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Original article

Efficacy of sequential methotrexate and 5-fluorouracil (MTX/5FU) in improving oral intake in patients with advanced gastric cancer with severe peritoneal dissemination

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Abstract

Background. **Although peritoneal dissemination of gastric cancer is common and often causes deterioration of the patient's condition and quality of life (QOL), these patients are usually excluded from clinical trials. We retrospectively investigated the clinical benefit and toxicity of sequential methotrexate and 5-fluorouracil (MTX/5FU) therapy for patients with peritoneal dissemination.**

Methods. **The subjects were 31 patients with severe peritoneal dissemination of gastric cancer who were treated with MTX/5FU. The treatment schedule comprised weekly administration of MTX (100 mg/m^2) followed by 5FU (600 mg/m^2). Leucovorin (10 mg/m^2) was administered six times, every 6 h, starting 24 h after MTX administration.**

Results. **The median survival time was 255 days, and the median progression-free survival was 127 days. Of the 21 patients with measurable lesions, 4 (19%) patients achieved a partial response. Ascites volume decreased markedly in 14 (54%) of the 26 patients with ascites. Seventeen patients had adequate oral intake, but the other 14 patients had required nutritional support before treatment. The median drip-infusion free survival was 100 days in the former 17 patients, and oral intake improved in 3 (21%) of the latter 14 patients. Grade 3 or 4 neutropenia was observed in 26% of the patients and anemia was observed in 45%. The grade 3 nonhematological toxicities were vomiting (6%) and fatigue (10%). Early death, within 30 days of the last administration of MTX/5FU, occurred due to disease progression in 2 patients, but there were no treatment-related deaths.**

Conclusion. **MTX/5FU chemotherapy may be effective in treating peritoneal dissemination of gastric cancer and might improve the patient's condition in terms of reducing ascites and improving oral intake.**

Key words Gastric cancer · Peritoneal dissemination · Sequential MTX/5FU therapy · Oral intake

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Introduction

About 50000 deaths from gastric cancer occurred in Japan in 2003 [1], and gastric cancer remains a major cause of cancer deaths in Japan. Although gastric cancer is potentially curable by surgery in the early stages, the prognosis of patients with unresectable or recurrent disease is very poor, and their median survival time is about 3 months when they are treated by best supportive care. Chemotherapy has been shown to improve the survival of these patients significantly [2–4]. More recently, randomized trials performed in various regions of the world have reached some conclusions regarding standard chemotherapy for unresectable or recurrent cancer. However, patients without measurable lesions, such as those suffering only from peritoneal metastasis, were often excluded from such clinical trials in the West; this patient group has been neglected, because most trials have considered the response rate as one of the important parameters for evaluation. Thus, data regarding an optimal therapy for this population remains sparse.

In Japan, a combination of S-1 (an oral formulation of tegafur, 5-chloro-24-dyhydropyridine, and potassium oxonate, at a molar ratio of 1:0.4:1) and cisplatin has been recognized as a standard of care after the results of two randomized trials, Japan Clinical Oncology Group (JCOG) 9912 [5] and S-1 Plus cisplatin versus S-1 In RCT In the Treatment for Stomach cancer (SPIRITS) trial [6]. Patients without a measurable lesion had been eligible to enter these trials and those with mild peritoneal metastasis were actually shown by a subset analysis to have benefited from the combination therapy. However, patients with severe peritoneal metastasis with clinical symptoms such as massive ascites and bowel obstruction had been ineligible for both the trials, because patients had to be treated by oral agents in at least one of the treatment arms.

Treatment with sequential methotrexate and 5-fluorouracil (MTX/5FU) has been widely used in Japan

to treat gastric cancer patients, particularly those with peritoneal metastasis. Although a phase II study to test its efficacy (JCOG 9207) had shown a disappointing response rate of 9.0% [7], this trial included only pretreated patients with measurable disease. The same study group launched another phase II trial in which only gastric cancer patients with malignant ascites were eligible (JCOG 9603) and, despite the overall response rate of only 5.7% among patients who had measurable disease, clinical response against the ascites was shown in 35.1% of the patients [8]. This finding, along with the findings of another, retrospective, study from the same country in which malignant ascites disappeared completely in 5 of 26 patients [9], suggests the benefit of MTX/5FU for patients with peritoneal carcinomatosis.

In the present study, the efficacy and toxicity of MTX/5FU were evaluated in previously untreated gastric cancer patients with severe peritoneal metastasis. In addition, the study focused on survival time without drip infusion for nutritional support, because this is considered to be a clinically relevant endpoint in patients who suffer from peritoneal carcinomatosis and are ultimately incurable.

Subjects and methods

Subjects

The subjects were recruited from patients who received MTX/5FU therapy at Shizuoka Cancer Center between October 2002 and August 2006, according to the following criteria: (1) histologically confirmed gastric adenocarcinoma; (2) unresectable or recurrent disease; (3) severe peritoneal dissemination with bowel stenosis confirmed by barium enema and/or ascites beyond the pelvic cavity detected by computed tomography (CT) scan; (4) age 20–75 years; (5) performance status (PS) 2 or less on the Eastern Cooperative Oncology Group (ECOG) scale; (6) no history of chemotherapy or radiation therapy; (7) white blood cell count between 3000 and 10000/mm^3; (8) platelet count 100000/mm^3 or more; (9) adequate liver function, as indicated by serum concentrations of bilirubin 2.0 mg/dl or less, aspartate aminotransferase 100 IU/l or less, and alanine aminotransferase 100 IU/l or less; (10) serum creatinine concentration 1.5 mg/dl or less; and (11) no blood transfusion within 14 days before the start of chemotherapy. The presence of a measurable metastatic lesion was not mandatory. Patients with active bleeding from the gastrointestinal tract, ileus requiring insertion of an ileus tube, other active synchronous carcinoma, central nervous system metastasis or concurrent uncontrolled medical illness, and massive ascites requiring drainage were excluded.

Treatment schedule

The treatment schedule comprised weekly administration of MTX (100 mg/m^2, i.v. bolus) followed by 5FU (600 mg/m^2, i.v. bolus) after a 3-h interval. Leucovorin rescue (10 mg/m^2 p.o. or i.v. every 6 h, six times) was commenced 24 h after MTX administration. To prevent toxicity from MTX, patients were hydrated with 500 ml of solution containing sodium bicarbonate (33.3 mEq) during the interval between the administration of MTX and 5FU, and acetazolamide (250 mg) was given intravenously immediately after the infusion of MTX, for urine alkalinization. Treatment was repeated until disease progression, development of unacceptable toxicity, or the patient's refusal to continue. When grade 4 hematological or grade 3 or 4 nonhematological toxicity occurred, or when the attending physician judged it appropriate, the dose of MTX and 5FU was reduced to 80%. Once serious toxicity was observed, treatment was suspended until recovery.

Response and toxicity evaluation

Objective responses of measurable metastatic lesions were evaluated according to the response evaluation criteria in solid tumors (RECIST) guideline. The tumor response was evaluated using a CT scan every 4–8 weeks after the initiation of treatment. A nontarget lesion was defined as a lesion smaller than 1 cm at the primary site, bone metastasis, pleural effusion, or ascites. Progressive disease (PD) was defined as the unequivocal progression of nontarget lesions, the appearance of any new lesions, or deterioration in clinical status that was considered to have been caused by disease progression.

Toxicity was monitored weekly and evaluated according to the common terminology criteria for adverse events, version 3.0 JCOG/JSCO (Japan Society of Clinical Oncology) (CTCAE ver.3 JCOG/JSCO) [10]. The response of ascites was evaluated using an abdominal CT scan, based on the following criteria specified in the *Japanese classification of gastric carcinoma* (13th edition) [11]: (1) complete response (CR), complete disappearance of ascites for 4 weeks, confirmed by CT scan; (2) partial response (PR), dramatic decrease in ascites volume for 4 weeks, confirmed by CT scan; (3) no response, other than criteria (1) or (2) including an unequivocal increase in ascites.

Statistical analysis

Overall survival was calculated from the initiation of treatment to the date of death or the last follow-up day in survivors. Progression-free survival was calculated from the initiation of treatment to the date of detection of disease progression or death from any cause. In patients who had not required nutritional support before

chemotherapy, drip-infusion-free survival was calculated from the initiation of treatment to the date when drip infusion for nutritional support was first started. The oral intake of patients who had initially required nutritional support was considered to have improved when nutritional support could be stopped for at least 1 week. Overall survival, progression-free survival, and the drip-infusion-free survival were calculated using the Kaplan-Meier method and the StatView version 5.0.1 software program (SAS Institute, Cary, NC, USA).

Results

Patients' characteristics and treatment

Between October 2002 and August 2006, 76 patients with unresectable or recurrent gastric cancer received MTX/5FU at our hospital. Forty-five patients were excluded, for the following reasons: absence of peritoneal metastasis ($n = 7$), poor PS ($n = 7$), age more than 75 years ($n = 3$), disseminated intravascular coagulation ($n = 3$), afferent loop syndrome ($n = 2$), ileus ($n = 1$), cardiac tamponade ($n = 1$), respiratory failure due to lymphangitis carcinomatosa ($n = 1$), deep venous thrombosis ($n = 2$), another active malignancy ($n = 1$), liver dysfunction ($n = 1$), massive ascites requiring drainage ($n = 11$), massive pleural effusion ($n = 2$), blood transfusion ($n = 1$), and loss to follow up ($n = 2$).

The remaining 31 patients were the subjects of this study. Their characteristics are shown in Table 1. The median age was 62 years (range, 25–74 years). A high percentage of patients (84%) had a good PS of 0 or 1. Twenty-three patients had histologically diffuse-type adenocarcinoma (poorly differentiated adenocarcinoma in 11, signet ring cell carcinoma in 11, mucinous carcinoma in 1. All patients were assessable for overall survival, progression-free survival, and toxicity. Twenty-one patients had at least one measurable lesion whose objective responses were assessable. Twenty-six patients had ascites whose responses were assessable. Drip-infusion-free survival could be assessed in 17 patients who had adequate oral intake, and 14 patients who had received nutritional support by total parental nutrition before treatment were assessable for improved oral intake.

