PDGFRα, and BCR-ABL proteins, respectively.[5-8] Imatinib has potent antiproliferative and apoptotic effects on GIST cells.[10,15,16] Studies in the United States and Europe have shown that 5% of GIST patients show a CR; 45%–54%, a PR; and 24%–28%, SD.[10,17] Recent update data from a European Union-United States study suggest that the response fraction may be increased with prolonged use of imatinib; two patients (1%) achieved a complete response; 67%, a PR; and 16%, SD.[18] Although no objective reduction of tumor size was reported in patients with SD, the treatment was beneficial in maintaining disease stability for more than several months in the majority of patients, and in increasing survival. In fact, the study indicated that the 4-year overall survival rate of patients with a PR (62%) was similar to that of patients with SD (64%). High-dose imatinib (800 mg/day) has been shown to provide longer progression-free survival (PFS) than the conventional dose (400 mg/day).[17] In the present study, the two dose groups (400 mg/day and 600 mg/day) did not show significant differences in either PFS or overall survival (OS).

In the present study, 69% of Japanese patients with advanced and/or metastatic GIST treated with imatinib had a confirmed PR, and 26% had SD. The median time to response was 12 weeks. The median PFS was 96 weeks and the estimated 3 year OS (Kaplan-Meier) rate for all patients was 73.6%. These efficacy results obtained in Japanese patients are very similar to those previously obtained in Caucasians.[10,17,18] Furthermore, sunitinib malate, an oral multitargeted tyrosine kinase receptor inhibitor, was developed and has been evaluated in patients with imatinib-resistant GIST and in patients who could not tolerate imatinib. A recent study has shown that the drug has significant clinical activities in imatinib-resistant GIST, with acceptable tolerability.[19]

In vitro, the inhibitory activity of imatinib on wild-type and mutated KIT and PDGFRα kinases depends on mutational status.[15,16] The clinical response to the drug has also been shown to be related to the mutation status of the c-*kit* and *PDGFRα* genes.[14] In the present study, imatinib was most effective for GIST patients with exon 11 mutations in the c-*kit* gene, followed by those with exon 9 mutations, then by those with no c-*kit* mutation. Patients with either exon 11 or exon 13 mutations had a significantly superior PFS compared with those who had exon 9 mutation or no mutation.

In the present study, imatinib was well tolerated, especially compared with conventional chemotherapy. The adverse events probably due to the drug were generally limited to nonserious (grade 1 or 2) cases of nausea, diarrhea, dermatitis, facial edema, edema of the lower limbs, vomiting, or eyelid edema. No difference in adverse events between the dose groups was shown in this study. Severe adverse effects (grade 3 or 4) included neutropenia (21.6%), anemia NOS (17.6%), dermatitis NOS (6.8%), and anorexia (5.4%). A total of eight patients (10.8%) were reported as having gastrointestinal or intratumoral hemorrhage. The hemorrhage may have been caused by the study drug through the following mechanisms: (1) direct local irritation of the gastrointestinal tract, (2) a systemic effect via thrombocytopenia, or (3) breakdown of tumor-associated blood vessels concomitant with tumor-cell death.

In our study, imatinib was detectable in the blood soon after oral intake, with a mean half-life of 18 h, and blood levels of imatinib and its metabolites appeared to be dose-dependent in a dose range between 400 and 600 mg/day. The median weight and body surface area of our Japanese patients were 56.4 kg (range, 35.9–95.8 kg) and 1.59 m^2 (range, 1.23–2.15 m^2), values which were less than those of the corresponding parameters in Caucasians, but the pharmacokinetic data in our patients were similar to those obtained in United States and European Union clinical trials.[10,20] This suggests that body weight does not have a major influence on the plasma concentration of the drug, in accordance with findings described elsewhere.[21] No clear correlation was observed between Cmax/AUC and body weight in our study.

In summary, imatinib has positive therapeutic effects in Japanese patients with unresectable and/or metastatic GIST, and it is safe and well tolerated. Its pharmacokinetic parameters are similar to those in Caucasians. The inhibitory activity of imatinib on the KIT and PDGFRα-signaling pathways depends on the genotype of the c-*kit* and *PDGFRα* genes, and imatinib mesylate provides a promising treatment modality for advanced GIST.

Acknowledgments The authors are grateful to the many investigators involved in this clinical trial, who include Shusaku Tsutsui[1], Yasuhide Yamada[2,a], Kenji Yamao[3,a], Masahiko Taniguchi[4], Yasushi Toh[5], Toshihiko Doi[6,a], and Yoshito Komatsu[7,a]. We also wish to thank Novartis Pharma K.K.

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Impact of Insulin-Like Growth Factor Type 1 Receptor, Epidermal Growth Factor Receptor, and HER2 Expressions on Outcomes of Patients with Gastric Cancer

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Abstract

Purpose: Expression levels of insulin-like growth factor type 1 receptor (IGF-IR), epidermal growth factor receptor (EGFR), and HER2 expressions have been linked to clinical outcomes in several solid tumors. However, the clinical significance of these biomarkers in gastric cancer (GC) remains unclear. This study was designed to delineate the clinical implications of these three biomarkers in GC.

Experimental Design: The study group comprised 87 patients who underwent gastrectomy at National Cancer Center Hospital and subsequently received chemotherapy for recurrent or residual tumors. Using immunohistochemical techniques, we analyzed the expressions of IGF-IR, EGFR, and HER2 on formalin-fixed paraffin-embedded specimens of surgically removed primary tumors.

Results: IGF-IR expression (defined as >10% membranous staining) was found in 67 tumors (77%), EGFR expression in 55 (63%), and HER2 expression in 16 (18%). Positive coexpression of IGF-IR and EGFR was found in 48 tumors (55%), that of IGF-IR and HER2 in 16 (18%), and that of EGFR and HER2 in 13 (15%). Multivariate survival analysis showed that IGF-IR – positive expression [hazard ratio (HR) 2.14, 95% confidence interval (95% CI) 1.20-3.82; $P = 0.01$], performance status 1 or 2 (HR 1.83, 95% CI 1.15-2.91; $P = 0.01$), and diffuse type tumors (HR 1.71; 95% CI 1.08-2.70; $P = 0.02$) were significant predictors of poor survival.

Conclusions: IGF-IR expression in surgical GC specimens, poor performance status, and diffuse type tumors are significant predictors of poor outcomes in patients with GC. Our data suggest that anti – IGF-IR strategies may prove valuable in such patients.

Globally, gastric cancer (GC) is the third most prevalent malignancy. Although its incidence is declining, ~930,000 cases are newly diagnosed each year (1). Despite the identification and development of several new classes of anticancer agents, GC remains an aggressive malignancy, with a median survival of 7 to 10 months in patients with metastatic or unresectable disease (2, 3). Once metastatic, GC is incurable, and chemotherapy is palliative. Increasing emphasis on the need for improved techniques for the prediction of treatment response and survival may facilitate the tailoring of chemotherapy and risk-related therapy, resulting in significantly better survival.

Insulin-like growth factor type 1 receptor (IGF-IR) is a cell membrane receptor that is activated by its ligands, IGF-I and IGF-II. IGF-IR participates in cell proliferation, differentiation, and prevention of apoptosis (4, 5). Because IGF-IR is also involved in malignant transformation (5), development of IGF-IR–directed cancer therapy has been initiated. IGF-IR is frequently overexpressed in human cancers, and the association between IGF-IR expression and outcomes has been assessed for breast cancer and other solid tumors (6, 7). However, IGF-IR expression in GC remains poorly understood.

Previous studies have indicated that IGF-IR can interact with epidermal growth factor receptor (EGFR) to augment the malignant behavior of tumors (8, 9). EGFR and its homologues HER2 (also known as erbB-2) are members of the *erbB* gene family. These receptors encode for transmembrane receptor–type tyrosine kinases, for which therapeutic approaches do exist. They play a crucial role in tumor cell proliferation, survival, adhesion, migration, and differentiation and also participate in tumor angiogenesis (10). On ligand stimulation, these receptors form either homodimers or heterodimers, which activate their cytoplasmic domain. Through EGFR, epidermal growth factor and transforming growth factor-α stimulate DNA synthesis and cell growth in various systems, including the gastrointestinal tract (11). HER2 is the preferred co-receptor for the formation of dimers with EGFR, HER3, or HER4; the heterodimers consisting of HER2 and these other receptors have a greater capacity for translating mitogenic signals than the homodimers and act synergistically to promote cellular transformation (12). In many cancers, the expression of

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Received 8/2/07; revised 12/12/07; accepted 2/7/08.

