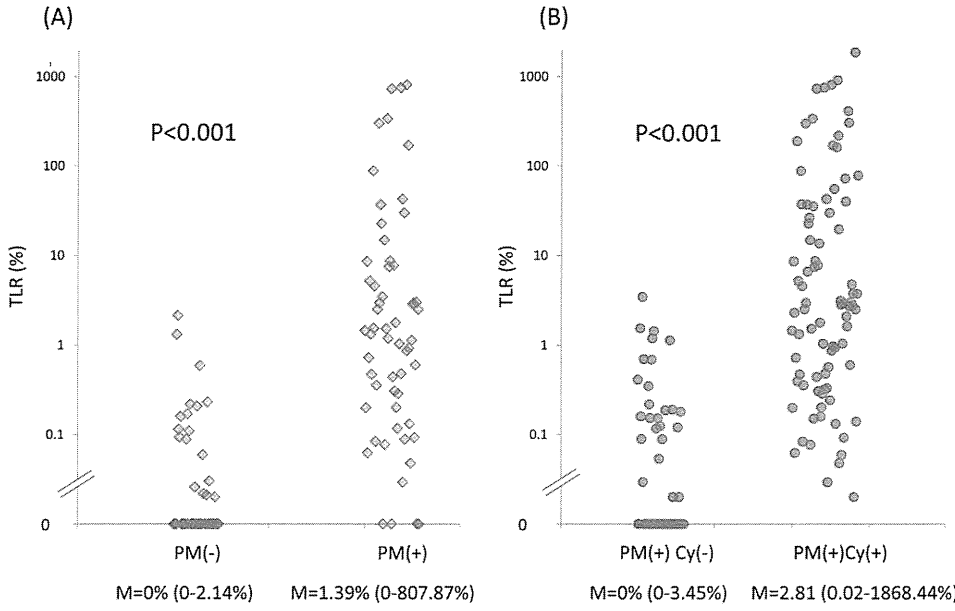

FIG. 3. **A:** TLR of the peritoneal fluids obtained from patients with ($n = 58$) or without ($n = 48$) peritoneal metastasis at laparotomy. **B:** TLR of peritoneal fluids with positive ($n = 86$) or negative ($n = 61$) cytology in patients with peritoneal metastasis. Out of 86 CY (+) samples, 49 were obtained at laparotomy and 37 from a peritoneal catheter or access port, while in 61 CY (−) samples, 9 were obtained intraoperatively and 52 from a port.

of human cancers (20–23) and are purportedly an ideal antigen for clinical application in diagnosis of circulating tumor cells. Since CD326 is not expressed in mesothelial cells (24), the numbers of CD326 (+) cells in peritoneal fluids are supposed to reflect the total number of tumor cells of epithelial origin in abdominal cavity. Since the cell density was highly variable in each sample recovered from peritoneal cavity, we needed to count other cell types as internal control. For this aim, we used pan-leukocyte marker CD45, since leukocytes were most predominant cell types in most cases and the number of the whole leukocytes was relatively stable, although changeable by peritoneal inflammation, than other cell types. Then, we counted the number of CD45 (+) leukocytes as well as CD326 (+) cells in total of 10^4 living cells with flow cytometry, and calculated the relative frequency of CD326 (+) tumor cells against CD45 (+) leukocytes.

Tumor cells are often detected as forming clusters in classical cytology examination. In fact, we found some CD326(+) clusters if we fixed the cell pellets just after centrifugation of peritoneal fluid. However, after repeated washing with EDTA-containing media and Ficoll treatment, few clusters were observed in all samples as shown in Figure 3. This suggests that most of the tumor cells in peritoneal fluids form clusters in Ca2+ dependent manner, which are easily dissociated to single cells during the staining procedure.

Another difficult problem in detecting the accurate number of tumor cells is defining the threshold for

Table 1
Correlation Between CY(+) and TLR(+)

	CY (−)	CY (+)
TLR (−)	34	0
TLR (+)	27	86

CY was determined by cytology.
TLR (−); TLR = 0 and TLR(+); TLR > 0.
$P < 0.001$ by chi-square test.