Table 1. Patients' characteristics ($n = 31$)

Characteristic	
Sex	
Male	15
Female	16
Age, years, median (range)	62 (25–74)
ECOG performance status score	
0	10
1	16
2	5
Primary tumor	
Yes	23
No	8
Macroscopic type (Japanese classification)	
0	2
1	1
2	4
3	14
4	9
Unknown	1
Histological type	
Intestinal type	8
Diffuse type	23
Metastatic sites	
Lymph nodes	18
Ovary	3
Liver	2
Bone	2
Adrenal gland	1
Muscle	1
Number of metastatic sites	
1	10
2	16
3	4
4	1
Target lesion	
Yes	21
No	10
Ascites	
Yes	26
No	5
Oral intake	
Possible	17
Impossible	14

ECOG, Eastern Cooperative Oncology Group

Treatment course

In total, 557 administrations of sequential MTX/5FU were performed in 31 patients. The median number of administrations was 17 (range, 2–57). The median follow-up period was 496 days in survivors at the time of analysis. The dose was reduced in two patients; one patient had experienced grade 3 fatigue, and the other patient had experienced grade 4 neutropenia. One patient was treated with a three-times-weekly administration and 1-week-off schedule because of grade 2 diarrhea and appetite loss. The other patient requested and received a biweekly treatment schedule. The most frequent reason for treatment termination was disease progression (27 patients; 87%). In one patient treatment was terminated because of severe toxicity: grade 4 increase in serum creatinine concentration.

Toxicity

The worst grades of toxicity per patient during the treatment course are summarized in Table 2. Anemia was the most common hematological toxicity, and 14 of the 31 patients (45%) experienced grade 3 or 4. Grade 3 or 4 neutropenia occurred in 26% of the patients and grade 3

Table 2. Toxicity profiles ($n = 31$)

Toxicity	Grade (NCI-CTC, version 3.0; %)		Grade 3/4 (%)
	3	4	
Hematological			
Leukopenia	6	3	29
Neutropenia	7	1	26
Anemia	9	5	45
Thrombocytopenia	2	0	6
Nonhematological			
Nausea	0	0	0
Vomiting	2	0	6
Diarrhea	1	0	3
Fatigue	3	0	10
Anorexia	1	0	3
Mucositis	1	0	3
Rash	1	0	3

Two patients (6%) died within 30 days of the last methotrexate/5-fluorouracil (MTX/5FU) chemotherapy administration
NCI-CTC, National Cancer Institute common toxicity criteria

or 4 leukopenia in 29%. Grade 3 nonhematological toxicities were vomiting (6%), fatigue (10%), anorexia (3%), mucositis (3%), and rash (3%). One patient experienced a grade 4 increase in serum creatinine concentration after six courses of the treatment; this increase resolved after appropriate treatment. Early death within 30 days from the last administration of MTX/5FU occurred in two patients. One patient died of severe aspiration pneumonia caused by ileus due to disease progression 13 days after treatment. The other patient died on day 16 after changing to another hospital, and detailed information was not available after the change.

Efficacy

The responses to treatment and responses of ascites are shown in Table 3. No patients achieved a CR, and 4 patients achieved a PR, with a response rate of 19% (95% confidence interval [CI], 2.25%–35.84%). Objective improvement of ascites was seen in 14 of 26 patients (54%). The overall survival is shown in Fig. 1. The median survival time was 255 days, and the median progression-free survival was 127 days. The drip-infusion-free survival of the 17 patients with initially adequate oral intake is shown in Fig. 2. The median drip-infusion-free survival time was 100 days. The rate of improvement in oral intake in the 14 patients who initially required nutritional support was 21% (3 of 14). In these 3 patients, the drip-infusion-free durations were 9 days, 118 days, and 145 days, respectively.

Discussion

In clinical practice, the clinician must select an optimal regimen for each patient based on the medical condition

Table 3. Response to treatment

Response	Number of patients (%)
Assessable for response ($n = 21$)	21 (68)
Objective response	
PR	4 (19)
SD	12 (57)
PD	5 (24)
Response rate	4 (19)
Response of ascites ($n = 26$)	
Assessable for response	26 (84)
CR	10 (38)
PR	4 (15)
No response	12 (46)
Response rate	14 (54)

PR, partial response; SD, stable disease; PD, progressive disease; CR, complete response

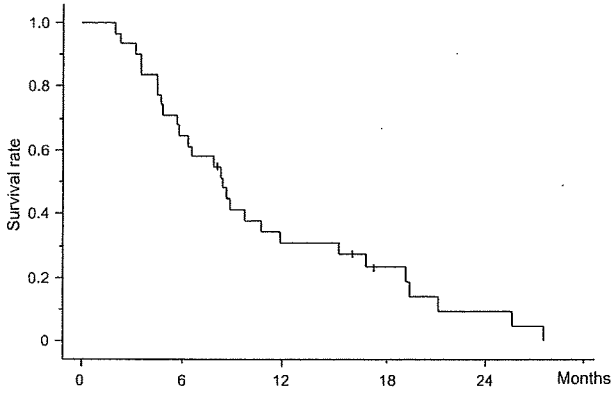

Fig. 1. Overall survival

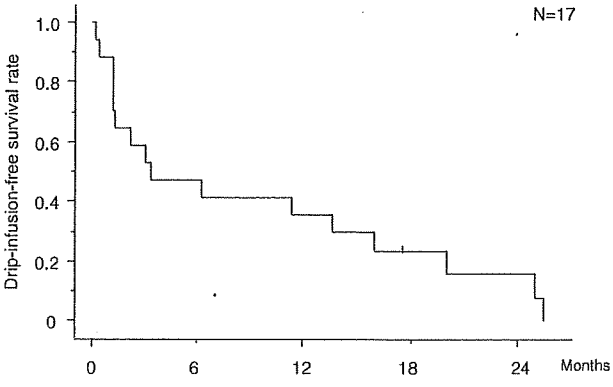

Fig. 2. Drip-infusion-free survival of the patients with initially adequate oral intake

and other factors such as age, performance status (PS), oral intake, organ functions, and extent of the disease. Cisplatin-based regimens are usually considered inappropriate for patients with severe peritoneal dissemination and retention of ascites, because these patients

have potential renal impairment or poor PS, which makes it difficult for them to tolerate the large-volume hydration needed to prevent cisplatin-induced renal injury. Oral agents such as S-1 are also not suitable.

Many studies have suggested that MTX/5FU is effective in patients with advanced gastric cancer. We found a 19% response rate, disease stabilization in 76% of the patients with measurable metastatic lesions, and 54% response rate of ascites. The median survival time was about 8.5 months. These results are similar to those of previous reports and support the notion that MTX/5FU is effective for patients with peritoneal dissemination and that this treatment improves oral intake.

With regard to toxicity, 26% of patients experienced grade 3 or 4 neutropenia, and 10% experienced grade 3 fatigue. These results suggest that MTX/5FU has substantial toxicities in patients with severe peritoneal dissemination. Although we observed no treatment-related deaths, there were two early deaths caused by disease progression. We recommend that when using MTX/5FU to treat patients with peritoneal dissemination from gastric cancer, the clinician should be alert to the signs of toxicity and to the sudden deterioration in a patient's medical condition because of disease progression.

Patients with peritoneal dissemination may have difficulty maintaining adequate oral intake during disease progression because of intestinal obstruction or massive ascites, and nutritional support is indicated. Previous reports showed rates of improvement in oral intake of 29%–38% with MTX/5FU regimens [8, 9]. Three of our 14 patients (21%) who had required nutritional support before the treatment achieved improved oral intake. The median drip-infusion-free survival time in the present study was 100 days. Tahara et al. [9] reported a median drip-infusion-free survival of 178 days. Yamao et al. [8], in reporting the results of JCOG 9603, did not report the rates of improvement in oral intake, or the data for drip-infusion-free survival. Oral intake is important for quality of life (QOL), and these results suggest that MTX/5FU chemotherapy can improve and maintain QOL in patients with peritoneal dissemination from gastric cancer. However, our study was retrospective and its sample size was small. The indication for nutritional support depends on the physician's judgment and the patient's background and requests. Differences in these factors may explain the differences in rates of improvement in oral intake and drip-infusion-free survival between our study and previous reports. The efficacy of MTX/5FU should ultimately be investigated in a phase III trial with a sufficient sample size. In fact, a randomized study comparing continuous 5FU infusion with MTX/5FU in gastric cancer patients with clinically apparent peritoneal metastasis (JCOG 0106) has completed accrual and awaits final survival analysis. It is expected that this trial will establish a tentative standard of care for this population. However, patients with severe peritoneal metastasis generally suffer from short life expectancy and such patients were often found to be unsuitable for registration even for the JCOG 0106 study. In the present study, the analysis of a consecutive series of patients implies that even those with severe peritoneal disease could benefit from chemotherapy, provided that they had not been pretreated and that the utmost care is taken to manage adverse events.

In conclusion, MTX/5FU chemotherapy has potential for improving and maintaining oral intake in patients with severe peritoneal dissemination from gastric cancer.

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Original article

Weekly paclitaxel for heavily treated advanced or recurrent gastric cancer refractory to fluorouracil, irinotecan, and cisplatin

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Abstract

Background. **Although triweekly administration of paclitaxel is approved for gastric cancer in Japan, currently, the drug is often delivered with a weekly schedule because of the equivalent efficacy and lesser toxicity of this dosing schedule as compared with the triweekly administration schedule. Weekly administration of paclitaxel as second-line or first-line chemotherapy for gastric cancer has been reported to yield a response rate of about 20%. Because there has been no report of the efficacy of weekly paclitaxel in the third-line setting, this retrospective study investigated the efficacy and toxicities of weekly paclitaxel used in the third-line setting for the treatment of gastric cancer refractory to all three key drugs, fluorouracil, irinotecan, and cisplatin, used in clinical practice.**

Methods. **In 85 patients with advanced or recurrent histologically confirmed gastric adenocarcinoma who had failed to respond to prior chemotherapy regimens containing fluorouracil, irinotecan, and cisplatin, paclitaxel (80 mg/m^2) was administered weekly, three times, for 3 weeks out of 4.**

Results. **The median number of courses was 3 (range, 1–38). The overall response rate was 23.2% (19/82) in the patients with measurable lesions, and ascites disappeared in 15 of 48 patients (31.3%). Progression-free survival was 105 days and the median survival time was 201 days from the initiation of paclitaxel administration. Grade 3 or 4 leukopenia, neutropenia, anemia, and thrombocytopenia were observed in 25 (29%), 25 (29%), 37 (44%), and 3 (4%) patients. Other, nonhematological, toxicities were nausea, vomiting, anorexia, sensory neuropathy, fatigue, and febrile neutropenia.**

Conclusion. **Weekly paclitaxel administration shows activity against advanced gastric cancer also in the third-line setting.**

Key words Metastatic gastric cancer · Weekly paclitaxel · Third-line chemotherapy

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Introduction

Gastric cancer remains the second leading cause of cancer death worldwide, with more than 700000 deaths per year [1]. In Japan, although markedly improved survival has been achieved, its mortality each year remains at approximately 50000, resulting in gastric cancer being the second-leading cause of cancer death in 2007 [2].

