS8*, *TACSTD1*, and *BCAS1*. However, 6 (*MDK*, *SOX9*, *CDX1*, *PRSS2*, *PRSS8*, and *BCAS1*) of the 15 genes showed clear false-positive signals in representative clinical samples with an earlier version of a focused microarray containing the 15 genes and a most popular marker gene *CEA*. Thus, these six genes were excluded. Finally, we selected 10 markers, *CK20*, *FABP1*, *MUC2*, *TFF1*, *TFF2*, *MASPIN*, *GW112*, *PRSS4*, *TACSTD1*, and *CEA*.

Manufacturing Microarrays for Detecting Minimal Gastric Cancer Cells in Peritoneal Washings

Focused microarray is constructed by fixing 50–60 mer of oligonucleotide probes on a slide glass by Bubble Jet technology.¹⁴ The microarray contains a single spot for each sequence of 10 marker genes and β -actin as a control gene. Each probe sequence used for the microarray is listed in Table 1.

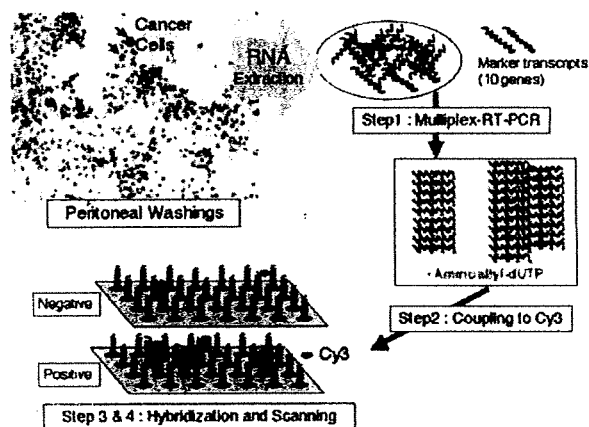


FIG. 1. Schematic flow diagram of MiniChip assay. Microscopic photo depicts one peritoneal wash cytology slide stained by anti-MASPIN antibody. Atypical cells are rare (arrows). Marker transcripts among extracted total RNAs were amplified and labeled with amino allyl-dUTP by multiplex reverse transcriptase-polymerase chain reaction (step 1) and stained by coupling to Cy3 dye (step 2), followed by hybridization to focused microarray (step 3) and fluorescence intensity scanning (step 4).

Marker Gene Detection by Focused Microarray

As shown in a schematic flow diagram (Fig. 1), the MiniChip assay consists of four steps: (1) amino allyl-dUTP labeling by multiplex RT-PCR; (2) coupling the labeled RT-PCR products to Cy3 dye; (3) hybridization Cy3-labeled cDNA to microarray; and (4) fluorescence scanning. Representative microarray