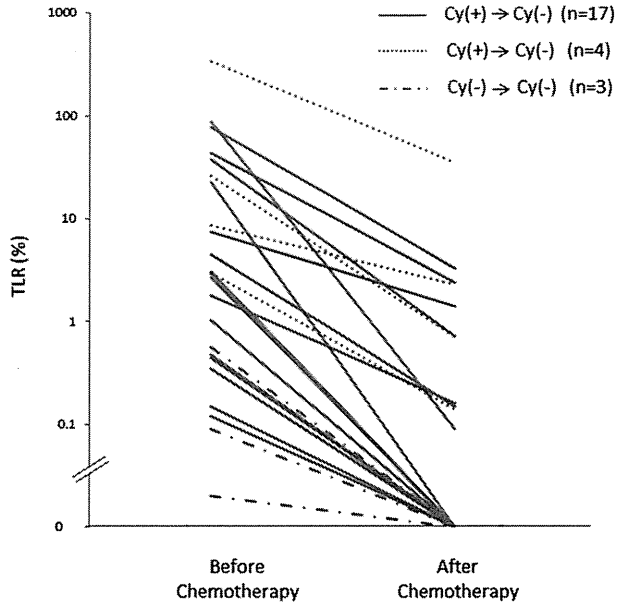

FIG. 4. The change of TLR before and after IP chemotherapy. In 24 cases, CY was initially positive in 21 and negative in 3 cases. In 17 of the 21 CY(+) cases (Blue continuous line), CY turned to be negative, while in 4 cases (Red dotted line) CY remained positive.

negative control for CD45 and CD326. This is especially important in quantitative analysis when the number of CD326 (+) cells is very low, compared with CD45 (+) cells. In our flow cytometric analysis, the borderline was usually defined as the value at which a positive result was detected in less than 0.3% of the total cell population for the negative control (mouse IgG). However, in our analysis of intraperitoneal cells, the threshold varies among the different cells. In fact, the level of binding of control murine IgG in tumor cells tended to be higher than that in leukocytes. In this study, therefore, we defined the threshold line in a FL1/SCC or FL2/SSC profile and the dot number was counted in regions that were positive for CD45 and CD326. By this method, cells were clearly divided into CD45 (+) leukocytes and CD326 (+) tumor cells, for most cases. In some cases, cells were located in CD45 (+) and CD326 (+) areas, although the number was very few. The double-positive cells may be so called "cancer-leukocyte fusion cells" (25), but further study is necessary to characterize the origin and pathological relevance of these cells.

TLR calculated by this method was strongly correlated with the presence of peritoneal metastasis, especially according to cytology status. In the majority of the cases without peritoneal metastasis, TLR was calculated as 0% or less than 0.1%, except for one case with a TLR of 2.14%. However, this sample was obtained from a patient with advanced gastric cancer with serosal exposure and he developed peritoneal metastasis at 6 months after surgery, suggesting that the CY (-) diagnosed by pathology was a false negative.

In contrast, cases with peritoneal metastasis showed significantly higher TLR. Among them, TLR of CY (+) samples was significantly greater than that of CY (-) samples, which showed similar TLR as those obtained from the patients without peritoneal metastasis. However, even in CY (+) cases, TLR was largely different, ranging from less than 0.1% to more than 100%. This implies that the amount of FTC in the abdomen was highly variable among CY (+) cases with peritoneal metastasis and thus the quantification of FTC is clinically important. Follow-up of patient outcome may enable a new classification of disease severity in peritoneal metastasis by FTC. In addition, comparison of TLR in the same patients before and after IP chemotherapy showed significant reduction in all cases. It is notable that in 4 cases, cytology status remained CY (+) even after chemotherapy, despite the fact that the TLR had clearly decreased. This may be related the fact that diagnosis of cytology status after cytoreductive treatment is technically difficult and thus TLR should be more useful to monitor the effectiveness of chemotherapy.

