

Fig. 2 Follow-up findings in patients with side-branch intraductal papillary mucinous neoplasm (SB-IPMNs).

invasive IPM carcinoma ($n = 1$), and an unresectable invasive IPM carcinoma ($n = 1$). The only unresectable carcinoma occurred in a patient 7 years after the initial diagnosis of IPMN. This patient was not regularly examined by EUS. The 5-year and 10-year actuarial rates of pancreatic cancer development were 2.4% and 20.0%, respectively (● Fig. 3).

● **Table 2** shows the characteristics of patients who developed pancreatic cancers.

Incidence of extrapancreatic cancers

Overall, 24 cancers of other organs occurred concurrently or subsequently in 18 patients (17.5%). These included five gastric, five colon, three prostate, two breast, two thyroid, two laryngeal, one bladder, one lung, one uterine, and one renal cell carcinoma, and one malignant lymphoma.

Predictors of IPM carcinoma

For evaluating the predictive factors for development of IPM carcinoma, we excluded two patients with ductal adenocarcinoma unrelated to SB-IPMNs, one patient who rejected surgery, and one patient who could not undergo operation because of respiratory failure.

There was no difference in the baseline parameters of gender, age, symptoms, initial MPD diameter, and initial cyst and mural nodule size between patients who developed IPM carcinoma during follow-up and those who did not. However, at the initial diag-

nosis, the frequency of extrapancreatic cancers was significantly higher in the group who went on to develop IPM carcinoma (● **Table 3**).

At the latest diagnosis, cyst size, MPD diameter, and mural nodule diameter were significantly larger in the patient group who had developed IPM carcinoma than in the group who had not, but there was no difference in the frequency of symptoms and the age of patients between the two groups. The frequency of extrapancreatic cancers was also significantly higher in the patients who had developed IPM carcinoma than in those who had not (● **Table 4**).

Discussion

▼ The International Association of Pancreatology (IAP) guidelines suggest that management of patients with SB-IPMN should be either follow-up or operation, depending upon the MPD diameter, and the cyst size and mural nodule size.

Although, according to the abovementioned guidelines, cysts of diameter ≥ 30 mm should be surgically resected [9], in practice the cutoff size for operating on cysts remains controversial. Schmidt et al. [20] operated on 103 patients with SB-IPMN and found that there was no difference in the size of cysts between the different pathological types of adenoma, borderline, carcinoma in situ (CIS), and invasive carcinoma. Salvia et al. [21] followed

Table 2 Clinical features of patients with side-branch intra-ductal papillary mucinous neoplasm (SB-IPMN) who developed pancreatic cancers during follow-up.

Patient no	Age in years at latest diagnosis, gender	Cyst size, mm		Main pancreatic duct size, mm		Mural nodule size, mm		Follow-up, months	Malignancy type	Diagnostic method	JPS stage	Resectability	Treatment
		At initial diagnosis	At latest diagnosis	At initial diagnosis	At latest diagnosis	At initial diagnosis	At latest diagnosis						
1	75, M	6	5	3	9.3*	0	0	44	Ductal adenocarcinoma	EUS	III	Resectable	Resection
2	71, M	20	35	4	8*	0	0	24	Ductal adenocarcinoma	EUS	II	Resectable	Resection
3	73, M	35	50	2	5	0	8	101	IPM carcinoma (non-invasive)	EUS	0	Resectable	Resection
4	68, F	18	20	3	5	0	5†	67	IPM carcinoma (non-invasive)	EUS	0	Resectable	Resection
5	71, M	15	30	4	3.3	0	11.8	109	Minimally invasive IPM carcinoma	EUS	I	Resectable	Resection
6	80, F	24	43.5	3	7	3	15	85	Invasive IPM carcinoma	EUS	IVa	Unresectable	Best supportive care

IPM, intra-ductal papillary mucinous; EUS, endoscopic ultrasonography; JPS, Japan Pancreatic Society

* Upstream dilatation

† New appearance of mural nodules in main pancreatic duct

Table 3 Comparisons at initial diagnosis in 99* patients with side-branch intraductal papillary mucinous neoplasm (SB-IPMN) who did or did not go on to develop IPM carcinoma.

Parameter	Patients with IPM carcinoma n = 4	Patients without IPM carcinoma n = 95†	P value
Age, median (range), years	64 (62–73)	62 (38–84)	0.3882
Gender (male : female)	2:2	52:43	> 0.9999
Symptoms, n patients	0	9	> 0.9999
Cyst size, median (range), mm	21.0 (15.0–35.0)	18.0 (5.0–50.0)	0.3101
Main pancreatic duct size, median (range), mm	3.0 (2.0–4.0)	3.0 (1.0–7.0)	0.6395
Mural nodule size, median (range), mm	0 (0–3.0)	0 (0–5.0)	0.6949
Extrapancreatic malignancies, n	4	11	0.0004

IPM, intraductal papillary mucinous

* 103 patients at initial diagnosis: excludes two patients with ductal adenocarcinomas unrelated to SB-IPMN, one patient who rejected surgery, and one patient who could not undergo operation because of respiratory failure.