For patients with unresectable or recurrent gastric cancer, the main therapeutic option is palliative chemotherapy. Clinical trials of systemic chemotherapy for gastric cancer have shown significantly prolonged survival with the chemotherapy as compared to best supportive care [3–5]. Recently, two phase III studies have been reported from Japan. One was the Japan Clinical Oncology Group (JCOG) 9912 trial, which revealed the noninferiority of S-1 (a combined preparation of tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxonate) alone to 5-fluorouracil (FU) alone and failed to demonstrate the superiority of irinotecan plus cisplatin (CDDP) to 5-FU alone in terms of the overall survival [6]. This trial concluded that 5-FU could be replaced by S-1 in the chemotherapy for advanced gastric cancer. The other study was the S-1 plus cisplatin versus S-1 in RCT in the treatment for stomach cancer (SPIRITS) trial, which showed the superiority of S-1 plus CDDP to S-1 alone in terms of the overall survival [7]. Based on these trials, S-1 plus CDDP has been recognized as the standard first-line therapy for unresectable and recurrent gastric cancer in Japan.

No standard chemotherapy regimen has been established for use after the failure of first-line chemotherapy. For patients with failure in S-1+CDDP therapy, irinotecan and paclitaxel are considered as the key drugs for the subsequent chemotherapy. In clinical practice, two treatment strategies have generally been adopted after the failure of first-line chemotherapy; irinotecan or irinotecan combination chemotherapy as the

second-line treatment followed by paclitaxel or paclitaxel combination chemotherapy as the third-line treatment, or vice versa.

A phase II study of paclitaxel for chemotherapy-naive patients with gastric cancer showed a response rate (RR) of 23% and median survival time (MST) of 340 days, and this result led to the approval of paclitaxel for treating gastric cancer in Japan [8–11]. Paclitaxel may be administered by two methods, in a triweekly schedule and in a weekly schedule (3 weeks on and 1 week off). Studies of paclitaxel for ovarian, breast, and lung cancer treatment showed milder toxicities and equivalent activity of the drug when administered in the weekly schedule as compared with the triweekly schedule [12–16]. In Japan, weekly administration of paclitaxel for gastric cancer after the failure of first-line chemotherapy is very common, and a few trials have reported on the efficacy of weekly paclitaxel in patients with advanced or recurrent gastric cancer, especially in the second-line setting [17–23]. However, no clinical trials have investigated the effect of weekly paclitaxel in the third-line setting.

At our hospital, irinotecan was preferred for the second-line setting (unless the patient had a contraindication for the use of irinotecan (such as intestinal obstruction due to peritoneal dissemination) and subsequently weekly paclitaxel was selected for the third-line setting. In the present retrospective study, we investigated the efficacy and safety of weekly paclitaxel as third-line chemotherapy in patients with gastric cancer who were refractory to all the three key drugs, fluorouracil, CDDP, and irinotecan used in earlier settings.

Patients and methods

Subjects

In total, 119 patients with advanced or recurrent gastric cancer were treated with weekly paclitaxel in the third-line setting between September 2002 and September 2008 at the Shizuoka Cancer Center, Shizuoka, Japan. Among them, the subjects of this retrospective study were 85 patients who were selected according to the following criteria: (1) histologically confirmed adenocarcinoma of the stomach; (2) failure of prior chemotherapy with at least two regimens, including 5-FU or its derivatives (S-1, capecitabine, tegafur/uracil [UFT]), irinotecan, and CDDP; (3) no history of prior chemotherapy with paclitaxel or docetaxel; (4) age 75 years or less; (5) performance status of 2 or less on the Eastern Cooperative Oncology Group scale; (6) adequate bone marrow, hepatic, and renal functions; (7) no synchronous double cancer or other serious disease; and (8) availability of informed consent before the start of treatment. The reasons that the remaining 34 patients were excluded from this study were: age in 3 patients, performance status in 3, double cancer in 3, nonadenocarcinoma in 3, prior history of docetaxel in 2, history of local chemotherapy (CDDP intraperitoneal therapy) in 6, and unknown details in 14.

Treatment

Paclitaxel at 80 mg/m^2 in 250 ml normal saline was administered by intravenous infusion over 1 h, and this was repeated weekly for 3 weeks out of 4, on an outpatient basis as a rule. Short-term premedication was used to prevent paclitaxel-associated hypersensitivity reactions; dexamethasone 8 mg, diphenhydramine 50 mg, ranitidine 50 mg, and granisetron 3 mg were administered 30 min before the infusion of paclitaxel. Treatment was repeated until disease progression, the occurrence of unacceptable toxicities, or the patient's refusal. In the event of serious hematological toxicity, treatment was suspended until recovery.

Although weekly administration of paclitaxel has not been approved in Japan, this schedule is widely used in clinical practice as a community standard. The clinical practice review committee in this hospital reviewed and approved this regimen for gastric cancer, and informed consent to receive this treatment was obtained from each patient.

Response and toxicity evaluation

Response was assessed every 2 months by computed tomography (CT). Objective responses in measurable metastatic lesions were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.0) [25]. Survival time was calculated from the date of initiation of paclitaxel to the date of death or last confirmation of survival. The efficacy for treating ascites was evaluated by the criteria of the *Japanese classification of gastric carcinoma* (13th edition). Symptomatic toxicity and laboratory data were monitored every week at the outpatient clinic. Toxicity was evaluated according to the Common Toxicity Criteria for Adverse Events, version 3.0 (CTCAE 3.0) [25].

Results

Patients' backgrounds

The patients' characteristics are shown in Table 1. Of the 85 patients, 63 (74%) were male. The median age was 61 years (range, 21–75 years). Sixty-nine patients (81%) showed a performance status of 0 or 1. Forty-one patients (48%) had primary lesions, 13 had pleural effu-

Table 1. Patient characteristics ($n = 85$)

	Number	%
Age (years)		
Median (range)	61 (21–75)	
Sex		
Male	63	74
Female	22	26
Performance status (ECOG)		
0	26	31
1	43	51
2	16	18
Histology		
Differentiated	35	41
Undifferentiated	47	55
Unknown	3	4
Primary lesion		
(+)	41	48
(–)	44	52
Metastatic sites		
Lymph node	49	58
Peritoneum	41	48
Liver	34	40
Ovary	12	14
Lung	11	13
Bone	5	6
Number of metastatic sites		
One	35	41
Two	29	34
Three or more	21	25
Pleural effusion	13	15
Ascites	48	56
Prior chemotherapy		
First-line regimen ($n = 85$)		
Oral fluoropyrimidine	57	67
5-FU (i.v.)	14	16
CPT-11	14	16
Second-line regimen ($n = 85$)		
Oral fluoropyrimidine	16	19
5-FU (i.v.)	3	4
CPT-11	66	77
Third-line regimen ($n = 5$)		
CPT-11	4	80
Other	1	20
Number of prior regimens		
One	80	94
Three	5	6
Subsequent chemotherapy after paclitaxel		
None (best supportive care)	38	45
One regimen	36	42
Two regimens	7	8
Three regimens or more	4	5

ECOG, Eastern Cooperative Oncology Group; 5-FU, 5-fluorouracil; CPT-11, irinotecan

sion, and 48 had ascites. The number of sites affected by metastasis, including lymph node, peritoneum, liver, ovary, lung and bone, was one in 35 patients, two in 29 patients, and three or more in 21 patients. All patients had received prior chemotherapies with regimens containing fluorouracil or its derivatives, irinotecan, and CDDP.

Table 2. Toxicity ($n = 85$)

	Grade				
	1	2	3	4	3/4 (%)
Hematological					
Leukopenia	21	17	23	2	29
Neutropenia	4	14	19	6	29
Anemia	13	36	23	12	41
Thrombocytopenia	9	6	1	2	4
Nonhematological					
Nausea	10	2	2	0	2
Vomiting	5	7	2	0	2
Diarrhea	12	2	0	0	0
Anorexia	16	11	2	0	2
Mucositis	6	0	0	0	0
Sensory neuropathy	33	9	1	0	1
Motor neuropathy	1	3	2	0	2
Edema	7	0	0	0	0
Allergic reaction	1	0	0	0	0
Fatigue	19	10	2	0	2
Febrile neutropenia	—	—	7	0	8

Dose intensity

The total number of paclitaxel infusions was 870. The median number of courses per patient was 3 (range, 1–38). The dose intensity was calculated as 51.4 mg/m^2 per week, which corresponded to 86% of the planned dose. The dose was reduced in 9 patients; because of myelosuppression in 6, hepatic and renal dysfunction in 1, mucositis in 1, and poor general condition in 1. The treatment was discontinued in all patients: due to disease progression in 79 patients, development of neutropenia in 4, development of neuropathy in 1, and comorbidity (cerebral infarction) in 1 patient.

Toxicity

The hematological and nonhematological toxicities encountered are shown in Table 2. Hematological toxicities were common, and 25 (29%) patients experienced grade 3 or 4 leukopenia and neutropenia. Seven (8%) patients developed febrile neutropenia. Anemia was the most common adverse event, because 82 of the 85 (96%) patients had had anemia before the initiation of paclitaxel, including 18 patients with grade 3 or 4 anemia. Thirty five patients (41%) experienced grade 3 or 4 anemia, with an incidence of grade 3 or 4 thrombocytopenia of only 4%. As nonhematological toxicities, 2 patients (2%) experienced grade 3 nausea/vomiting and anorexia. Grade 3 sensory neuropathy was observed in 1 patient (1%) and motor neuropathy occurred in 2 patients (2%). No severe allergic reactions were reported. One patient (1%) died within 30 days of the last administration of paclitaxel after disease progression was confirmed.