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doi:10.1158/1078-0432.CCR-07-1898

multivariate Cox proportional hazard models were used to estimate the relations of protein expressions and clinical characteristics to overall survival. All reported P values are two-sided, and the level of significance was set at $P < 0.05$. Variables for multivariate analysis were selected by means of a forward stepwise approach, using a significance level of $P < 0.10$ for entering into or remaining in the model. All analyses were done with the use of the statistical software package StatView, version 5.0 (SAS Institute, Inc.).

Results

A total of 87 patients were eligible for the study. Chemotherapy began in July 1997 in the first patient and May 2004 in the last patient. The demographic characteristics of the patients at the start of first-line chemotherapy are shown in Table 1. There were 70 men (80%) and 17 women (20%), with a median age of 64 years. The associations of IGF-IR, EGFR, and HER2 expression on immunohistochemical assay to clinical outcome could be assessed in all patients. At the time of analysis, 79 patients (91%) had died and eight patients (9%) were alive.

The chemotherapy regimens received by the patients and the response rates are also listed in Table 1. The response rates to first-line chemotherapy in our study are comparable with those reported previously (2, 17–19).

Expression frequencies of IGF-IR, EGFR, and HER2 in primary tumors and associations with clinicopathologic features. All 87 of the samples showed positive immunohistochemical staining compared with the negative controls, i.e., without the primary antibodies. Semiquantitative data are summarized in Table 2, and typical examples of positive staining are shown in Fig. 1. Membranous expression was evaluated to be positive for IGF-IR in 67 tumors (77%), positive for EGFR in 55 (63%), and positive for HER2 in 16 (18%).

IGF-IR expression was significantly more common in intestinal type tumors than in diffuse type ($P = 0.002$, Mann-Whitney U test; Table 2). HER2-positive tumors were uncommon among diffuse type cancers.

We evaluated the association between protein expression levels and the anatomic extent of disease at the time of gastrectomy using the Japanese classification (20) to define pathologic stage. Pathologic stage did not correlate with the expression of IGF-IR, EGFR, or HER2 in primary tumors (Table 2).

Expressions of IGF-IR, EGFR, and HER2 according to response to first-line chemotherapy. In patients given S-1 monotherapy as first-line treatment, no significant associations were found between tumor response and the protein expressions of the primary tumors assessed according to a four-grade scale (IGF-IR, $P = 0.87$; EGFR, $P = 0.23$; HER2, $P = 0.50$; Mann-Whitney U test). In patients who received cisplatin + irinotecan as first-line chemotherapy, there were also no associations between tumor response and protein expressions (IGF-IR, $P = 0.91$; EGFR, $P = 0.39$; HER2, $P = 0.48$; Mann-Whitney U test). Other first-line regimens were not examined because the number of patients who responded to treatment was too small.

Expressions of IGF-IR, EGFR, and HER2, clinical characteristics, and overall survival since the start of first-line chemotherapy in all patients. The overall median survival time in our study was 14.1 months. Patients with advanced GC who had IGF-IR–positive tumors had slightly poorer survival (Fig. 2A). EGFR expression was unrelated to overall survival (Fig. 2B). HER2 expression was also unrelated to overall survival (Fig. 2C).

On univariate Cox regression analyses, no clinical characteristic significantly correlated with overall survival. A multivariate

Table 2. Results of immunohistochemical analysis and associations of protein expressions with histologic type and pathologic stage at gastrectomy

	Total no. of patients (%)	Histologic type			Pathologic stage* at gastrectomy					
		Intestinal ($n = 40$)	Diffuse ($n = 47$)	$P^\dagger$	Stage I ($n = 2$)	Stage II ($n = 11$)	Stage III ($n = 23$)	Stage IV ($n = 51$)	$r^\ddagger$	P
IGF-IR				**0.002**					0.128	0.24
Negative	20 (23)	5	15		1	3	9	7		
Positive	67 (77)	35	32		1	8	14	44		
Low	21 (24)	6	15		1	0	6	14		
Moderate	21 (24)	13	8		0	3	3	15		
High	25 (29)	16	9		0	5	5	15		
EGFR				0.33					0.133	0.22
Negative	32 (37)	12	20		1	6	9	16		
Positive	55 (63)	28	27		1	5	14	35		
Low	16 (18)	9	7		0	1	5	10		
Moderate	18 (21)	8	10		0	2	5	11		
High	21 (24)	11	10		1	2	4	14		
HER2				**0.001**					0.016	0.88
Negative	71 (82)	27	44		2	8	20	41		
Positive	16 (18)	13	3		0	3	3	10		
Low	5 (6)	3	2		0	1	1	3		
Moderate	2 (2)	2	0		0	0	0	2		
High	9 (10)	8	1		0	2	2	5		

NOTE: Significant P values are shown in bold.
*According to Japanese classification.
†Mann-Whitney U test.
‡Spearman's rank-correlation coefficient.

in 48 tumors (55%), that of IGF-IR and HER2 in 16 (18%), and that of EGFR and HER2 in 13 (15%; Table 4). Although a definite correlation was not found among the four-grade scores of these protein expressions, IGF-IR expression weakly correlated with EGFR and HER2 (Table 4).

No combination of protein expressions was significantly related to overall survival. Coexpression of IGF-IR and EGFR (n = 48) was associated with a median overall survival of 13.2 months, and patients with negative expression of either or both of these proteins (n = 39) had a median overall survival of 14.7 months (P = 0.50, log-rank test). Coexpression of IGF-IR and HER2 (median overall survival, 16.0 months n = 16 versus 13.6 months in other patients n = 71; log-rank P = 0.31), and that of EGFR and HER2 (median, 13.2 months n = 13 versus 14.1 months in other patients n = 74; log-rank P = 0.65) were also not significantly related to overall survival.

Discussion

In our study, 67 of the 87 cases (77%) of advanced GC were positive for IGF-IR expression on immunohistochemical assay, and such expression was a significant independent predictor of poor outcomes, as were well-recognized prognostic factors, such as performance status and the histologic type of tumor. In contrast, the expression of EGFR or HER2 was unrelated to overall survival. The frequency of EGFR expression (63%) in the present study was higher than those in previous studies (range, 31-47%) using immunohistochemical techniques to evaluate GC (21–23), whereas the frequency of HER2 expression (18%) was in agreement with previous findings (range, 10-23%; refs. 24–27). Although EGFR expression in GC remains poorly understood, a possible reason for our high frequency of EGFR expression was that our study was underpowered. Differences in EGFR expression among studies may also be ascribed to the lack of an established immunohistochemical scoring system commonly used to evaluate GC.

Because reports that the IGF system is involved in cancer progression, angiogenesis, metastasis, and resistance to apoptosis, IGF-IR has received considerable attention as a potential target for cancer therapy (28–31). During the past few years, intensive efforts have been directed toward the development of anti–IGF-IR drugs, such as receptor-specific blocking monoclonal antibodies and small molecule tyrosine kinase inhibitors. Two phase I/phase II clinical trials of new monoclonal antibodies are now under way (32, 33), as are many phase I and preclinical trials of other monoclonal antibodies and tyrosine kinase inhibitors. In GC, evidence supporting an association of IGF-IR expression with clinicopathologic characteristics and survival remains scant thus far. Our study showed a high rate of IGF-IR–positive expression in GC and provided evidence that such expression is related to poor outcomes. Our findings suggest that membranous IGF-IR