† Includes five patients with adenomas and one patient with borderline lesion

Table 4 Comparisons at latest diagnosis in 99* patients with side-branch intraductal papillary mucinous neoplasm (SB-IPMN) who had or had not developed IPM carcinoma during follow-up.

Parameter	Patients with IPM carcinoma n = 4	Patients without IPM carcinoma n = 95†	P value
Age, median (range), years	72 (68–80)	68 (48–87)	0.1822
Symptoms, n patients	2	11	> 0.0825
Cyst size, median (range), mm	36.8 (20.0–50.0)	19.0 (4.0–49.0)	0.0193
Main pancreatic duct size, median (range), mm	5.0 (3.3–7.0)	3.0 (1.0–6.3)	0.0056
Mural nodule size, median (range), mm	9.9 (5.0–15.0)	0 (0–24.0)	< 0.0001
Extrapancreatic malignancies, n	4	13	0.0006

IPM, intraductal papillary mucinous

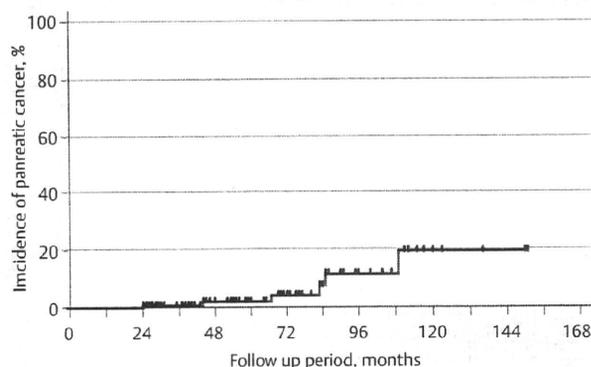
* 103 patients at initial diagnosis: excludes two patients with ductal adenocarcinomas unrelated to SB-IPMN, one patient who rejected surgery, and one patient who could not undergo operation because of respiratory failure.

† Includes five patients with adenomas and one patient with borderline lesion

89 patients with SB-IPMN without malignant signs. Only five received surgery because of enlarging cystic lesions, and none of them had malignancies. Tanno et al. [22] reported that among 82 patients with SB-IPMN unaccompanied by mural nodules who were followed up, only one developed CIS. Seven of 10 patients with cystic lesions of ≥ 30 mm had no changes; the remaining three patients were diagnosed with two adenomas and one borderline lesion after resection. Therefore these reports suggest that cyst size is not a reliable predictor of malignancy. On the other hand, when Sadakari et al. [23] operated on 73 SB-IPMNs without mural nodules they found six malignant IPMNs; five out of six malignant IPMNs were noninvasive IPM carcinomas. They also reported that there was no significant difference in cyst size (< 30 mm or ≥ 30 mm) between benign and malignant IPMNs, although MPD diameter and cytological findings from pancreatic juice showed significant differences. Although in our study, at the time of the latest diagnosis the cyst size, MPD diameter, and mural nodules were significantly larger in those who developed IPM carcinomas than in those who did not, all the IPM carcinomas had mural nodules and/or dilated MPD. In the four cases where SB-IPMNs without mural nodules or dilated MPD were resected because of acute pancreatitis and synchronous carcinoid of papilla, the pathological findings were three adenomas and one borderline IPMN; in two of these four cases the cyst diameter was ≥ 30 mm at the last diagnosis. Therefore, the treatment strategy for SB-IPMN should not be based on cyst size alone, but should also recognize the importance of mural nodules and MPD diameter.

Currently many investigators regard the size of mural nodules and even simply their presence, as a predictor of malignancy in

IPMN [9, 24]. However, no consensus has been reached about an appropriate cutoff value for the size of mural nodules. A cutoff size for malignancy of 5.4 mm [25] or 10 mm [26] has been suggested. In the present study, mural nodules ≥ 10 mm, and/or rapid increase in size of mural nodules (≥ 5 mm than at the previous imaging), and/or the new appearance of mural nodules in MPD (which indicates progression to the mixed type in the consensus guideline [9]) were taken as indicative of a higher likelihood of malignancy. Based on this strategy, all the cases of IPM carcinoma where there had been regular EUS were resectable. Several investigators have reported that EUS is the most effective

**Fig. 3** Incidence of pancreatic cancer development during follow-up of patients with side-branch intraductal papillary mucinous neoplasm (SB-IPMN), analyzed using the Kaplan-Meier method.

imaging modality for distinguishing benign IPMN from malignant tumors with assessment of protruding lesions (identical to mural nodules) [25,27]. We found unresectable invasive IPM carcinoma in only one patient who was annually assessed by plain CT for 7 years after initial EUS, and this reinforces the importance of regular EUS during follow-up.