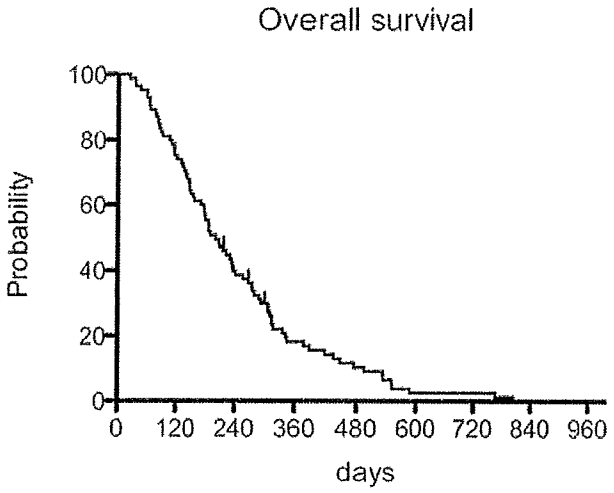

Fig. 1. Overall survival

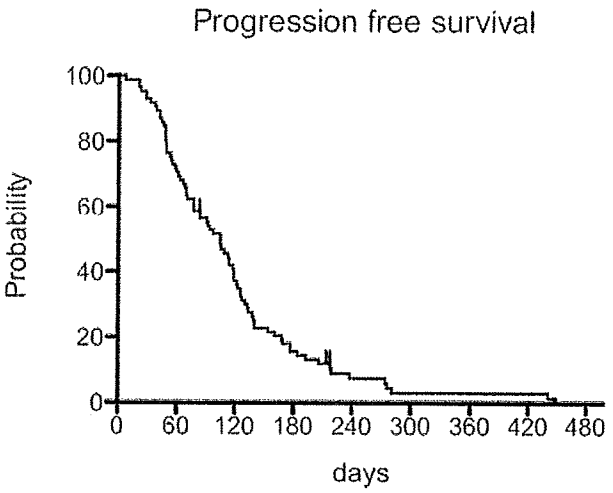

Fig. 2. Progression-free survival

Table 3. Response to weekly paclitaxel ($n = 82$)

Response	Number of patients	%
CR	0	
PR	19	23.2
SD	35	42.7
PD	27	32.9
NE	1	

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable

Responses and survival

Eighty-two of the 85 patients were assessable for response, and the remaining 3 patients did not have measurable disease. Nineteen of the 82 patients (23%) showed a partial response, yielding a response rate of 23.2% (Table 3) and a disease control rate (partial response + stable disease) of 65.9%. Ascites disappeared in 7 patients and decreased in size in 8 (31.3%) of the 48 patients who had been noted to have ascites before the start of treatment. The median follow-up period was 561 days when the survival data were updated in March 2009. The median survival time was 201 days after the initiation of paclitaxel administration (Fig. 1). The median progression-free survival (PFS) was 105 days (Fig. 2). After the failure of paclitaxel, 38 patients (45%) received no further chemotherapy or showed disease progression, 36 received one regimen of subsequent chemotherapy, 7 received two regimens, and 4 received three or more regimens.

Discussion

This retrospective study investigated the efficacy and tolerability of weekly paclitaxel in patients with heavily treated advanced gastric cancer. Weekly paclitaxel is one of the most commonly used regimens in the second-line or later setting for gastric cancer in Japan. Five reports of weekly paclitaxel for gastric cancer have revealed similar response rates, of about 15.9%-33.3%, and similar median survival times, of 5.0–7.8 months, despite the differences in the patient background characteristics in each of the studies. The present study, in which all the patients had received two or more treatment regimens containing 5-FU, irinotecan, and cisplatin, showed a response rate of 23% and median survival time of 6.7 months. These results seem to be similar to those of previous studies in the first- or second-line setting. Thus, it is speculated that paclitaxel may show consistent efficacy, irrespective of the previous chemotherapy.

Peritoneal dissemination is a major and serious complication of advanced gastric cancer, often resulting in the development of ascites, intestinal obstruction, and hydronephrosis, especially after failure of chemotherapy. Most patients with peritoneal dissemination or malignant ascites are usually excluded from clinical trials because of the lack of a measureable lesion or their poor general medical condition; hence, the efficacy of chemotherapy for peritoneal dissemination has not yet been confirmed. It has been reported that paclitaxel reaches an effective concentration for the treatment of ascites (8.5 ng/ml) when administered by a weekly schedule [26–29]. In line with several reports of the efficacy of paclitaxel for malignant ascites, showing consistent efficacy [26–28], the present study showed a proportion of patients with decrease or remission of ascites (31.3%) similar to that shown in previous studies [19, 21]. These results may lend support to the notion that paclitaxel still remains effective against malignant ascites in the third-line setting, while irinotecan cannot

be used for patients with severe peritoneal dissemination for fear of severe toxicities.

The greatest concern in third-line chemotherapy for cancer is drug toxicity. The incidences of grade 3 or 4 leukopenia, neutropenia, and anemia in the present study were 29%, 29%, and 44%, with 8% developing febrile neutropenia. These percentages appear to be rather high as compared to those reported previously [19], while the incidence of severe nonhematological toxicities of 2% or less appeared to be consistent with that in previous studies. Especially, anemia was the most frequently encountered severe toxicity in the present study. All of the subjects in this study had been heavily treated before, and chronic anemia due to bleeding from the primary lesion may have occurred in some of the 41 patients (48%) with primary lesions. Indeed, 82 of the 85 (96%) patients were found to have grade 1 or more severe anemia and 18 (22%) had grade 3 or 4 anemia immediately before the start of treatment. Yamada et al. [11] reported an incidence of grade1 or 2 peripheral neuropathy of 73% (44/60) with triweekly paclitaxel therapy. In our present study, the incidence of grade 3 peripheral neuropathy was as low as 4%, and that of grade 1 or 2 was 54%. Short duration of treatment and administration by a weekly schedule may explain the low incidence of neurotoxicity in this study. It is considered that the use of weekly paclitaxel in the third-line setting may be feasible, but it requires careful management of any hematological toxicities.

In conclusion, weekly paclitaxel administration seems to be feasible and show activity for advanced gastric cancer also in the third-line setting. Although careful management of hematological toxicities is required, this therapy can be applied even for patients with severe peritoneal dissemination. Weekly paclitaxel therapy in the third-line setting may be one of the feasible therapeutic strategies for metastatic and recurrent gastric cancer.

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Short Communication

Close Association of *UGT1A9* IVS1+399C>T with *UGT1A1*28*, **6*, or **60* Haplotype and Its Apparent Influence on 7-Ethyl-10-hydroxycamptothecin (SN-38) Glucuronidation in Japanese

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ABSTRACT:

The anticancer prodrug, irinotecan, is converted to its active form 7-ethyl-10-hydroxycamptothecin (SN-38) by carboxylesterases, and SN-38 is inactivated by UDP-glucuronosyltransferase (UGT)1A1-mediated glucuronidation. UGT1A9 also mediates this reaction. In a recent study, it was reported that the *UGT1A9* IVS1+399 (I399)C>T polymorphism is associated with increased SN-38 glucuronidation both in vitro and in vivo. However, its role in UGT1A9 expression levels and activity is controversial. Thus, we evaluated the role of I399C>T in SN-38 glucuronidation using 177 Japanese cancer patients administered irinotecan. I399C>T was detected at a 0.636 allele frequency. This polymorphism was in strong linkage disequilibrium (LD) with *UGT1A9*1b* (−126_−118T_9>T_{10}, |D′| = 0.99) and *UGT1A1*6* (211G>A, 0.86), in moderate LD with *UGT1A1*60* (−3279T>G, 0.55), but weakly associated with *UGT1A1*28* (−54_−39A(TA)$_6$TAA>A(TA)$_7$TAA, 0.25). Haplotype analysis showed that 98% of the I399C alleles were linked with low-activity haplotypes, either *UGT1A1*6*, **28*, or **60*. On the other hand, 85% of the T alleles were linked with the *UGT1A1* wild-type haplotype **1*. Although I399T-dependent increases in SN-38 glucuronide/SN-38 area under concentration-time curve (AUC) ratio (an in vivo marker for UGT1A activity) and decreases in SN-38 AUC/dose were apparent ($P < 0.0001$), these effects were no longer observed after stratified patients by *UGT1A1*6*, **28*, or **60* haplotype. Thus, at least in Japanese populations, influence of I399C>T on SN-38 glucuronidation is attributable to its close association with either *UGT1A1*6*, **28*, or **60*.

Irinotecan is an important drug for treatment of various tumors including lung, colon, and gastric (Smith et al., 2006). The infused drug is metabolized to its active form 7-ethyl-10-hydroxycamptothecin (SN-38) by carboxylesterases, and SN-38 is inactivated by glucuronidation. At least four UDP-glucuronosyltransferase (UGT) isoforms, namely UGT1A1, UGT1A7, UGT1A9, and UGT1A10, are known to glucuronidate SN-38 (Gagné et al., 2002; Saito et al., 2007).

The UGT1A gene complex consists of 9 active first exons including *UGT1A10*, *1A9*, *1A7*, and *1A1* (in this order) and common exons 2 to 5. One of the 9 first exons can be used in conjunction with the common exons (Tukey and Strassburg, 2000). The UGT1A N-terminal domains (encoded by the first exons) determine substrate-binding specificity, and the C-terminal domain (encoded by exons 2 to 5) is important for binding to UDP-glucuronic acid. The 5′- or 3′-flanking region of each exon 1 is presumably involved in regulation of its expression. Substantial interindividual differences have been detected in mRNA and protein levels and enzymatic activity of the UGT1A isoforms (Fisher et al., 2000; Saito et al., 2007).

SN-38 glucuronidation is thought to be mediated mainly by UGT1A1, and its genetic polymorphisms affecting irinotecan pharmacokinetics and adverse reactions have been already identified. The TA-repeat polymorphism, −54_−39A(TA)$_6$TAA>A(TA)$_7$TAA (*UGT1A1*28* allele), is associated with lower promoter activity, resulting in reduced SN-38 glucuronidation (Beutler et al., 1998; Iyer et al., 1999). The single nucleotide polymorphism (SNP) 211G>A (Gly71Arg, **6* allele), found mainly in East Asians, causes reduced protein expression levels and SN-38 glucuronidation activity (Gagné et al., 2002; Jinno et al., 2003). Another SNP in the enhancer region of *UGT1A1*, −3279T>G (**60* allele), is also a causative factor for reduced expression (Sugatani et al., 2002). Allele frequencies have been reported for **28* (0.09–0.13), **6* (0.15–0.19), and **60* (0.26–0.32) in Japanese and Chinese populations and for **28* (0.30–0.39), **6* (~0), and **60* (0.44–0.55) in whites (Saito et al., 2007). In a previous study, in the Japanese population, we defined haplotype **28* as the haplotype harboring the **28* allele, haplotype **6* as that harboring the**6* allele, and haplotype **60* as that harboring the **60* allele (and without the **28* or **6* allele) (Sai et al., 2004; Saeki et al., 2006). Note that most of the **28* haplotypes concurrently harbored the **60* alleles, and that the **28* and **6* alleles were exclusively present on the different chromosomes (Sai et al., 2004; Saeki et al., 2006). We have also revealed that the haplotype **28*, **6*, or **60* was associated with reduced SN-38 glucuronide (SN-38G)/SN-38 area under concentration-time curve (AUC) ratios, an in vivo parameter for UGT1A activity (Minami et al., 2007).