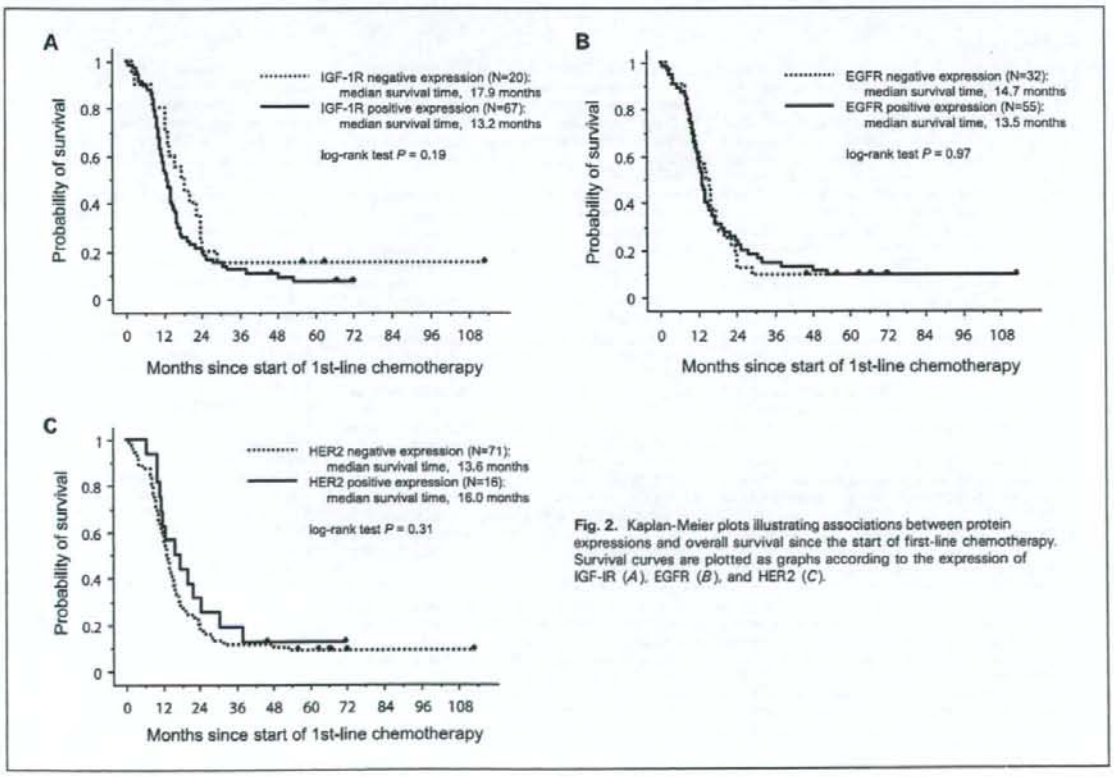

Fig. 2. Kaplan-Meier plots illustrating associations between protein expressions and overall survival since the start of first-line chemotherapy. Survival curves are plotted as graphs according to the expression of IGF-IR (*A*), EGFR (*B*), and HER2 (*C*).

our study. The trend toward poorer survival in patients with diffuse type GC is consistent with the findings of previous studies (45). Other possible reasons why the relation between IGF-IR expression and survival was insignificant on univariate analysis, but significant on multivariate analysis, were an underpowered study or the fact that only a single methodology of immunohistochemistry was used.

On the other hand, patients with HER2-positive tumors showed a slight but insignificant trend toward better survival (Fig. 2C). This finding might be related to the fact that HER2-positive expression was uncommon among diffuse type tumors (a well-known fact) which are significantly associated with poorer survival (Tables 2 and 3). Some studies have indicated that HER2 expression is an independent predictor of poor survival in GC (26, 46), whereas others have not (24, 25). Expression of EGFR in GC has also been linked to shorter overall survival, more advanced tumor stage, and lymph node metastases in some studies, but not in others (23, 47–49). EGFR expression was not related to any of these factors in our study. The results of immunohistochemical assays can be affected by many variables, including tissue fixation, choice of primary antibodies, and scoring systems, potentially leading to conflicting relations between the expressions of growth factor receptors and clinical outcomes. Immunohistochemical scoring systems of IGF-IR and EGFR differ among studies (16, 23, 49). Even for HER2, it remains unclear whether the scoring system used for breast cancer is valid for GC. The utilization of fluorescent *in situ* hybridization as an adjunct in GC or the automated quantification of immunohistochemical results may circumvent the subjective nature of immunohistochemical analyses. Nonetheless, a common scoring system for GC should be urgently established to accurately predict the clinical response to antagonists of these receptors, as well as to predict patient survival.

In conclusion, our study provides evidence that IGF-IR expression in GC specimens, poor performance status, and diffuse type cancer are significant predictors of poor survival in patients with advanced GC. We also showed that coexpression of IGF-IR and EGFR or IGF-IR and HER2 is relatively common in GC. Because the expression of IGF-IR has been associated with resistance to anti-EGFR and anti-HER2 therapies (35, 36), the potential therapeutic benefits of simultaneously targeting such receptors in patients with GC should be critically evaluated. Taken together, our findings suggest that anti–IGF-IR strategies may prove valuable in patients with GC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Hideko Morita, Hiromi Orita, and Mari Araake for help in collecting and organizing the clinical samples and Hideko Morita for providing excellent technical assistance.

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Jpn J Clin Oncol 2008;38(5)354–359
doi:10.1093/jjco/hyn035

Clinical Efficacy and Safety of Octreotide (SMS201-995) in Terminally Ill Japanese Cancer Patients with Malignant Bowel Obstruction

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Received February 28, 2008; accepted April 10, 2008

Objective: In patients with advanced cancer, malignant bowel obstruction (MBO) causes gastrointestinal symptoms such as nausea and vomiting leading to severely impaired oral food intake. Thus, MBO markedly diminishes the quality of life (QOL) of these patients because placement of a nasogastric tube becomes necessary. Many studies have shown that octreotide (SMS201–995; SMS), a synthetic analog of somatostatin, is effective for controlling the symptoms of MBO. This study was conducted to assess the efficacy and safety of 300-μg/day initial dose of SMS in Japanese patients with MBO and to investigate the clinical benefit of patients achieved by the improvement of nausea/vomiting based on subjective assessment.

Methods: The subjects were patients with MBO that was refractory to other medical treatment and who had suffered at least two vomiting episodes per day for two consecutive days or had required a nasogastric tube. After enrollment, patients received SMS (300 μg/day) subcutaneously as a continuous injection for 6 days. Patients who responded to this 6-day course of treatment continued to receive the drug.

Results: Among 25 patients who were enrolled, 11 (44.0%) responded to treatment with resolution or improvement of nausea/vomiting. Their symptomatic improvement was assessed by quantitatively measuring the level of control of nausea/vomiting and by using a self-administered QOL questionnaire that evaluated the frequency and severity of nausea/vomiting, the proportion of patients enjoying recreational activities and the overall patient satisfaction with the therapy. SMS was well tolerated, and nausea and agitation were the only adverse events potentially related to this drug.

Conclusion: The results of the study confirmed that the 300-μg/day dose of SMS is safe and effective for patients with MBO uncontrolled by other therapies and suggested that the relief of symptoms with nausea/vomiting by SMS could contribute to improvement of the QOL of patients.

Key words: malignant bowel obstruction – octreotide – terminally ill cancer patients

INTRODUCTION

In patients with advanced cancer, malignant bowel obstruction (MBO) occurs primarily due to carcinomatosis because of the recurrence of gastrointestinal or ovarian cancer. Surveys performed at palliative care facilities of Japan have shown that the incidence of MBO is 10–16% (1,2), which is similar to that reported in the USA and Europe (3–5). Current treatments for MBO include (i)

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surgery to bypass/remove the obstruction; (ii) gastrointestinal drainage via a nasogastric tube or other means and (iii) medication such as antiemetics or other drugs. Surgical treatment is often contraindicated to the poor general condition of the patient, and placement of a nasogastric tube may be the only treatment available for inoperable cases. Some patients with MBO fail to respond to conventional drugs, such as antiemetics. A nasogastric tube can achieve symptomatic relief in such patients, but common complications include mucosal erosion and hemorrhage, esophagitis and aspiration pneumonia. Therefore, a tube is not recommended for terminally ill cancer patients in whom the quality of life (QOL) should hopefully be improved by palliative care.

As Mercadante et al. (6) first reported the use of SMS, a synthetic analog of somatostatin, for the management of MBO in 1993, several clinical studies have been conducted to evaluate its efficacy for nausea/vomiting due to MBO (5,7–10), primarily at palliative care facilities.