Recent reports of tumor cells that lack the expression of CD326 have appeared, although the frequency generally seems to be rare (26-29). In our series, we stained some samples with anti-cytokeratin (CAM5.2), showing the presence of cytokeratin (+), CD45 (−), and CD326 (−) cells in peritoneal cells, especially in cases with high TLR (data not shown). These cells may be CD326 (-) tumor cells since they also lacked mesothelial antigens such as calretinin or HBME-1. Triple staining with CD326 and cytokeratin, as well as CD45, may provide more accurate information for FTC.

In summary, we have demonstrated a new method to quantify the relative frequency of FTC in PM(+) patients using flow cytometry. The TLR calculated in this method is highly reproducible and accurately reflects the volume of intraperitoneal FTC, and thus could be a useful biomarker to evaluate the effectiveness of IP chemotherapy, as well as to predict patient outcome. Recently, it has been reported that relative number of CD326 (+) tumor cells examined with a computerized image analysis system can be used for monitoring the efficacy of IP administration of catumaxomab for malignant ascites (30,31). Our method is based on the same concept. However, since immunostaining is performed in cell suspensions, the whole process is completed with about an hour. Moreover, analysis is possible without specialized equipment. Thus, it can be widely practiced in various institutes.

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Original Article

A Phase 2 Trial of Intravenous and Intraperitoneal Paclitaxel Combined With S-1 for Treatment of Gastric Cancer With Macroscopic Peritoneal Metastasis

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BACKGROUND: The prognosis of patients with gastric cancer with peritoneal metastasis is extremely poor. This phase 2 study evaluated the benefits and tolerability of weekly intravenous and intraperitoneal paclitaxel (PTX) treatment combined with oral S-1 in patients with gastric cancer who had macroscopic peritoneal metastasis. **METHODS:** Patients with gastric cancer who had primary tumors with macroscopic peritoneal metastasis were enrolled. PTX was administered intravenously at 50 mg/m^2 and intraperitoneally at 20 mg/m^2 on days 1 and 8, respectively. S-1 was administered at 80 mg/m^2 per day for 14 consecutive days, followed by 7 days of rest. The primary endpoint was the 1-year overall survival (OS) rate. The secondary endpoints were the response rate, efficacy against malignant ascites, and safety. **RESULTS:** Thirty-five patients were enrolled. The median number of treatment courses was 11 (range, 2-35). The 1-year OS rate was 77.1% (95% confidence interval, 60.5-88.1). The overall response rate was 71% in 7 patients with target lesions. Malignant ascites disappeared or decreased in 15 of 22 (68%) patients. The frequent grade 3/4 toxic effects were neutropenia (34%), leukopenia (23%), and anemia (9%). **CONCLUSIONS:** Combination chemotherapy consisting of intravenous and intraperitoneal PTX with S-1 is well-tolerated and effective in patients with gastric cancer who have macroscopic peritoneal metastasis. ***Cancer*** **2013;119:3354-8.** *© 2013 American Cancer Society.*

KEYWORDS: phase 2 study; paclitaxel; S-1; gastric cancer; peritoneal metastasis; intraperitoneal chemotherapy.

INTRODUCTION

Gastric cancer is the fourth most common cancer worldwide and the second leading cause of cancer-related deaths.[1] Multimodal therapy combined with systemic chemotherapy, radiation therapy, and surgery has been developed in the treatment of advanced gastric cancer.[2] Even for noncurable gastric cancer, many clinical trials have been conducted using various newly developed anticancer drugs. In a phase 3 study using docetaxel (DTX), cisplatin, and fluorouracil for recurrent or metastatic gastric cancer patients, the 1-year overall survival (OS) rate and median survival time (MST) were 40% and 9.2 months, respectively.[3] In another phase 3 study using S-1 and cisplatin for unresectable or recurrent gastric cancer, the 1-year OS rate and MST were 54.1% and 13.0 months, respectively.[4]

Among patients with noncurable gastric cancer, the prognosis of patients with peritoneal metastasis is extremely poor,[5] and no standard treatment for peritoneal metastasis has been established.