In a recent study, an intronic SNP of *UGT1A9*, IVS1+399 (I399)C>T, has been shown to be associated with increased UGT1A9 protein levels and glucuronidation activities toward SN-38 and the UGT1A9 probe drug propofol (Girard et al., 2006). Elevation of

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ABBREVIATIONS: SN-38, 7-ethyl-10-hydroxycamptothecin; UGT, UDP-glucuronosyltransferase; SNP, single nucleotide polymorphism; SN-38G, SN-38 glucuronide; AUC, area under concentration-time curve; I399, *UGT1A9* IVS1+399; LD, linkage disequilibrium.

SN-38 glucuronidation activity by this SNP is significant among subjects without *UGT1A1*28*. Sandanaraj et al. (2008) have also reported that I399C/C patients showed higher SN-38 AUC than C/T and T/T patients. With the same *UGT1A1* diplotypes, patients with I399T/T (and *UGT1A9* $-126\_-118T_{10}/T_{10}$) have shown higher SN-38G C_{max} than I399C/T (and T_9/T_{10}) patients. *UGT1A9*1b* (*UGT1A9* $-126\_-118T_9>T_{10}$) has been shown to have no affect on UGT1A9 expression levels (Girard et al., 2006; Ramírez et al., 2007; Sandanaraj et al., 2008). Thus, two groups did suggest that I399T allele was associated with higher glucuronidation activity. However, using human liver microsomes, Ramírez et al. (2007) showed that I399C>T had no significant effect on both UGT1A9 mRNA levels and glucuronidation activities for two UGT1A9 substrates. Therefore, the roles of I399C>T in UGT1A9 activities as well as SN-38 glucuronidation remain inconclusive.

In the present report, we reveal the linkage of I399C>T with *UGT1A1*, *UGT1A7*, and *UGT1A9* polymorphisms and analyze its association with the SN-38G/SN-38 AUC ratio and SN-38 AUC/dose (per dose) to clarify its role in SN-38 glucuronidation.

Materials and Methods

Patients. One hundred and seventy-seven patients (81 lung, 63 colon, 19 stomach, and 14 other cancer patients) administered irinotecan at the National Cancer Center were enrolled in this study as described previously (Minami et al., 2007). This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants. Eligibility criteria, patient profiles, and irinotecan regimens are summarized in our previous report (Minami et al., 2007). In brief, patients consisted of 135 males and 42 females with a mean age of 60.5 (26–78 years old), and their performance status was 0 (84 patients), 1 (89 patients), or 2 (4 patients). Irinotecan administrations were conducted according to the standard protocols in Japan as follows: i.v. 90-min infusion at a dose of 100 mg/m^2 weekly or 150 mg/m^2 biweekly in irinotecan monotherapy; and 60 mg/m^2 weekly with cisplatin in most combination therapies.

Genotyping and Haplotype Analysis. Genomic DNA was extracted from whole blood of 177 irinotecan-administered patients (Saeki et al., 2006). *UGT1A9* IVS1+399C>T (rs2741049) was genotyped using the TaqMan SNP Genotyping Assay kit (C_9096281_10) according to the manufacturer's instructions (Applied Biosystems, Foster City, CA). The *UGT1A1*28* allele [$-54\_-39A(TA)_6TAA>A(TA)_7TAA$], *UGT1A1*6* allele [211G>A (Gly71Arg)], *UGT1A1*60* allele (−3279T>G), *UGT1A7*2* haplotype [387T>G, 391C>A and 392G>A (Asn129Lys and Arg131Lys)], *UGT1A7*3* haplotype [387T>G, 391C>A, 392G>A, and 622T>C (Asn129Lys, Arg131Lys, and Trp208Arg)], and *UGT1A9*1b* allele ($-126\_-118T_9>T_{10}$) were determined previously (Saeki et al., 2006). Hardy-Weinberg equilibrium analysis of I399C>T, linkage disequilibrium (LD) analysis of the *UGT1A9*, *UGT1A7*, and *UGT1A1* polymorphisms, and haplotype estimation with an expectation-maximization algorithm were performed using SNPAlyze version 7.0 software (Dynacom, Chiba, Japan).

Pharmacokinetics. Pharmacokinetic data for the 176 irinotecan-treated patients (data for one patient was unavailable) were described previously (Minami et al., 2007). In brief, heparinized blood was collected before irinotecan administration and at 0, 0.33, 1, 2, 4, 8, and 24 h after termination of the first infusion of irinotecan. SN-38 and SN-38G plasma concentrations were determined by high-performance liquid chromatography, and AUC was calculated using the trapezoidal method in WinNonlin version 4.01 (Pharsight, Mountain View, CA).

Statistical Analysis. Gene dose effects of I399C>T and *UGT1A1* haplotypes (**28*, **6*, *or *60*) were assessed by the Jonckheere-Terpstra test using StatExact version 6.0 (Cytel Inc., Cambridge, MA). Multiplicity adjustment was conducted with the false discovery rate. The significant difference was set at $p = 0.05$ (two-tailed).

Results

Linkages of *UGT1A9* IVS1+399 (I399)C>T with Other Polymorphisms. In our patients, I399C>T was detected at a 0.636 allele frequency, which is almost the same as those in the HapMap data (rs2741049) for Japanese (0.663) and Han Chinese (0.633) populations, but higher than those for Europeans (0.383) and Sub-Saharan Africans (Yoruba) (0.417). Genotype distribution for this SNP was in Hardy-Weinberg equilibrium ($p = 0.418$). LD analysis was performed between I399C>T and the previously determined genotypes, *UGT1A9*1b*, *UGT1A7*2* and **3*, and *UGT1A1*28*, **6*, and **60*, which were detected at >0.1 frequencies in Japanese populations (Saeki et al., 2006). When assessed by the |D'| value, I399C>T was in complete LD with *UGT1A7* 387T>G, 391C>A and 392G>A (*UGT1A7*2*, |D'| = 1.000); in strong LD with *UGT1A9* $-126\_-118T_9>T_{10}$ (*UGT1A9*1b*, 0.987), *UGT1A7* 622T>C (*UGT1A7*3*, 0.977), and *UGT1A1* 211G>A (*UGT1A1*6*, 0.864); and in moderate LD with *UGT1A1* −3279T>G (*UGT1A1*60*, 0.554), but weakly associated with *UGT1A1* $-54\_-39A(TA)_6TAA>A(TA)_7TAA$ (*UGT1A1*28*, 0.252). In r^2 values, the I399C>T was in strong LD with *UGT1A7*2* ($r^2 = 0.976$) and *UGT1A9*1b* (0.916), in moderate LD with *UGT1A7*3* (0.478), but in weak LD with *UGT1A1*6* (0.261) and *UGT1A1*60* (0.208), and in little LD with *UGT1A1*28* (0.018).

Haplotype Analysis. Haplotype analysis was performed using the 9 polymorphisms including I399C>T. As shown in Fig. 1, 95% (123/129) of the I399C alleles were linked with the *UGT1A9* -126_-118T_9 alleles, and 100% (225/225) of the T alleles were linked with the T_{10} alleles (*UGT1A9*1b*). The I399C alleles were completely (129/129) linked with the *UGT1A7* 387G, 391A, and 392A alleles, and most T alleles (223/225) were linked with the 387T, 391C, and 392G alleles. The 40% (51/129) and 60% (78/129) of the I399C alleles were linked with *UGT1A7*2* and *UGT1A7*3* haplotypes, respectively. We also found that 98% (126/129) of the I399C alleles were linked with the *UGT1A1*6* (211G>A), **28* [$-54\_-39A(TA)_6TAA>A(TA)_7TAA$], or **60* (−3279T>G). According to the *UGT1A1* haplotype definition by Sai et al. (2004), 42% (54/129), 36% (46/129), 19% (25/129), and 1% (1/129) of the I399C alleles were linked with the *UGT1A1* haplotypes **6a* (harboring **6* allele), **60a* (harboring **60* allele), **28b* (harboring **60* and **28* alleles), and **28d* (harboring **28* allele), respectively. On the other hand, 85% (191/225) of the T alleles were linked with the *UGT1A1* wild-type haplotype **1*.

Association Analysis. The associations of I399C>T with irinotecan pharmacokinetic parameters were then analyzed using the estimated haplotypes. First, association with SN-38G/SN-38 AUC ratio, an in vivo parameter of UGT1A activity (Sai et al., 2004; Minami et al., 2007; Sandanaraj et al., 2008), was analyzed. *UGT1A7*2* had unchanged activity for SN-38 glucuronidation (Gagné et al., 2002), and neither *UGT1A9*1b* nor *UGT1A7*3* had significant effects on the SN-38G/SN-38 AUC ratio in our previous study (Minami et al., 2007). On the other hand, the *UGT1A1*6*, **28*, and **60* haplotypes were associated with the reduced SN-38G/SN-38 AUC ratios (Minami et al., 2007). Although effects of the haplotype **28* and **6* were more striking, haplotype *UGT1A1*60*, harboring only the **60* allele without the **28* allele, was weakly associated with the reduced ratio. To remove even this weak effect and clarify the real effect of I399C>T, *UGT1A1*60* was also considered as low-activity haplotype in this analysis. Namely, we analyzed the associations of I399C>T with the AUC ratio within the groups stratified by the *UGT1A1* haplotypes, *UGT1A1*28* (**28b* and **28d*), **6* (**6a*), and **60* (**60a*) (combined and shown as *UGT1A1*"+").