Accordingly, we conducted the first clinical study to assess the efficacy and safety of SMS for the control of nausea/vomiting in Japanese patients with MBO who were unlikely to respond to any other therapy. This study was also designed to evaluate the clinical benefit controlling nausea/vomiting for improvement of the QOL by patient self-assessment.

PATIENTS AND METHODS

Patients in this study were required to be hospitalized, to be between 20 and 74 years of age, to suffer from MBO that was refractory to medical treatment and to have a life expectancy of at least 3 weeks. Before being enrolled in this study, the patients also had at least two episodes of vomiting per day on two designated days or had marked drainage of bowel contents ($\geq$500 ml/day) from a nasogastric tube. Patients who retained hepatic function, as indicated by a total bilirubin $\leq$2.0 mg/dl, were eligible for the study. The study excluded patients with serious complications (e.g. active infection, pleunal effusion and gastrointestinal hemorrhage) and those with symptomatic brain metastasis.

After enrollment, patients received SMS (300 μg/day) subcutaneously as a continuous injection for 6 days. Patients who responded to this 6-day course of treatment continued to receive the drug. The dose of SMS was to be decreased to 150 μg/day in the event of Grade 2 or worse adverse events or if there was marked aggravation of nausea/vomiting.

Patients were assessed daily to determine the number of vomiting episodes, the severity of their nausea and (if relevant) the volume of fluid draining from the nasogastric tube. The volume of intravenous and oral fluid was also measured daily because of a probable influence on the volume of vomitus and tube drainage. The clinical benefit of treatment was also assessed daily during the 6-day treatment period using a self-administered QOL questionnaire (see Appendix 1). Patients were asked about the frequency and severity of nausea/vomiting, the intensity of pain, the amount and quality of sleep and the extent of their enjoyment of recreational activities (TV/radio, reading and conversation).

Table 1. Grading of vomiting by Japan Clinical Oncology Group Toxicity Criteria

Grade	Vomiting
0	No vomiting episodes
1	Nausea only
2	One to five vomiting episodes per 24 h
3	Six or more vomiting episodes per 24 h

Response criteria were based on the change from baseline (24 h before the start of treatment) to Day 6 in the severity of nausea/vomiting graded using the Toxicity Criteria of the Japan Clinical Oncology Group (JCOG) (11). Grading of vomiting by JCOG Toxicity Criteria is shown in Table 1. As shown in Table 2, the response to treatment was graded using three categories ['complete control' (CC), 'partial control' (PC) and 'no control' (NC)]. Patients with JCOG Grade 0 nausea/vomiting on Day 6 were assigned a rating of CC. The rating was PC if the JCOG grade for nausea/vomiting was decreased by one grade or more from baseline on Day 6. No change or an increase of JCOG grade was regarded as NC.

In patients with a nasogastric tube at baseline, extubation was allowed if drainage was reduced to >500 ml/day. After extubation, the response to the treatment was graded according to the following three categories defined by the JCOG grade of nausea/vomiting on Day 6: CC (JCOG Grade 0), PC (only one episode of vomiting per day or nausea only) and NC (nausea/vomiting $\geq$JCOG Grade 2).

The occurrence of adverse events and abnormal laboratory findings were considered for the evaluation of safety, and the severity of adverse drug reaction was grades in accordance with JCOG criteria.

With regard to the clinical laboratory testing, hematology, biochemistry and urine tests were performed just before the start of the treatment with study medication and after 7 days of treatment.

This study was approved by the institutional review board of the National Cancer Center and was conducted in compliance with the Japanese Good Clinical Practice Guidelines. In

Table 2. Criteria for the response to treatment on Day 6

Nasogastric tube (GT)	Complete control (CC)	Partial control (PC)	No control (NC)
Without GT	Grade 0	One grade or more decrease from baseline	No charge or increase grades
With GT*	Grade 0	Only one vomiting per 24 h or nausea only	

*Evaluated after removal of nasogastric tube.

Table 3. Demographic and baseline characteristics of the patients

	Variable	Patients (%)
Sex	Male	11 (44.0)
	Female	14 (56.0)
Age (years)	Range	41–67
	Median	53
Diagnosis of primary tumor	Gastric cancer	14 (56.0)
	Colon cancer	5 (20.0)
	Ovarian cancer	3 (12.0)
	Pancreatic cancer	2 (8.0)
	Cervical cancer	1 (4.0)
Complication(s)	No	16 (64.0)
	Yes	9 (36.0)
Nasogastric tube at baseline	No	17 (68.0)
	Yes	8 (32.0)
Previous gastrectomy	No	18 (72.0)
	Partial	2 (8.0)
	Total	5 (20.0)
Surgical treatment of bowel obstruction	No	21 (84.0)
	Yes	4 (16.0)
PS at baseline	PS 1	1 (4.0)
	PS 2	3 (12.0)
	PS 3	17 (68.0)
	PS 4	4 (16.0)

PS, performance status.

accordance with the Declaration of Helsinki, written informed consent was obtained from all patients prior to enrollment.

RESULTS

Twenty-five patients were enrolled and treated with SMS. Their demographic and baseline characteristics are summarized in Table 3. There were 11 men and 14 women with ages ranging from 41 to 67 years (median: 53 years). Gastric cancer was the most frequent type of malignancy ($n = 14$; 56.0%), followed by colon, ovarian and pancreatic cancer. At baseline, a nasogastric tube was already inserted in eight patients (32.0%) but not in 17 patients (68.0%). The baseline performance status (PS) was 3–4 in 21 patients (84.0%).

None of the subjects needed dose reduction to 150 μg/day according to the protocol criteria.

Response

The response to treatment is summarized in Table 4. Among the 25 patients treated, five (20%) had a response of CC and six (24%) had a response of PC with an overall response rate (CC plus PC) of 44% (95% confidence interval: 24.4–65.1%). Among the 17 patients without a nasogastric tube at baseline, three (17.6%) achieved CC and five (29.4%) achieved PC with an overall response rate of 47.1%. Among the eight patients with a nasogastric tube at baseline, two (25%) achieved CC and one (12.5%) achieved PC with an overall response rate of 37.5%.

In the entire study population, the median number of vomiting episodes per day was significantly reduced from 6.0 (range: 2.0–55) at baseline to 2.5 (range: 0–29) on Day 6 ($P = 0.0024$). Among the eight patients with a nasogastric tube at baseline, four showed a marked decrease in drainage on Day 2 (Fig. 1). All of the three responders (with a rating of CC or PC) in this subgroup showed a reduction in drainage that was sufficient for extubation and achieved symptomatic improvement.

After 6 days (144 h) of continuous therapy, 14 patients were judged to require further treatment with SMS. Treatment was continued for up to 46 days, with the median duration being 8 days.

Clinical Benefit (Subjective Assessment)

Subjective clinical assessment was done by an investigation of four categories (Appendix 1). Twenty-three of the 25 patients completed the self-administered questionnaire. The other two patients were unable to complete this questionnaire because of their poor general condition. In addition, the number of responders decreased to 16 patients on Day 6 because of disease progression. The efficacy of SMS was reflected by an improvement of QOL in terms of nausea/vomiting and enjoyment in activities.

Severity of Nausea/Vomiting

At baseline, 11 of 23 patients (47.8%) had moderate or severe nausea/vomiting. On Day 6, only six of 16 patients (37.5%) still had moderate nausea/vomiting and no patient

Table 4. Response

	Rating	CC	PC	NC	Not evaluated	Total	Response rate (CC + PC), % (95% CI)
	No. of patients (%)	5 (20.0%)	6 (24.0%)	13 (52.0%)	1 (4.0%)	25	44.0 (24.4–65.1)
At baseline	Nasogastric tube (−)	3 (17.6%)	5 (29.4%)	8 (47.1%)	1 (5.9%)	17	47.1 (23.0–72.2)
	Nasogastric tube (+)	2 (25.0%)	1 (12.5%)	5 (62.5%)	0 (0%)	8	37.5 (8.5–75.5)

CI, confidence interval.