One potential treatment approach is the introduction of paclitaxel (PTX), which has been shown to produce a high response rate (29%-36%) for undifferentiated-type adenocarcinoma[6,7] and can be expected to show high efficacy for peritoneal dissemination. The intraperitoneal (IP) administration of PTX was developed to reinforce the drug's effect on peritoneal metastasis. This would be accomplished by making the drug act directly on the nodules at a high concentration. In fact, IP administration of PTX has been used together with systemic chemotherapy for the peritoneal dissemination of ovarian cancer, which has shown significant survival benefits.[8] These results inspired us to use IP PTX for peritoneal metastasis of gastric cancer, and therefore we designed a study involving IP and intravenous (IV) PTX combined with oral S-1 chemotherapy. The phase 1 study determined the optimum dose[9] of IP PTX to be 20 mg/m^2. We then conducted a phase 2 study in 40 patients with gastric cancer who had peritoneal metastasis, which showed a 1-year OS rate of 78% and MST of 23.6 months.[10] In this previous study, however, 6 patients with gastric cancer who had positive peritoneal wash-

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ing cytology but negative macroscopic peritoneal disseminated nodules (Cy1 P0) were included. In the current study, therefore, we identified peritoneal metastasis macroscopically with investigative laparoscopy and re-evaluated the efficacy and tolerability of the same regimen (weekly IV and IP PTX combined with oral S-1) for patients with apparent peritoneal metastasis (P1).

MATERIALS AND METHODS

Patient Eligibility

The eligibility criteria were as follows: 1) histologically proven unresectable or recurrent gastric adenocarcinoma; 2) peritoneal dissemination diagnosed by staging laparoscopy or computed tomography (CT); 3) age > 20 years; 4) performance status (Eastern Cooperative Oncology Group) of 0 to 2; 5) adequate bone marrow function (leukocyte count, 3000-12,000/mm^3); 6) hemoglobin, > 8.0 g/dL and platelet count > 100,000/mm^3; 7) adequate liver function (total serum bilirubin level < 2.0 mg/dL and serum transaminases < 100/UI); 8) adequate renal function (serum creatinine level within the upper limit of the normal range); 9) an expected survival period of > 3 months; 10) the absence of metastasis to distant organ sites (liver, lungs, or bone) except the ovary; and 11) no other active concomitant malignancies or other severe medical conditions. Patients who had previously received chemotherapy were also eligible. Written informed consent was obtained from all patients. This study was carried out in accordance with the Declaration of Helsinki and was approved by the institutional review board of the University of Tokyo. This study was registered in the UMIN Clinical Trials Registry (UMIN000002850).

Treatment

Patients newly diagnosed with advanced gastric cancer underwent staging laparoscopy and were enrolled in this study when peritoneal dissemination was confirmed. Palliative gastrectomies were performed when patients had gastric bleeding or obstruction due to tumors, but non-curative gastrectomy for tumor reduction was not performed. A peritoneal access port was implanted in the subcutaneous space of the lower abdomen, with a catheter placed in the pelvic cavity. The Peritoneal Cancer Index (PCI), which quantitatively combines the distribution of intraperitoneal tumors,[11] was determined at the time of the staging laparoscopy.

S-1 was administered orally twice daily at a dose of 80 mg/m^2 per day for 14 consecutive days, followed by 7 days of rest. PTX was administered IV at a dose of 50 mg/m^2 and IP at a dose of 20 mg/m^2 on days 1 and 8, respectively. PTX was diluted in 1 L normal saline and administered through the implanted peritoneal access port over 1 hour concurrent with IV infusion following standard premedication. The treatment course was repeated every 3 weeks until observation of disease progression or unacceptable toxicity. Gastrectomy for cytoreduction was performed when peritoneal washing cytology was negative, and second-look laparoscopic examination revealed peritoneal metastatic nodules disappeared or decreased in number and size in response to chemotherapy.