When stratified by the I399C>T genotype, a T allele-dependent

Gene		*UGT1A9*		*UGT1A7*[2]				*UGT1A1*[3]			Number	Frequency
Nucleotide change		-126_-118 T_9>T_{10}	IVS1+399 C>T	387 T>G	391 C>A	392 G>A	622 T>C	-3279 T>G	$(TA)_6$>$(TA)_7$	211 G>A		
Allele name		**1b*		**2, *3*	**2, *3*	**2, *3*	**3*	**60, *28*	**28*	**6*		
Haplotypes[1]	**1C-*3-*6a*										47	0.133
	**1C-*2-*60a*										44	0.124
	**1C-*3-*28b*										21	0.059
	**1C-*2-*28b*										4	0.011
	**1C-*3-*60a*										2	0.006
	**1C-*3-*28d*										1	0.003
	**1C-*2-*6a*										1	0.003
	**1bC-*3-*6a*										6	0.017
	**1C-*2-*1*										2	0.006
	**1C-*3-*1*										1	0.003
	**1bT-*1-*1*										190	0.537
	**1bT-*3-*1*										1	0.003
	**1bT-*1-*28b*										22	0.062
	**1bT-*1-*60a*										5	0.014
	**1bT-*1-*6a*										5	0.014
	**1bT-*1-*28d*										1	0.003
	**1bT-*2-*60a*										1	0.003
Alelle frequency		0.653	0.636	0.370	0.370	0.370	0.223	0.280	0.138	0.167	354	1.000

FIG. 1. Haplotypes assigned by using common *UGT1A9*, *UGT1A7*, and *UGT1A1* polymorphisms. [1]Haplotypes were shown as *UGT1A9* haplotypes − *UGT1A7* haplotypes − *UGT1A1* haplotypes. Major allele, white blocks; minor allele, gray blocks. **1C*, T_9 and I399C: **1bC*, T_{10} and I399C: **1bT*, T_{10} and I399T in *UGT1A9*. [2]*UGT1A7*2* and **3* are the haplotypes harboring the three and four *UGT1A7* alleles, respectively. [3]*UGT1A1* $(TA)_6$>$(TA)_7$ indicates −54_−39A$(TA)_6$TAA>A$(TA)_7$TAA.

increase in the SN-38G/SN-38 AUC ratio was observed ($p < 0.0001$, Jonckheere-Terpstra test) (Fig. 2A). However, this trend was obviously dependent on biased distributions of *UGT1A1* haplotypes; e.g., 96% of the I399C/C patients were homozygotes for *UGT1A1*28*, **6*, *or *60*; and "*UGT1A1*28*, **6*, or **60*"-dependent reduction of SN-38G/SN-38 AUC ratio was found within the I399T/T genotypes ($p < 0.05$). As shown in Fig. 2B, *UGT1A1*28*, **6*, or **60* (*UGT1A1*+)-dependent reduction in the SN-38G/SN-38 ratio was observed when patients were stratified by these three haplotypes. However, no significant effect of I399C>T was found within the stratified patients ($p > 0.05$ within the −/−, −/+, or +/+ patient group in Fig. 2B). As for SN-38 AUC/dose (SN-38 AUC values adjusted by the doses used), a similar *UGT1A1* haplotype dependence was observed. Although the I399T-dependent reduction of SN-38 AUC/dose was detected ($p < 0.0001$), biased distributions of the *UGT1A1*28*, **6*, or **60* were again evident, and the *UGT1A1* + haplotypes-dependent increase was significant within the I399 C/T and T/T patients ($p < 0.01$ and $p < 0.05$, respectively) (Fig. 2C). Moreover, no significant effect of I399C>T on SN-38 AUC/dose was found when stratified by the *UGT1A1* haplotypes ($p > 0.05$ within the −/−, −/+, or +/+ patient group in Fig. 2D).

Discussion

In the present study, LD between I399C>T and *UGT1A1*, *UGT1A7*, or *UGT1A9* polymorphisms in Japanese populations was shown for the first time. Moreover, the apparent effect of I399C>T on SN-38 glucuronidation in Japanese cancer patients was suggested to result from its close association with *UGT1A1*28*, **6*, or **60*.

As for the influence of I399C>T on UGT1A9 activity, conflicting results have been reported. Girard et al. (2006) have shown that I399C>T was associated with increased UGT1A9 protein levels and enzyme activity toward an UGT1A9 probe drug propofol using 48 human liver microsomes derived mainly from whites. In contrast, using human liver microsomes from 46 white subjects, Ramírez et al. (2007) have revealed that the I399C>T had no significant effects on UGT1A9 mRNA levels and in vitro glucuronidation activities toward the two UGT1A9 substrates, flavopiridol and mycophenolic acid. Furthermore, another report has demonstrated that I399C>T had no influence on the pharmacokinetic parameters (such as AUC and C_{max}) of mycophenolic acid in 80 Japanese renal transplant recipients (Inoue et al., 2007). Thus, these latter two studies did suggest that the I399C>T polymorphism has no effect on UGT1A9 enzymatic activity. Note that, at least for Japanese populations, no study has reported that I399C>T affects UGT1A9 activity.

As for the influence of I399C>T on SN-38 glucuronidation, a possible enhancing effect has been suggested. Girard et al. (2006) have shown an increasing effect of I399C>T on SN-38 glucuronidation, and that this SNP did not show any close linkages with the *UGT1A1*28* or **60* allele ($r^2 < 0.06$). In addition, Sandanaraj et al. (2008) have reported that in 45 Asians consisting of Chinese (80%), Malay (18%), and others (2%), I399C/C patients had higher SN-38 AUC than C/T and T/T patients. Again, this SNP was not in LD with the *UGT1A1*28*, **6*, or **60* allele (r^2 were <0.09). Furthermore, association of I399T with increased SN-38G C_{max} has been observed even after stratified patients by *UGT1A1* genotypes, although the study sample size was small. These findings suggest that the I399T

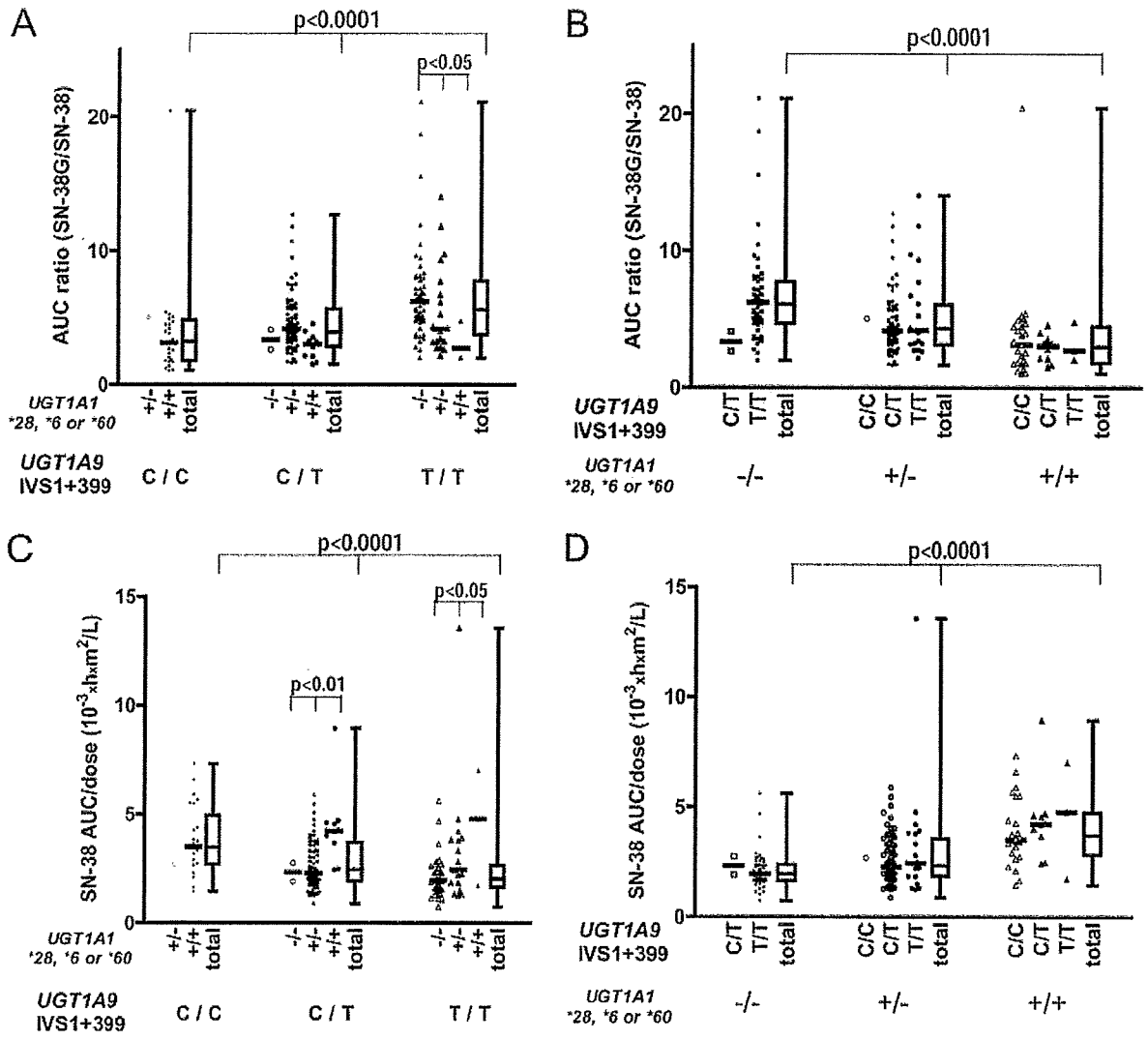

FIG. 2. Association analysis of *UGT1A9* IVS1+399 (I399)C>T with SN-38G/SN-38 AUC ratio (A and B) and SN-38 AUC/dose (C and D). A and C, I399 C/C, C/T, and T/T patients were further divided by the presence of *UGT1A1*28*, **6*, or **60* haplotypes: −/−, no *UGT1A1*28*, **6*, or **60*; −/+, heterozygotes for either *UGT1A1*28*, **6*, or **60*; +/+, homozygotes or compound heterozygotes for either *UGT1A1*28*, **6*, or **60*. B and D, *UGT1A1* −/−, −/+, and +/+ patients were further divided by I399 C/C, C/T, and T/T genotypes. Gene dose effects of I399C>T and the *UGT1A1* + haplotype were assessed by the Jonckheere-Terpstra test.

allele was associated with increased glucuronidation activity for SN-38 without linkages with the *UGT1A1* polymorphisms. Our data demonstrate that an increase in SN-38G/SN-38 AUC ratio (i.e., increased glucuronidation activity) was also found with I399C>T; however, after stratified patients by the *UGT1A1*6*, **28*, or **60* haplotypes (haplotype+) showing reduced SN-38 glucuronidation activity (Sai et al., 2004; Minami et al., 2007), any significant effect of the I399C>T was no longer observed. Thus, no direct effect of I399C>T on SN-38 glucuronidation was shown in the current study in Japanese populations. The discrepancy between our study and others might be derived from ethnic and/or population differences in haplotype distribution. In fact, in our Japanese population, 98% of the I399C alleles were linked with either *UGT1A1 *6*, **28*, or **60*, whereas 85% of the T alleles were linked with *UGT1A1*1*. On the other hand, in Sandanaraj's report (in Chinese + Malay), 84% of the I399C alleles were linked with *UGT1A1 *6*, **28*, or **60*, whereas only 67% of the T alleles were linked with *UGT1A1*1* (Sandanaraj et al., 2008).