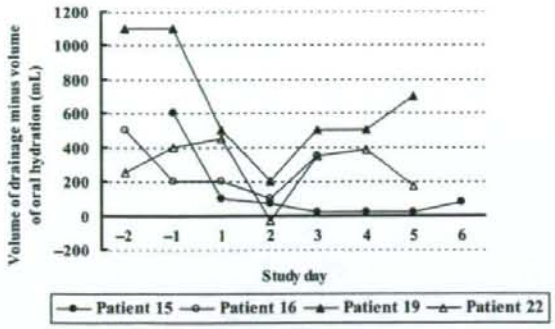

Figure 1. Changes in the net volume of nasogastric tube drainage in four patients who showed a substantial reduction.

rated nausea/vomiting as severe. Nausea/vomiting was absent or slight in nine of 16 patients (56.3%) on Day 6 compared with eight of 23 patients (34.8%) at baseline.

Change of Nausea/Vomiting Relative to Baseline

Nausea/vomiting was markedly or moderately alleviated in 11 of 23 patients (47.8%) on Day 1 and in 10 of 16 patients (62.5%) on Day 6.

Enjoyment of Recreational Activities

Recreational activities were never or hardly ever enjoyed by seven of 16 patients (43.8%) on Day 6 compared with 15 of 23 patients (65.2%) at baseline. The percentage of patients with fair or modest enjoyment of recreational activities increased from 17.4% (four of 23 patients) at baseline to 43.8% (seven of 16 patients) on Day 6. Figure 2 shows the changes in patients in the individual rating categories during SMS treatment (Days 1–6).

Safety

Adverse events occurred in nine patients. Among these, only two events in two patients (8.0%) (Grade 1 nausea and Grade 2 agitation) were judged to be potentially related to SMS. Potential treatment-related laboratory adverse events occurred in six patients (26.1%), including thrombocythemia, leukocytosis, increased ALP and increased γ-GTP. Thus, treatment with SMS was well tolerated and did not cause any serious or clinically significant adverse reactions.

DISCUSSION

Many recent studies have shown that SMS is useful for controlling gastrointestinal symptoms due to MBO in patients who have advanced cancer. In this study, we evaluated the efficacy and safety of 300-μg/day initial dose of SMS in Japanese patients with MBO. The primary efficacy endpoint was the change in vomiting episodes after treatment. To ensure objectivity of assessment, we used the JCOG Toxicity Criteria to grade the severity of emesis. In contrast, previous clinical studies have often used the World Health Organization (WHO) Toxicity Criteria (7) (Grade 1: nausea; Grade 2: transient vomiting; Grade 3: vomiting requiring therapy and Grade 4: intractable vomiting). Compared with the WHO criteria, the JCOG criteria (Grade 1: only nausea; Grade 2: 1–5 vomiting episodes per 24 h; Grade 3: ≥6 vomiting episodes per 24 h) seem to provide a more quantitative assessment of the severity of emesis. In this study, we also assessed the clinical benefit of treatment by using a self-administered QOL questionnaire. No previous clinical studies of SMS have included subjective assessment of efficacy by the patients. This is probably because of only a few eligible patients (e.g. terminally ill cancer patients) were in a satisfactory condition to answer a self-administered questionnaire. This was emphasized by the smaller number of respondents on Day 6 ($n = 16$) compared with baseline ($n = 23$) in our

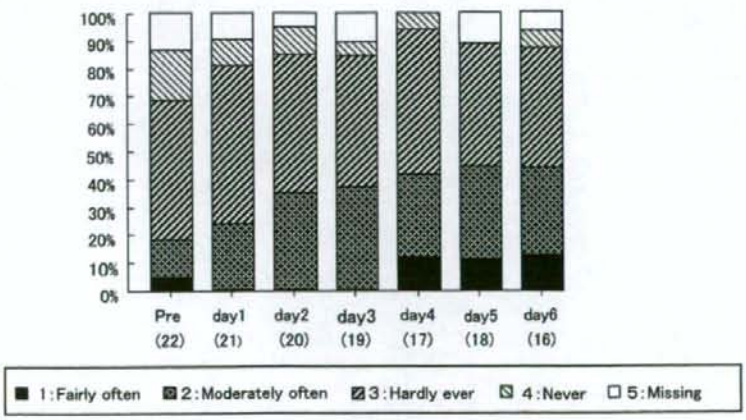

Figure 2. Changes of patients who could enjoy recreational activities rate (%).

study. As a result of previous findings and in consideration of the target disease (MBO refractory to other medical treatment), SMS was administered at 300 μg/day (the maximum effective dose) as a 24-h continuous subcutaneous injection using a pump. Dose escalation was not planned or performed because previous studies have shown that there is no significant additional benefit of higher doses.

Forty-four percent of the patients in this study responded to the treatment with SMS, and the overall response rate was slightly lower than that reported previously (5–10,12–14). Possible explanations for the less favorable response to SMS in the present study include differences in the general condition (poor PS), the underlying malignancies and the timing for assessment of the response to treatment.

Regarding the type of underlying malignancies, more than half of the patients enrolled in the present study had gastric cancer ($n = 14$; 56.0%). Analysis of response data obtained in this study revealed that five of the 14 patients with gastric cancer (35.7%) and six of the 11 patient with other cancer (54.5%) had a response of PC or better. Gastric cancer patients tended to have a lower response rate. Gastric cancer is a common malignancy in the Japanese population, but few earlier studies of SMS have involved patients with gastric cancer. Thus, the lower response rate of gastric cancer patients was partly responsible for the lower overall response rate in the present study.

The timing of assessment might also have affected the difference in the response rate. In this study, we assessed an estimate of response to SMS after 6 days of treatment by comparing the severity of nausea/vomiting as graded according to JCOG Toxicity Criteria between Days 0 (24 h before the starting treatment) and 6. The timing of assessment was based on the results of previous studies (6,7), which suggested no significant difference between the response on Day 6 (144 h) and the response observed over a longer treatment period. However, *post hoc* analysis of our data revealed that the comparison between Days 0 and 6 did not provide an accurate estimate of the response to SMS in some patients. In fact, the benefit of SMS could not be assessed correctly by Day 6 (the study endpoint) in some of the patients because of worsening of their symptoms due to disease progression. In overseas clinical studies (12,13), the response to SMS was assessed after only 3 days of treatment, so the longer treatment period before examination in this study might also have contributed to the lower response rate.

The patients' assessment of clinical benefit of treatment in terms of relief of gastrointestinal symptoms showed that the nausea/vomiting status tended to improve similarly to the assessments on JCOG Toxicity Scale, and the percentage of patients enjoying TV, radio, reading and conversation with others was particularly increased. The increase became progressively greater on each day of the 6-day treatment period (Fig. 2), suggesting that symptomatic improvement achieved by SMS may be associated with an improvement of QOL.

Initial treatment with 300 μg/day of SMS for 6 days was confirmed to be effective and safe for the controlling nausea/vomiting in Japanese patients with MBO. Further studies will be performed to evaluate the SMS therapy with respect to the duration of treatment, effect of higher doses and the usefulness of SMS treatment in relation to the location of obstruction in the upper or lower gastrointestinal tract, and investigation should be performed in more patients.

Further studies may also include assessment on Day 4 after 3 days of SMS treatment as done by Ripamonti coworkers (12–13) in overseas clinical study of SMS.

Acknowledgements

The authors are grateful to the patients and physicians who participated in this study, and are particularly indebted to Drs. E. Ariga, H. Minami, H. Fujii, Y. Matsumura and K. Muro from the National Cancer Center East/Central Hospital, Japan.

Funding

This study was sponsored by Novartis Pharma, Japan.

Conflict of interest statement
None declared.