Assessment of Response and Toxicity

The primary endpoint was the 1-year OS rate. Secondary endpoints were the overall response rate, efficacy against malignant ascites, and safety. The 1-year OS rate was estimated according to the Kaplan-Meier method. Overall survival curves were compared by the log-rank test. Prior to each course of treatment, a medical history, physical examination, laboratory studies (blood cell count, electrolytes, liver and renal function tests, and urinalysis), and chest radiography were performed. Gastroendoscopy, upper gastrointestinal radiography, and CT were conducted in order to define the extent of disease and response. Tumor responses were evaluated after every 3 courses of treatment and categorized based on the RECIST (Response Evaluation Criteria in Solid Tumors) guidelines. The amount of malignant ascites and peritoneal cytology were also taken into account to assess the antitumor effects. In accordance with the Japanese Classification of Gastric Carcinoma,[12] the amount of ascites was assessed by radiologists, using CT. At the first day of each treatment course, ascites or peritoneal lavage fluid was collected through a peritoneal access port and cytologic analysis was carried out. Toxicity was graded according to the National Cancer Institute-Common Terminology Criteria for Adverse Events, version 3.0.

Statistical Methods

Because a standard treatment for gastric cancer with peritoneal metastasis has not been established, a threshold for the 1-year OS rate was determined to be 54% due to the result of phase 3 clinical trials in unresectable or recurrent gastric cancer with systemic administration of S-1 and cisplatin.[4] The expected 1-year OS rate was 74%, based on our previously reported phase 2 study.[10] We assumed a null hypothesis of 54% and an alternative hypothesis of 74%, with 1-sided, type I error of 0.05 and power of 0.8, with an accrual time of 2 years, and follow-up of 0.5 years after closure of recruitment. With these specifications, enrollment was deemed necessary in 27 patients, according to the Southwest Oncology Group One-Arm Survival program.

RESULTS

From December 2009 to November 2010, a total of 35 patients were enrolled in this study and were fully evaluated for OS and toxicity. Seven patients with measurable target lesions were assessed for overall response rate. PCI scores were obtained for 32 patients who underwent staging laparoscopy in our institution. Patient characteristics are listed in Table 1.

OS and Response

Because the actual accrual time was 11 months, more than 1 year shorter than expected, the follow-up period was prolonged from 0.5 to 1.5 years.

A median of 11 courses of treatment were performed, with a range of 2 to 35 courses. Combination chemotherapy was discontinued because of disease progression in 23 cases and severe adverse events in 5 cases. Gastrectomy with lymph node dissection as cytoreductive surgery was performed in 21 patients. Total gastrectomy was performed in 20 cases and distal gastrectomy in one case. Ovary with metastasis was resected in 2 cases, and the large intestine and small intestine with stenosis due to peritoneal metastasis were resected in 4 and 2 cases, respectively. Peritonectomy was not performed in any case. Figure 1 shows the OS time after the introduction of combination chemotherapy for all 35 patients enrolled in this study. The 1-year OS rate was 77.1% (95% confidence interval [CI], 60.5%-88.1%), whereas the 2-year OS rate was 44.8% (95% CI, 29.1%-61.6%) at a median follow-up time of 18.0 months. The MST was 17.6 months (95% CI, 13.4 months to not-reached). As shown in Figure 2, the 9 patients with PCI scores ≥ 20 had a lower survival rate than did the 23 patients with PCI scores < 20 (MST, 13.4 months versus not-reached); however, the difference in survival between the 2 groups was not statistically significant ($P = .13$).

Tumor responses are summarized in Table 2. Only 7 patients had measurable disease as shown by RECIST criteria, but 5 of these (71%) showed objective response. Malignant ascites disappeared or decreased in 15 (68%) of 22 patients. Peritoneal cytology turned to negative in 28 (97%) of 29 patients.