In irinotecan therapies, genetic polymorphisms leading to increases in SN-38 AUC, which closely correlates with increased risk of severe neutropenia (Minami et al., 2007), are clinically important. The current study also demonstrated no significant influence of I399C>T on SN-38 AUC/dose after stratified patients by *UGT1A1* haplotypes. Consistent with this finding, no influence of this SNP was observed on the incidence of grade 3 or 4 neutropenia after irinotecan therapy in our population (data not shown). Recently, genetic testing of *UGT1A1*6* and **28*, which are related to severe neutropenia in Japanese populations, has been approved for clinical application in Japan. This study indicates that there is no clinical necessity for additional genotyping of I399C>T, at least in Japanese populations.

In conclusion of this study, the apparent influence of I399 (*UGT1A9* IVS1+399)C>T on SN-38 glucuronidation is attributable to its close association with *UGT1A1*6*, **28*, or **60* in the Japanese population. Furthermore, additional genotyping of I399C>T for personalized irinotecan therapy seems to be clinically irrelevant for Japanese populations.

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Risk factors for skeletal-related events in patients with non-small cell lung cancer treated by chemotherapy

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ABSTRACT

The purpose of this study was to identify the risk factors for skeletal-related events (SREs) in patients with advanced non-small cell lung cancer (NSCLC). SREs were defined as pathologic fractures, spinal cord compression, requirement for radiation therapy, other radiological intervention, or surgery to the bone, and hypercalcemia of malignancy. Time-to-the first SRE and SRE-free survival, and their associations with the patient characteristics were evaluated retrospectively in 642 patients with metastatic NSCLC who received systemic chemotherapy. A total of 118 (18.4%) patients developed SREs during or after the initial chemotherapy. Of these, 107 required radiotherapy to the bone, 5 developed hypercalcemia of malignancy, 3 developed compression fracture of the vertebrae, 2 required surgical treatment of the bone, and 1 underwent radiofrequency ablation therapy to the bone. The first SRE occurred within 12 months in 80 (67.8%) of the 107 patients. The results of multivariate analysis revealed that male sex, performance status (PS) of 2–3 and multiple bone metastases were risk factors for the first SRE, with hazard ratios (HRs) (95% confidence interval [CI]) to the reference of 1.44 (0.98–2.11), 2.21 (0.97–5.03) and 4.43 (2.91–6.76), respectively. SRE-free survival showed a similar trend. The HRs (CI) of male sex, PS of 2 and multiple bone metastases were 1.64 (1.30–2.06), 3.72 (2.31–5.98) and 1.80 (1.40–2.31), respectively. In conclusion, the presence of multiple bone metastases was significantly associated with the development of SRE in patients with advanced NSCLC treated by systemic chemotherapy. Male sex and poor performance status may be additional risk factors for the development of SREs in these patients.

1. Introduction

The bone is a preferred site of metastasis in patients with advanced cancer, which is attributable to the abundant blood flow to the red marrow, high expression levels of adhesion molecules on the tumor cells that bind them to marrow stromal cells and the bone matrix, and a large amount of growth factors in the bone that provide fertile ground for tumor cell growth [1]. Bone metastases are encountered in 30–40% of patients with metastatic lung cancer and 30–55% of patients with lung cancer at autopsy [2,3]. Bone metastases are a serious complication leading to skeletal-related events (SREs), including bone pain requiring radiotherapy, pathologic fractures, and spinal cord compression [2,3]. There have been few reports on the frequency and risk factors of SREs in patients with advanced non-small cell lung cancer (NSCLC), probably because these patients with bone metastases also show systemic disease progression along with progression of the bone metastases, and had a poor prognosis with an estimated median survival time of less than 6 months [2]. With the recent advances in the systemic treatment of NSCLC, however, the median survival of advanced NSCLC patients has increased to approximately 1 year, increasing the period for which the patients are at risk for SREs. A recent phase III trial of zoledronic acid versus placebo in NSCLC patients with bone metastases showed that the frequency of SREs did not differ between the patients receiving zoledronic acid (42%) and those receiving placebo (45%), however, there was a trend toward a longer median time-to-the first SRE in patients receiving zoledronic acid (5.6 months versus 5.0 months, $p = 0.188$) [4]. These results suggest that zoledronic acid may delay the appearance of SREs in NSCLC patients with bone metastases, but that this effect may be limited to the subgroup of patients at a high risk for SREs. Thus, we tried to identify the risk factors for SREs in patients with advanced NSCLC who were treated with systemic chemotherapy.

2. Patients and methods

2.1. Patient selection

Patients were retrospectively selected for this study according to the following criteria: (1) a histological or cytological diagnosis of

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NSCLC; (2) stage IV disease or postoperative recurrence with distant metastases; (3) no prior chemotherapy; (4) chemotherapy prescribed by the National Cancer Center Hospital between December 2000 and June 2006. Patients with postoperative local recurrence without distant metastases were excluded.

2.2. Data collection and statistical analyses

The patients' baseline characteristics before the initial chemotherapy, including the age, sex, and performance status, histology, history of radiotherapy to the bone before chemotherapy, were obtained retrospectively from the medical charts. For the purposes of this study, SREs were defined as (1) pathologic fracture, (2) spinal cord compression, (3) required for radiation therapy to the bone, (4) requirement for surgery to the bone, (5) requirement for radiological intervention to the bone, and (6) hypercalcemia of malignancy that was either fatal or required emergent treatment. The occurrence of SREs and its association with the patients' baseline characteristics were evaluated by the chi-square test for categorical variables and Mann–Whitney's test for continuous variables. Sex, performance status, number of bone metastases, and history of radiotherapy to the bone before chemotherapy were included as the covariates in the multivariate logistic regression analysis. Time-to-the first SRE was measured from the start of initial chemotherapy to the first SRE developing during or after the chemotherapy. Patients who did not develop SREs at the last follow-up were censored at that time. For SRE-free survival, both the first SRE and death were counted as events, and a patient who did not develop any event at the last follow-up was censored at that time. For overall survival, death from any cause was counted as an event. Time-to-the first SRE, SRE-free survival, and overall survival were evaluated using the Kaplan–Meier method, the log-rank test and Cox's proportional hazard model. Sex, age, performance status, tumor histology, number of bone metastases, and history of radiotherapy to the bone before chemotherapy were included as covariates in the proportional hazards model for multivariate analysis. The Dr. SPSS II 11.0 for Windows software (SPSS Japan Inc., Tokyo, Japan) was used for the statistical analyses.

3. Results

A total of 642 patients fulfilled the eligibility criteria for this study. The first-line chemotherapy was platinum-based chemotherapy in 469 (73.1%) patients, gefitinib in 117 (18.2%) patients, third-generation monotherapy in 47 (7.3%) patients, and non-platinum doublets in 9 (1.4%) patients. Responses to these chemotherapies were complete response in 6 patients and partial response in 177 patients, yielding a response rate of 28.5%. Disease progression was observed in 580 (90.3%) patients. The initial progression site was the bone in 78 (12.1%) patients, and sites other than the bone in 502 (78.2%) patients. The overall median (95% confidence interval) survival time was 15.4 (14.0–16.9) months. Zoledronic acid was approved for use in the treatment of bone metastases in Japan in January 2005. This agent was administered before the development of SREs in 26 (4.0%) patients, and after the development of SREs in another 17 (2.6%) patients enrolled in this study.

A total of 118 (18.4%) patients developed SREs during or after the initial chemotherapy. Of these, 107 required radiotherapy to the bone, 5 developed hypercalcemia of malignancy, 3 developed compression fracture of the vertebrae, 2 required surgical treatment of the bone, and 1 underwent radiofrequency ablation therapy to the bone. The first SRE occurred within 6 months of the start of therapy in 48 (40.7%) patients, between 6 and 12 months after the start of treatment in 32 (27.1%) patients, and between 12 and 18 months after the start of treatment in 26 patients, and even later in 12 (10.2%) patients. The median (range) observation period for SREs was 10.4 (0.1–77.0) months.

Table 1
Patient characteristics.

Characteristics	Patients without SREs		Patients with SREs		p-Value
	N	(%)	N	(%)	
Number of patients	524	(81.6)	118	(18.4)	
Sex					
Male	325	(80.8)	77	(19.2)	0.53
Female	199	(82.9)	41	(17.1)	
Age median (range)	61	(24–86)	59.5	(26–77)	0.083
Performance status					
0	163	(82.7)	34	(17.3)	0.68
1	335	(81.5)	76	(18.5)	
2–3	26	(76.5)	8	(23.5)	
Histology					
Adenocarcinoma	419	(80.6)	101	(19.4)	0.16
Non-adenocarcinoma	105	(86.1)	17	(13.9)	
Bone metastases					
None	358	(89.7)	41	(10.3)	<0.001
Single	46	(73.0)	17	(27.0)	
Multiple	120	(66.7)	60	(33.3)	
Radiotherapy to the bone before chemotherapy					
No	499	(82.9)	103	(17.1)	0.001
Yes	25	(62.5)	15	(37.5)	

The percentage of patients who developed SREs was not influenced by the sex, age, performance status or cancer histology (Table 1). In contrast, the number of bone metastases at the time of the initial diagnosis strongly influenced the rate of occurrence of SREs; only 10.3% of patients who had no bone metastasis developed SREs, while 27% of patients with a single bone metastasis and 33% of patients with multiple bone metastases developed SREs during their clinical course ($p < 0.001$). History of radiotherapy to the bone before chemotherapy was also associated with SREs during and after the chemotherapy; only 17% of patients who did not require radiotherapy to the bone developed SREs, while 38% of patients who underwent radiotherapy to the bone developed SREs ($p = 0.001$) (Table 1). Multivariate analysis using a logistic regression model showed that the number of bone metastases was strongly associated with the occurrence of SRE (odds ratio [95% confidence interval] for single bone metastasis, 3.08 [1.60–5.94]; odds ratio for multiple bone metastases, 4.27 [2.66–6.86]), whereas a history of radiotherapy to the bone before the chemotherapy was not (odds ratio [95% confidence interval], 1.43 [0.69–2.97]).