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APPENDIX

QOL QUESTIONNAIRE

Date:

- Question 1: How intense is your pain?
  (1) None
  (2) Slight
  (3) Moderate
  (4) Severe
- Question 2: How many vomiting episodes do you have per day?
- Question 3: How severe is your nausea and vomiting?
  (1) None
  (2) Slight
  (3) Moderate
  (4) Severe
- Question 4: Did the severity of your nausea and vomiting change after the start of the clinical trail?
  (1) Markedly improved
  (2) Moderately improved
  (3) Unchanged
  (4) Worse
- Question 5: How is your sleep quality?
  (1) Good
  (2) Fair
  (3) Poor
  (4) No sleep
- Question 6: Can you enjoy watching TV, listening to the radio, reading a book, or talking with others?
  (1) Fairly often
  (2) Moderately often
  (3) Hardly ever
  (4) Never

Jpn J Clin Oncol 2008;38(6)432–437
doi:10.1093/jjco/hyn043

A Phase II Study of Sequential Methotrexate and 5-fluorouracil Chemotherapy in Previously Treated Gastric Cancer: A Report from the Gastrointestinal Oncology Group of the Japan Clinical Oncology Group, JCOG 9207 Trial

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Received February 26, 2008; accepted May 7, 2008; published online May 30, 2008

Objective: As prognosis of advanced gastric cancer is still poor, a standard regimen after first-line fluorouracil (FU)-based chemotherapy has not yet been established. Therefore, we conducted a phase II study to evaluate the efficacy and toxicity of sequential treatment with methotrexate (MTX) and also 5-FU as second-line chemotherapy in patients with advanced gastric cancer.
Methods: Treatment consisted of weekly doses of MTX (100 mg/m^2, i.v. bolus), followed by 5-FU (600 mg/m^2, i.v. bolus) 3 h after MTX administration. Leucovorin rescue therapy (six doses of 10 mg/m^2, given at 6-h intervals) was commenced 24 h after a treatment with MTX. The primary endpoint was the response rate.
Results: Between December 1992 and June 1995, 56 patients were registered in this study and one was ineligible. All registered patients were included in all analyses. The median age of the patients was 60 years (20–75 years). Most patients (75%) had a performance status of 0 or 1, and 51 (90%) received 5-FU-based chemotherapy as first-line treatment. The major adverse events were myelosuppression and gastrointestinal toxicity. Grade 4 neutropenia occurred in 6.3% of the patients. The overall objective response rate was 9.0% [five partial responses among 56 patients, 95% confidence interval (CI): 3.0–20%]. The median overall survival time was 237 days, and the 1-year survival proportion was 21.4%.
Conclusions: Sequential MTX/5-FU therapy provides good survival outcomes with tolerable toxicity despite a limited response in patients with previously treated advanced gastric cancer. This regimen is now being evaluated in a randomized study in patients with pretreated advanced gastric cancer, by the Japan Clinical Oncology Group.

Key words: methotrexate – 5-fluorouracil – gastric cancer – second-line chemotherapy

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INTRODUCTION

Gastric cancer is the most common cancer in Japan and many other Asian countries. Mortality statistics shows that around 50 000 patients die from gastric cancer every year in Japan (1). Primary tumor resection is the best strategy for the treatment of gastric cancer. In patients with curatively resected Stages I–III gastric cancer, the 5-year survival proportion is $\geq$50%, but remains $\leq$10% in Stage IV or recurrent disease. Some randomized trials have demonstrated that fluorouracil (FU)-based regimens improve survival proportions in patients with advanced gastric cancer, as compared with supportive care alone (2–4). Although this survival advantage is significant as first-line treatment, no randomized study has shown a survival benefit of any second-line regimen for patients with metastatic gastric cancer failed to first-line FU-based chemotherapy, as compared with best supportive care.

Methotrexate (MTX) enhances the cytotoxicity of 5-FU by inhibiting the synthesis of DNA, RNA or both when 5-FU is administered several hours after MTX (5,6). A meta-analysis of randomized trials of sequential MTX/5-FU therapy revealed a higher response rate and longer survival as compared with single-agent bolus 5-FU chemotherapy in patients with colorectal cancer. Toxicity with these sequential MTX/5-FU regimens was similar to that with 5-FU alone (i.e. vomiting, stomatitis, diarrhea and leukopenia) (7,8). The sequential MTX/5-FU therapy was also found to be effective against advanced gastric cancer as shown in phase II trials (9,10). One Japanese phase II trial of sequential MTX/5-FU therapy in advanced gastric cancer reported response rates of 23% [13 partial responses (PRs)/56 patients] and 41% (15 PRs/37 patients) with low- and intermediate dose MTX regimens, respectively (11). Several reports indicated that a 7- to 24-h interval between MTX and 5-FU may provide an advantage of efficacy. However, a longer than 7-h interval cannot be used on an outpatient basis. In Japan, a 3-h interval between MTX and 5-FU regimens has been verified to be safe and effective in patients with advanced gastric cancer (11). Therefore, we decided to adopt a 3-h interval in the present study.

In 1990s, the sequential MTX/5-FU therapy was widely used as an option for advanced gastric cancer in Japan. At that time, however, other active drugs, such as irinotecan (CPT-11) and taxanes, were unavailable. The results of *in vitro* studies showed that bolus and continuous administrations of FU had different mechanisms of cytotoxicity and resistance, thereby resulting in incomplete cross-resistance between pulse and prolonged exposure to FU (18,19). Therefore, the sequential MTX/5-FU therapy, in which 5-FU was given by bolus infusion, was used empirically without clinical evidence in patients who failed to respond to infusional FU-based regimens, such as 5-FU alone or 5-FU/cisplatin. This background led to the present phase II clinical trial assessing the efficacy and toxicity of sequential MTX/5-FU chemotherapy in patients with pretreated advanced gastric cancer, conducted by the Japan Clinical Oncology Group (JCOG 9207 study).

PATIENTS AND METHODS

Eligibility

All patients enrolled in this trial fulfilled the following eligibility criteria: (i) histologically confirmed gastric cancer, (ii) unresectable or recurrent disease, (iii) treated with one prior regimen until disease progression or unacceptable toxicity after discontinuing palliative or adjuvant chemotherapy, (iv) a wash-out period of at least 4 weeks since the last chemotherapy treatment, (v) measurable or assessable disease, (vi) age $\leq$75 years, (vii) performance status (PS) $\leq$3 on the Eastern Cooperative Group (ECOG) scale, (viii) adequate bone marrow function (WBC $\geq$ 4000/mm^3, platelets $\geq$ 100 000/mm^3), (ix) adequate liver function (serum total bilirubin level $\leq$2.0 mg/dl, transaminase level $\leq$2.5-fold the upper limit of normal), (x) adequate renal function [serum creatinine $\leq$ 1.5 mg/dl, blood urea nitrogen (BUN) $\leq$ 25 mg/dl, creatinine clearance $\geq$ 50 ml/min], (xi) a normal electrocardiogram, (xii) a life expectancy of at least 8 weeks and (xiii) provision of written informed consent. Patients with active gastrointestinal bleeding, synchronous carcinomas, large amounts of pleural effusion or ascites, central nervous system metastasis, concurrent uncontrolled disease, or severe psychiatric disease were excluded. Pregnant or nursing women were also excluded. The study protocol was approved by the JCOG Clinical Trial Review Committee and by the institutional review board of each participating center.

Treatment Plan

The treatment schedule consisted of a weekly dose of MTX (100 mg/m^2, i.v. bolus) followed by 5-FU (600 mg/m^2, i.v. bolus) after a 3-h interval. Leucovorin rescue therapy (10 mg/m^2 orally or i.v. every 6 h, for a total of six doses) was commenced 24 h after MTX administration. To prevent MTX-induced nephrotoxicity, acetazolamide (250 mg) was given intravenously immediately after MTX infusion, and sodium bicarbonate (33.3 mEq) dissolved in 500 ml of normal saline was administered by drip infusion for urinary alkalinization during the 3-h interval between the doses of MTX and 5-FU. Before each cycle, the patients had to meet the following criteria: WBC $\geq$ 3000/mm^3, platelet count $\geq$75 000/mm^3, adequate liver and renal function as defined in the eligibility criteria, a PS of 0–3 and no grade $\geq$2 toxicity. The treatment was terminated if the disease progressed within 4 weeks or if a complete response (CR), PR or minor response (MR) was not achieved within 8 weeks. Otherwise, the treatment was repeated until disease progression or severe toxicity was confirmed.

Evaluation of Response and Toxicity

Baseline evaluations included a complete medical history, physical examination, complete blood cell count, serum chemistry, creatinine clearance, urinary analysis, electrocardiography, gastroscopy, gastrography, abdominal computed

tomography, abdominal ultrasonography and chest radiography. Hematologic, serum chemical and urinary analyses and symptoms were monitored on a weekly basis during the treatment. The objective response was evaluated every 4 weeks. CR, PR, no change (NC), progressive disease (PD) or not evaluated (NE) were defined according to the response assessment criteria proposed by the Japanese Research Society of Gastric Cancer (12). The tumor response was confirmed by central review. Toxicity was evaluated according to the JCOG common toxicity criteria (13) that were established on the basis of the National Cancer Institute Common Toxicity Criteria, ver.1.