Safety

Adverse events are listed in Table 3. The frequent grade 3/4 toxic effects included neutropenia (34%), leukopenia (23%), and anemia (9%). Complications related to the peritoneal access device consisted of obstruction of the IP catheter in 3 patients and infection of the access port in 3 patients. None of the patients experienced abdominal pain or any other toxicity related to IP infusion. No chemotherapy-related mortality was reported.

TABLE 1. Patient Characteristics ($n = 35$)

Characteristic	No. of Patients	%
Sex		
Male	18	51
Female	17	49
Age, years		
Median	55	
Range	28-74	
ECOG performance status		
0	25	71
1	10	29
Prior treatment		
Palliative gastrectomy	3	9
chemotherapy	15	43
Histological type		
Intestinal	4	11
Diffuse	31	89
Metastasis		
Peritoneal cytology	29	83
Malignant ascites	22	63
Ovary (in 17 females)	4	24
Lymph node	11	31
PCI score ($n = 32$)		
1-9	10	31
10-19	13	41
20-29	7	22
30-39	2	6

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PCI, peritoneal cancer index.

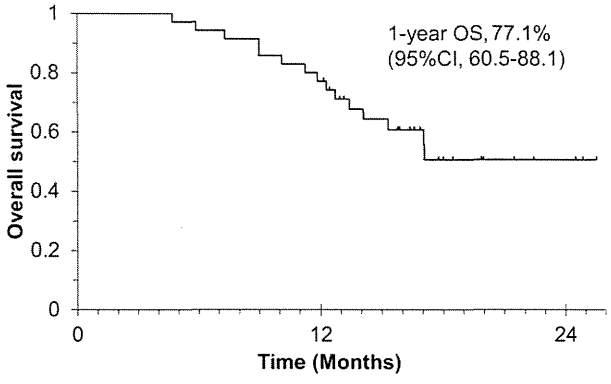

Figure 1. Kaplan-Meier plot is shown for overall survival (OS) with 95% confidence interval (CI) also shown (n = 35).

DISCUSSION

In this phase 2 study, we evaluated the efficacy and safety of the administration of IV and IP PTX with S-1 in 35 patients with gastric cancer who had macroscopic peritoneal metastasis. The 1-year OS rate and MST were 77.1% and 17.6 months, respectively.

Patients with metastasis in distant organs, except the ovary, were excluded in this study. The retrospective data

TABLE 2. Tumor Responses ($n = 35$)

Response	No. of Patients	%
RECIST guideline (n = 7)		
Complete response	0	0
Partial response	5	71
Stable disease	1	14
Progressive disease	1	14
Malignant ascites (n = 22)		
Disappeared	5	23
Decreased	10	45
Peritoneal cytology (n = 29)		
Turned negative	28	97

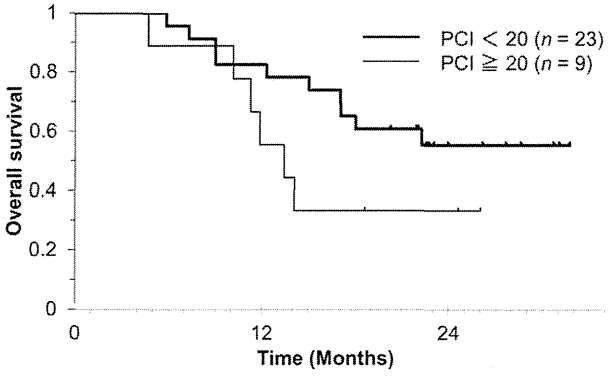

Figure 2. Kaplan-Meier plot is shown for overall survival, stratified according to peritoneal cancer index (PCI) scores

TABLE 3. Number of Patients Experiencing Toxic Effects ($n = 35$)