The results of univariate analysis revealed that the time-to-the first SRE was associated with the sex, performance status, number of bone metastases and history of radiotherapy to the bone before chemotherapy, but not with the age or histology. Multivariate analysis demonstrated that the history of radiotherapy to the bone was not associated with the development of SREs (Table 2). The median time-to-the first SRE was not reached in patients with no or only a single bone metastasis, but 19.7 (95% confidence interval, 14.5–24.9) months in patients with multiple bone metastases ($p < 0.001$) (Fig. 1).

A similar trend was observed for the SRE-free survival. Sex, performance status and the number of bone metastases were significantly associated with the SRE-free survival according to both univariate and multivariate analyses. History of radiotherapy to the bone, however, was associated with the SRE-free survival according to the univariate but not multivariate analysis (Table 2). The median (95% confidence interval) SRE-free survival was 23.5

Table 2
Risk factors influencing the time-to-the first skeletal-related event (SRE) and SRE-free survival.

Analysis	Hazard ratios (95% confidence interval)	
	Univariate	Multivariate
Time-to-the first SRE		
Sex		
Female	1	1
Male	1.47 (1.01–2.15)	1.44 (0.98–2.11)
Performance status		
0	1	1
1	1.43 (0.96–2.15)	1.15 (0.76–1.74)
2–3	3.73 (1.71–8.14)	2.21 (0.97–5.03)
Bone metastases		
None	1	1
Single	3.26 (1.85–5.75)	3.00 (1.68–5.35)
Multiple	4.98 (3.33–7.44)	4.43 (2.91–6.76)
RT to the bone[a]		
No	1	1
Yes	3.39 (1.97–5.86)	1.39 (0.77–2.49)
SRE-free survival		
Sex		
Female	1	1
Male	1.63 (1.29–2.05)	1.64 (1.30–2.06)
Performance status		
0	1	1
1	1.59 (1.24–2.03)	1.47 (1.15–1.89)
2–3	4.39 (2.78–6.93)	3.72 (2.31–5.98)
Bone metastases		
None	1	1
Single	1.36 (0.94–1.97)	1.27 (0.87–1.85)
Multiple	2.06 (1.63–2.61)	1.80 (1.40–2.31)
RT to the bone[a]		
No	1	1
Yes	1.92 (1.27–2.89)	1.10 (0.71–1.71)

[a] History of radiotherapy to the bone before chemotherapy.

(18.6–28.5) months in the patients with a performance status of 0, 13.1 (10.4–15.8) months in patients with a performance status of 1, and 5.2 (1.0–9.4) months in patients with a performance status of 2 or 3 ($p < 0.001$) (Fig. 2).

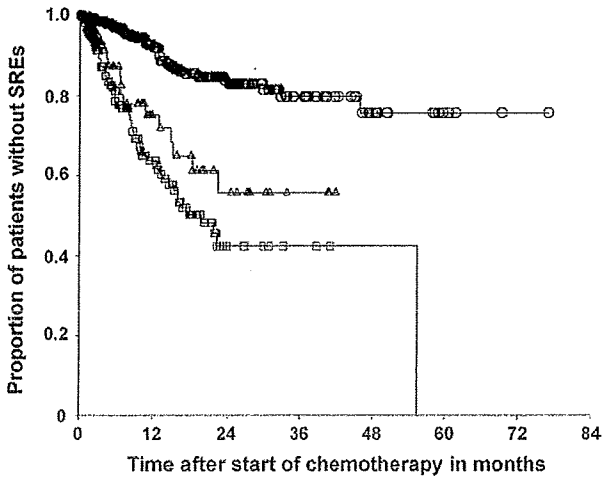

Fig. 1. The median time-to-the first SRE and the number of bone metastases at the initial diagnosis. (A) Graph line with open circles: patients without bone metastasis; (B) graph line with open triangles: patients with single bone metastasis; (C) graph line with open squares: patients with multiple bone metastases. These symbols represent censored cases.

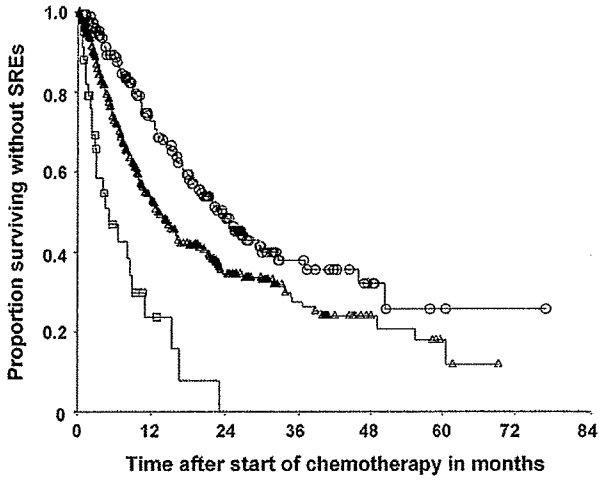

Fig. 2. SRE-free survival was significantly affected by the performance status. (A) Graph line with open circles: patients with performance status of 0; (B) graph line with open triangles: patients with a performance status of 1; (C) graph line with open squares: patients with performance status of 2 or 3. These symbols represent censored cases.

4. Discussion

This study showed that the male sex, a poor performance status and presence of multiple bone metastases were associated with a short time-to-the first SRE and poor SRE-free survival. To the best of our knowledge, this is the first study identifying risk factors for the development of SREs in patients with advanced NSCLC treated by administered systemic chemotherapy.

Hypercalcemia of malignancy has been included in the definition of SRE, and we adopted the traditional definition for this study. Hypercalcemia of malignancy was counted as SRE in only five cases in this study, and analyses using the SRE definition without hypercalcemia yielded almost the same results (data not shown). Hypercalcemia is commonly observed in patients with advanced cancer. In the case of breast cancer, 85% of patients having hypercalcemia had definite radiographic evidence of bone metastases, whereas 15% showed no evidence of skeletal involvement [5]. Parathyroid hormone-related peptides (PTHrP) from tumor cells contribute to the development of hypercalcemia, irrespective of whether or not bone metastases are present. Measurement of the plasma PTHrP concentrations by radioimmunoassay showed that PTHrP was detected in all advanced cancer patients with hypercalcemia without evidence of bone metastases on radionuclide scanning, but only in 65% of cancer patients with hypercalcemia who had bone metastases [6]. Thus, various mechanisms, including both osteolysis and PTHrP, appear to be involved in the pathogenesis of hypercalcemia in cancer patients with bone metastases. Whether hypercalcemia of malignancy should be included in the definition of SREs or not depends on the purpose of the study. In a phase III trial of zoledronic acid versus placebo in NSCLC patients with bone metastases, hypercalcemia of malignancy was excluded from the definition of SRE for the purposes of the primary efficacy analysis, because the efficacy of zoledronic acid in the treatment of hypercalcemia of malignancy has been established. However, secondary efficacy analyses were reported with and without hypercalcemia of malignancy as a SRE, to evaluate the overall benefit of zoledronic acid [4].

The three parameters, 'presence of SRE', 'time-to-the first SRE' and 'SRE-free survival', were evaluated as the outcome measures in this study. It remains unknown, however, which of the three parameters is the most relevant to the study objective. The 'presence of

SRE' is simple to determine, however, this parameter cannot differentiate between early- and late-onset SRE. 'Time-to-the first SRE' has been used in patients with breast cancer and prostate cancer. In this study population, however, this parameter may not reliably reflect the clinical situation, because many patients died of lung cancer progression before the development of SREs, and these cases were censored. As for 'SRE-free survival', both SREs and death are regarded as events. SREs developed in only 18% of the patients in this study, resulting in as many as 82% of patients sharing identical events between overall survival and SRE-free survival. Thus, the risk factors for SRE-free survival identified in this study may not be specific factors for SREs, but represent the risk factors for overall survival. The factor "multiple bone metastases" was identified as a risk factor for the development of SREs as assessed by all the three parameters, and was, therefore, considered as a definite risk factor for the development of SREs. Male sex and poor performance status were significant risk factors influencing the SRE-free survival, marginally significant in relation to the time-to-the first SRE, and not significant in relation to the presence of SRE. Thus, these two factors may be detected as risk factors for SRE-free survival only because of a strong bias confounding the results for the overall survival.

We observed the elevated odds ratios of "history of radiotherapy to the bone" in the univariate analyses. Patients with symptomatic bone metastasis, especially those who received radiotherapy to the bone before the chemotherapy, would have had a higher likelihood of having been examined for the presence of bone metastasis than patients without it. In addition, initiation of radiotherapy to the bone prior to the chemotherapy itself is one of the criteria for SRE. In this case, recurrence of SREs, but not the first SRE, was evaluated as an event in this study. These may lead to a possible bias toward increasing the frequency of SREs in these patients. Multivariate analyses, however, showed that history of radiotherapy to the bone was not a significant factor for the development of SREs. Thus, we believe "history of radiotherapy to the bone" is not a significant independent factor for the development of SREs.

The percentage of patients developing SREs was lower and the time-to-the first SRE was much longer than those in a phase III study of zoledronic acid versus placebo in NSCLC patients with bone metastases [4]. For example, as high as 45% of patients receiving placebo in that study developed SREs, whereas only 27% and 33% of patients with single and multiple bone metastases, respectively, showed SREs in this study. The median time-to-the first SRE was 5.5 months in that study, while it was not reached in patients with a single bone metastasis and 19.7 months in patients with multiple bone metastases in this study. This difference may be partly explained by the percentage of patients who received systemic chemotherapy, which was 100% in this study but only 80% in that study.

Little attention has been paid to SREs in patients with advanced NSCLC, because many patients die of systemic disease progression before the development of SREs. Recent 2nd-line chemotherapies, however, have yielded partial response rates in the range of 12–22% and extended survival to as long as 14 months in Japanese patients with NSCLC [7]. Thus, many patients with advanced NSCLC live longer after failure of first-line chemotherapy, and they are considered to be at a higher risk of SREs than before.

Bisphosphonates are routinely used to prevent SRE in breast cancer patients with bone metastases and in multiple myeloma patients, but the role of these agents in the treatment of advanced NSCLC has been investigated little. A phase III trial of zoledronic acid versus placebo in NSCLC patients with bone metastases showed only a trend toward a longer time-to-the first SRE in patients receiving zoledronic acid [4]. There have been no criteria to determine the indications of bisphosphonate therapy in patients with advanced NSCLC, but the risk factors for SREs identified in this study may be helpful to establish indications for zoledronic acid therapy in these patients.

In conclusion, multiple bone metastases was significantly associated with the development of SREs in patients with advanced NSCLC treated by systemic chemotherapy. Male sex and poor performance status may be additional risk factors for the development of SREs in these patients.

Conflict of interest statement

The authors indicated no potential conflicts of interest.

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