Statistical Methods

The primary endpoint of this study was the tumor response rate. The secondary endpoints were overall survival and toxicity. Sample size was determined by feasibility reasons. Within a reasonable length of time (1.5 year of accrual), 15 participating institutions can recruit 50 subjects. This produces the width of 25% of its 95% CI for a point estimate around 30%.

An interim analysis was planned to test for the treatment inefficacy by examining whether the 90% upper confidence limit of the response rate would exceed 25% for the first evaluable 20–25 patients. Overall survival was calculated from the date of registration until the date of death, using the Kaplan–Meier method, and the CIs were calculated using the Greenwood's formula.

All the analyses were conducted using SAS software (ver. 6.12; SAS Institute, Inc., Cary, NC, USA).

RESULTS

Patient Population and Study Treatment

Between December 1992 and June 1995, 56 patients were enrolled in the study at 15 hospitals. Although one patient was ineligible because of no previous chemotherapy, all analyses were conducted for all registered patients. Table 1 lists the demographic data, baseline disease and pretreatment characteristics. Thirty-eight men and 18 women were registered. The median age of the patients was 60 years (range, 25–75 years), and 42 of 56 patients (75%) had a good PS of 0 or 1. Fifty-one patients (91%) had received 5-FU-based chemotherapy as first-line treatment. A total of 419 doses of sequential MTX/5-FU therapy were administered to 56 patients. The median number of doses was 5 (range, 1–31). Forty-four of the 56 enrolled patients (78%) received four or more doses of sequential MTX/5-FU therapy. Treatment was terminated because of the disease progression in 38 patients, toxicity in seven, patient refusal in four, death in four and others in three.

Table 1. Patient characteristics

Characteristic	Total ($n = 56$)
Age (years)	
Median	60
Range	20–75
Gender	
Male	38
Female	18
ECOG performance status	
0	12
1	30
2	12
3	2
Macroscopic type of primary cancer	
1	2
2	14
3	22
4	11
Others	6
Histological type	
Intestinal type	27
Diffuse type	29
Metastatic sites	
Lymph nodes	39
Liver	20
Peritoneum	8
Lung	3
Krukenberg's tumor	3
Douglas' metastasis	2
Bone	1
Malignant ascites	8
Pleural effusion	1
Skin	1
Prior chemotherapy	
5-FU-based regimen	51
Non-FU regimen	4*

*One patient was ineligible due to no prior chemotherapy. ECOG, Eastern Cooperative Group.

Toxicity

Toxic effects occurring during the study are summarized in Table 2. The major toxicities were myelosuppression and gastrointestinal toxicity. Grade 3 and 4 neutropenia occurred in 10 and 6% of the patients, respectively. Severe thrombocytopenia was infrequent. The incidence of Grade 3/4 diarrhea was 3.6%. Mild nausea and vomiting (Grade 1 and 2) were frequent (63.6%). A Grade 4 elevation of total bilirubin was observed in one patient (2%) who was later found to have obstructive jaundice caused by disease progression. Early death, defined as death within 30 days from the last dose of chemotherapy, occurred in nine patients. The causal

Table 2. Toxicity profiles

Toxicity	JCOG grade					Total	Grade 3/4 (%)
	0	1	2	3	4		
Hematological toxicity							
Leucopenia	22	14	16	3	0	55	5.5
Neutropenia	16	15	9	5	3	48	16.7
Anemia	6	20	24	5	—	55	9.1
Thrombocytopenia	48	4	2	1	0	55	1.8
Non-hematological toxicity							
Nausea/vomiting	18	22	13	2	—	55	3.6
Diarrhea	35	12	6	2	0	55	3.6
Stomatitis	36	17	0	1	1	55	1.8
Alopecia	46	8	1	—	—	55	—
Allergic reaction	54	1	0	0	0	55	0
Fever	41	8		1	0	55	1.8
Peripheral neuropathy	53	2	0	0	0	55	0
Total bilirubin	39	—	7	4	1	51	9.8
AST	33	16	2	0	0	51	0
ALT	41	10	0	0	0	51	0
Creatinine	43	6	1	0	0	50	0
Hyponatremia	26	20	5	1	0	52	1.9
Hypokalemia	39	11	2	0	0	52	0
Body weight loss	11	12	6	0	0	29	0
ECOG PS	0	1	2	3	4	Total	
	6	19	18	9	2	54	

PS, performance status; JCOG, Japan Clinical Oncology Group; AST, aspartate aminotransferase; AL, alanine aminotransferase.

relationship between the early deaths and the study treatment was evaluated by the JCOG Data and Safety Monitoring Committee. Seven of the nine deaths were judged as 'death due to PD'. The other two deaths were evaluated to be treatment related. One of the treatment-related deaths was caused by cardiogenic shock, probably because of a 5-FU-related ischemic cardiac event.

Efficacy

The tumor responses of all registered patients were assessed and confirmed by central review (Table 3). The response of the patient who had not previously received chemotherapy was classified as NE. Only five of the 56 patients had an objective PR (response rate; 9.0%, 95% CI, 3–20%). The survival curve is shown in Fig. 1. The median overall survival time was 237 days (95% CI, 145–281 days). The 1-year survival proportion was 21.4% (95% CI, 10.7–32.2%), and the 2-year survival proportion was 3.6% (95% CI, 0–8.4%).

Table 3. Treatment response

CR	0
PR	5
NC	31
PD	14
NE	6
RR	9.0%; 95% CI, 3–20%

CR, complete response; PR, partial response; NC, no change; PD, progressive disease; NE, not able to be evaluated; RR, response rate; CI, confidence interval.

DISCUSSION

5-FU-based chemotherapy is considered a standard therapy for advanced gastric cancer. However, 5-FU-based combination regimens of chemotherapy have not been shown to prolong overall survival as compared with 5-FU alone (14–17). Furthermore, the potential benefits of second-line chemotherapy for patients with pretreated gastric cancer remain unclear, and few prospective studies have been conducted.

Although this study showed that the sequential MTX/5-FU therapy possessed limited antitumor activity as second-line chemotherapy, despite an MST of 237 days (95% CI, 145–281), and 1- and 2-year survival proportions of 21.4% (95% CI, 10.7–32.2%) and 3.6% (95% CI, 0–8.4%), respectively. These survival data were similar to those obtained for first-line chemotherapy with several regimens at that time. Possible reasons for the good survival may include good patients' clinical characteristics. At the baseline evaluation, the median age of the patients was 60 years (range, 20–75 years), and most patients had a good PS of 0 or 1. Another possible reason is a tumor stabilization effect of this combination regimen. Probably because nearly all patients had received 5-FU-based chemotherapy as first-line treatment, 56% of patients had NC, for a disease control rate (PR + NC) of 65%. The toxicity of the regimen can be considered tolerable. The proportion of patients with toxicity in our study was similar to that with the MTX/5-FU therapy used as first-line treatment (11).

Although the response rate of the present study was only 9%, the study regimen had good survival outcomes with tolerable toxicity. Given that survival with the best supportive care is ~3–4 months (3,4), this sequential MTX/5-FU therapy can be considered to be an option for standard second-line treatment.

Recently, second-line chemotherapy with paclitaxel or bi-weekly irinotecan has produced response rates of 27 and 18%, respectively (19,20), although these data were derived by subset analysis. Peritoneal dissemination of gastric cancer may cause serious complications, such as intestinal obstruction, ascites and hydronephrosis with renal dysfunction. In patients with these conditions, cisplatin or irinotecan, drugs active against gastric cancer, are difficult to use because of an

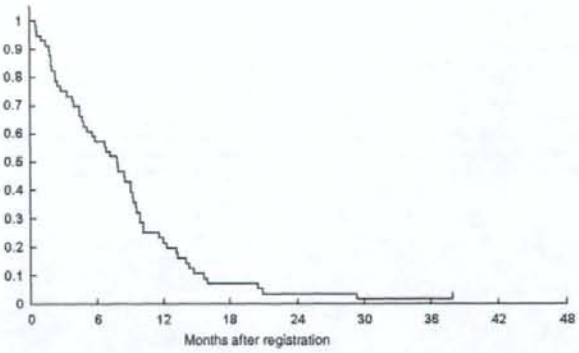

Figure 1. Overall survival.

increased risk of toxicity. The MTX/5-FU therapy is considered to be effective and safe as first-line treatment in patients who have the advanced gastric cancer with peritoneal dissemination, especially malignant ascites (21). On the basis of these results, a randomized phase II trial comparing the MTX/5-FU therapy with paclitaxel in patients with pretreated advanced gastric cancer who have mainly peritoneal disease is now being conducted by the JCOG (protocol JCOG 0407).