	Grade (Common Terminology Criteria for Adverse Events version 3.0)				
Toxicity	1	2	3	4	3/4 (%)
Leukopenia	8	13	7	1	23
Neutropenia	4	13	9	3	34
Anemia	14	16	3		9
Thrombocytopenia	8				0
Fatigue	23	2	1		2
Anorexia	16	8			0
Nausea	8	7			0
Vomiting	5	2	1		3
Diarrhea	4	4	1		2
Rash	6				0
Mucositis	6	2			0
Neuropathy	6	2			0

in our institution from 1978 to 2005, before IP chemotherapy was introduced, revealed that the 1-year OS rate and MST of 46 patients with gastric cancer who had distant metastasis only in the peritoneum were 32.9% and 9.3 months, respectively. Compared with our historical survival data, the patients enrolled in this study were considered to have favorable prognoses.

In this study, only patients with macroscopic peritoneal disseminated metastases (P1) were selected, whereas in the previously reported phase 2 study, 6 patients with positive peritoneal washing cytology but negative macroscopic peritoneal disseminated nodules (Cy1 P0) were also selected in addition to 34 P1 patients.[10] The 1- and 2-year OS rates of the current trial were 77.1% and 44.8%, respectively; the values were almost identical to those found in the previously reported study. This indicates that repeated IP administration of PTX permits direct interaction of the drug with the peritoneal disseminated nodules as well as free cancer cells in the peritoneal cavity, achieving significant effectiveness for both microscopic and macroscopic peritoneal metastasis.

When PTX is IP administered, after being dissolved in polyethoxylated castor oil, it is absorbed slowly because of its high molecular weight and lipophilic property.[13] The IP-administered anticancer drug directly exposes the disseminated cancer nodules in the peritoneal cavity to drug infiltration.[14] We demonstrated in a mouse model that IP-administered PTX infiltrates approximately at least 100 to 200 μm from the surface of the disseminated tumor within 24 hours.[15,16] In contrast, even if anticancer drugs are IV administered, most of them cannot cross the peritoneum-serum barrier and, therefore, cannot adequately reach the disseminated nodule.[17]

Repetition of IP PTX administration through the implanted peritoneal access port was considered to be one of the factors contributing to the favorable survival results of this study. The median number of IP administrations in this study was 22. Although IP-administered PTX penetrates only approximately 100 to 200 μm from the surface of the disseminated tumor, repetitive and direct exposure of PTX to the surface of the tumor nodules might induce extensive tumor reduction.

However, the effectiveness of IP chemotherapy may be reduced by the presence of large amounts of IP tumors. This is apparent because patients with massive peritoneal metastasis had a tendency to have a lower survival rate than did those with relatively small amounts of peritoneal metastasis (Fig. 2).

Yonemura et al reported bidirectional neoadjuvant chemotherapy in 79 patients with gastric cancer with macroscopic and/or microscopic peritoneal metastasis.[18] The patients were treated with oral S-1 and IP administration of DTX and cisplatin. The 1-year OS rate and MST were 67.4% and 15.0 months, respectively, in the 41 patients

who received cytoreductive surgery after chemotherapy. Fujiwara et al reported that 2 cycles of IP chemotherapy with DTX combined with S-1 were administrated in 18 patients with gastric cancer who had macroscopic and/or microscopic peritoneal metastasis.[19] Gastrectomy was performed in 16 patients after chemotherapy. The 1-year OS rate and MST were 76% and 24.6 months, respectively. Because DTX is known to show kinetics after IP administration similar to that of PTX, these results suggest that DTX is another promising agent for IP chemotherapy.

The main toxicities of this chemotherapy regimen were neutropenia and leukopenia. Nonhematological toxic effects were relatively mild and were similar to those reported in a previous study.[10]

In conclusion, S-1 combined with IP and IV administration of PTX chemotherapy is safe and effective for treatment of gastric cancer with macroscopic peritoneal metastasis. Further validation of IP chemotherapy for gastric peritoneal metastasis will be determined in the randomized phase 3 clinical trial (the Phoenix-gc trial) that started in November 2011.

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CONFLICT OF INTEREST DISCLOSURE

The authors made no disclosure.

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