Acknowledgements

We would like to thank Dr Kenichi Nakamura for his helpful comments, Dr Naoki Ishizuka for his help in statistical analysis and Ms Aya Kimura for data management.

Funding

This work was supported by Grants-in-aid for Cancer Research (8S-1, 11S-3 and 11S-4) and by the Second-term Comprehensive 10-year Strategy for Cancer Control (H10-Gan-027, H12-Gan-012) from the Ministry of Health, Labor and Welfare, Japan.

Conflict of interest statement

None declared.

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Drug Metab. Pharmacokinet. 23 (2): *SNP1* (139)–*SNP9* (147) (2008).

SNP Communication

Genetic Variations and Haplotypes of *ABCC2* Encoding MRP2 in a Japanese Population

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: The multidrug resistance-associated protein 2 (MRP2) encoded by the *ABCC2* gene is expressed in the liver, intestine and kidneys and preferentially exports organic anions or conjugates with glucuronide or glutathione. In this study, all 32 exons and the 5′-flanking region of *ABCC2* in 236 Japanese were resequenced, and 61 genetic variations including 5 novel nonsynonymous ones were detected. A total of 64 haplotypes were determined/inferred and classified into five *1 haplotype groups (*1A, *1B, *1C, *1G, and *1H) without nonsynonymous substitutions and *2 to *9 groups with nonsynonymous variations. Frequencies of the major 4 haplotype groups *1A (−1774delG), *1B (no common SNP), *1C (−24C>T and 3972C>T), and *2 [1249G>A (Val417Ile)] were 0.331, 0.292, 0.172, and 0.093, respectively. This study revealed that haplotype *1A, which has lowered activity, is quite common in Japanese, and that the frequency of *1C, another functional haplotype, was comparable to frequencies in Asians and Caucasians. In contrast, the haplotypes harboring 3972C>T but not −24C>T (*1G group), which are reportedly common in Caucasians, were minor in Japanese. Moreover, the allele 1446C>T (Thr482Thr), which has increased activity, was not detected in our Japanese population. These findings imply possible differences in MRP2-mediated drug responses between Asians and Caucasians.

Keywords: ABCC2; MRP2; genetic variation; haplotype; amino acid change

Received; October 15, 2007, Accepted; December 5, 2007

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As of October 7, 2007, the novel variations reported here are not found in the database of Japanese Single Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp/), dbSNP in the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or PharmGKB Database (http://www.pharmgkb.org/).

This study was supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation, and Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare.

Introduction

The multidrug resistance-associated protein 2 (MRP2) or canalicular multispecific organic anion transporter (cMOAT) is a 190–200 kDa transmembrane glycoprotein comprised of 1545 amino acids and belongs to the superfamily C of ATP-binding cassette (ABC) transporters. This transporter is expressed on hepatic canalicular membranes, intestinal apical membranes, luminal membranes of renal proximal tubules, placental epithelial cells, and the blood brain barrier.[1] MRP2 exports endogenous and exogenous substances, preferentially organic anions or conjugates with glucuronide, glutathione and sulfate.[1-3] This protein originally identified in cisplatin-resistant tumor cells[4] is shown to confer drug resistance to other anti-cancer drugs, such as vincristine and doxorubicin.[5,6]

MRP2 is encoded by the *ABCC2* gene located on chromosome 10q24 and consists of 32 exons (31 coding exons) and spans 69 kb. Several *ABCC2* genetic variations have been detected in patients with Dubin-Johnson syndrome (DJS), an autosomal recessive disease characterized by hyperbilirubinemia with conjugated bilirubin or increased coproporphyrin excretion in urine.[2,7] Recent studies on *ABCC2* have identified common single nucleotide polymorphisms (SNPs) such as −24C>T and −3972C>T (Ile 1324Ile) among several ethnic populations, and several studies have suggested their association with altered MRP2 expression or function.[8-17] In more recent studies on *ABCC2* haplotypes covering an extended 5′-flanking region, close linkages were found among −1549A>G in the 5′-flanking region and two common SNPs −24C>T and −3972C>T (Ile1324Ile).[8] In addition, as possible functional SNPs, −1774delG in Koreans[8] and −1019A>G in Caucasians[10] were reported. However, there is little information on detailed haplotype structures throughout the gene, and comprehensive haplotype analysis in Japanese has not yet been conducted.

We previously analyzed *ABCC2* genetic variations within all 32 exons and the proximal 5′-flanking region (approximately 800 bp upstream of the translation initiation site) using established cell lines derived from Japanese cancer patients to obtain preliminary information on *ABCC2* SNPs in Japanese.[18] In this study, to reveal *ABCC2* haplotype structures in Japanese, we resequenced the *ABCC2* gene including the distal 5′-upstream region (approximately 1.9 kb upstream from the translation initiation site) as well as all 32 exons in 236 Japanese subjects and conducted haplotype analysis using the detected genetic polymorphisms.

Materials and Methods

Human DNA samples: Genomic DNA samples were obtained from blood leukocytes of 177 Japanese cancer patients at two National Cancer Center Hospitals (Tokyo and Chiba, Japan) and Epstein-Barr virus-transformed lymphoblastoid cells prepared from 59 healthy Japanese volunteers at the Tokyo Women's Medical University under the auspices of the Pharma SNP consortium (Tokyo, Japan). Written informed consent was obtained from all subjects. Ethical review boards of all participating organizations approved this study.

PCR conditions for DNA sequencing: We sequenced all 32 exons of the *ABCC2* gene and approximately 800 bp upstream of the translation initiation codon (proximal 5′-flanking region) as described previously and also extended the sequenced region to 1.9 kb upstream of the translation initiation site (distal 5′-flanking region). Briefly, for amplification of the proximal 5′-flanking region and 32 exons, 5 sets of multiplex PCR were performed from 200 ng of genomic DNA using 1.25 units of Z-taq (Takara Bio. Inc., Shiga, Japan) with 0.3 uM each of the mixed primers as shown in **Table 1** [1st PCR]. The first PCR conditions consisted of 30 cycles of 98°C for 5 sec, 55°C for 5 sec, and 72 °C for 190 sec. Next, each exon was amplified separately using the 1st PCR product by Ex-Taq (0.625 units, Takara Bio. Inc.) with appropriate primers (0.3 uM) (**Table 1**) [2nd PCR]. The conditions for the second round PCR were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min. For amplification of the distal 5′-flanking region, multiplex PCR was performed from 25 ng of genomic DNA using 1 unit of Ex-Taq (Takara Bio. Inc.) with 0.4 uM each of the 2 sets of primers as shown in **Table 1** [PCR]. The PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min, and 72°C for 2 min, and then a final extension at 72°C for 7 min.

Following the PCR, products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) with the sequencing primers listed in **Table 1** (Sequencing). Excess dye was removed by a DyeEx-96 kit (Qiagen, Hilden, Germany), and the eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). All variations were confirmed by sequencing PCR products generated from new amplifications from genomic DNA. Genbank NT_030059.12 was used as the reference sequence.

Linkage disequilibrium (LD) and haplotype analyses: Hardy-Weinberg equilibrium and LD analyses were performed using SNPAlyze 3.1 software (Dynacom Co., Yokohama, Japan). Pairwise LDs were shown as rho square (r^2) and |D′| values in **Figure 1**. Diplotype configurations (haplotype combinations) were inferred by LDSUPPORT software, which determined the posterior probability distribution of diplotype configurations for each subject based on estimated haplotype frequencies[19].

Results and Discussion

In this study, sixty-one *ABCC2* genetic variations including 36 novel ones were detected in 236 Japanese subjects