Extrapancratic malignancies have been reported in 23.6% to 35% of IPMN patients, and have included gastric, colorectal, biliary, esophageal and lung cancers [28–30]. In the present study, 17.5% of our followed-up patients developed extrapancreatic malignancies, and the frequency of extrapancreatic cancer was significantly higher in patients with IPM carcinomas compared with patients without pancreatic cancers, at both the initial and the latest diagnosis. Hence patients with SB-IPMN should be scheduled for close follow-up examination of susceptible extrapancreatic sites as well as of the pancreas.

A few reports have described a high incidence of development of ductal carcinoma of the pancreas with concomitant SB-IPMN [14–16]. Tada et al. described the development of four pancreatic cancers (5%) in 80 SB-IPMN patients, including two ductal carcinomas (2.5%) concomitant with SB-IPMN. The cysts in these patients were 10 mm and 11 mm (i.e., both < 30 mm), respectively [14]. Uehara et al reported five ductal carcinomas of the pancreas distinct from SB-IPMN in 60 patients during follow-up; two of these patients had no significant change in cyst size (or four patients according to our definition of ≥ 10 mm enlargement) [15]. Tanno et al. reported four ductal carcinomas during follow up in 89 patients with SB-IPMNs, and in none of these cases were there symptoms, mural nodules, or a cyst size larger than 30 mm [16]. All ductal carcinomas of the pancreas distinct from IPMN in these reports occurred in patients with SB-IPMN. Such carcinomas sometimes developed in patients with SB-IPMN without established indications for resection as advocated in the international consensus guidelines of 2006, with relatively small and static cyst size. We found two ductal carcinomas of the pancreas with concomitant SB-IPMN; one of these patients had a cyst of 6 mm that did not change during follow-up. Although we detected ductal carcinomas at a resectable stage, other studies report unresectable or advanced stage ductal carcinomas in follow-up cases [15,16]. These observations stress the importance of modifying our current follow-up strategies for SB-IPMN in view of the additional risk of ductal carcinoma, and the importance of scanning not only the cystic lesions, but also the entire pancreas in these patients.

In the present study, the 5-year and 10-year rates for pancreatic cancer development were 2.4% and 20.0%, respectively, and the reason for the high 10-year rate might be that we included IPM carcinomas, which grow slowly, and often develop after a long follow-up period. In fact, IPM carcinomas developed in our patients after a median follow-up of 93 months (range 67–109). Furthermore, the studies described above [14–16] and our study together found that 13 ductal carcinomas of the pancreas concomitant with SB-IPMN developed after a median follow-up duration of 44 months (14–112 months). Hence patients should be regularly assessed by imaging over the long term, even if SB-IPMN does not change for 2 years, whereas the international consensus guidelines of 2006 state that the follow-up interval can be lengthened after 2 years with no change [9].

We need to bear in mind that our current concepts about the natural history of SB-IPMN are derived mainly from retrospective series that have included patients who were followed up after surgery [31]. Only a few studies have prospectively followed up

patients with SB-IPMN who were conservatively managed [21,22]. The patient cohorts included in these natural history studies, including ours, have small numbers of patients, and most studies suffer from selection bias. In addition, many of the patients at our center were referred from other hospitals; this also might cause selection bias. In this study, 31 patients were followed up at other hospitals, 36 dropped out, and in 29 the final status was unknown because there was no reply to the questionnaire. Hence, we do not know the final outcome of the SB-IPMN for 96 patients. However, according to the questionnaire responses, amongst those followed up at other hospitals and those who eventually dropped out, there were four pancreatic cancers (ductal or IPM carcinomas) in 67 patients (6.0%), so the rate of pancreatic cancers in our follow-up cases was nearly equal to that in these two groups. This seems to indicate the patients followed up at our hospital did not have a higher risk than the patients followed up in other hospitals or those who dropped out. Although our study did not include cyst size as an indication for resection, the European study of Salvia et al. did. This could cause different results, but in the European study, only five of 89 followed-up patients had surgery because of enlarging cystic lesions, and none of them had malignancies; our study also found no pancreatic malignancies in two patients with cysts ≥ 30 mm who had surgery because of acute pancreatitis. Therefore, the cyst size alone is not a reliable predictor of malignancy. As with other follow-up studies [14–16,22], we cannot state with absolute certainty that none of the followed-up patients had CIS, because imaging modalities cannot detect a CIS lesion itself. But the surgical case studies mentioned above [20,23] seem to indicate little possibility of pancreatic malignancy in the patients with no progression and lower likelihood of malignancy in our study.

In conclusion, the results of our long-term observations of conservatively managed SB-IPMN patients show that the incidence of ductal carcinoma of the pancreas and of IPM carcinoma is high. Although conservative management is appropriate for selected patients, regular and continued clinical and imaging follow-up, especially by EUS, is essential, even if an SB-IPMN does not change for 2 years. Mural nodules and dilatation of the MPD seem to be more appropriate than cyst size regarding the indication for resection of these neoplasms. SB-IPMNs should be resected in any of the following circumstances: mural nodule(s) and/or MPD ≥ 10 mm in size; rapid increase in size of mural nodule(s) and/or MPD (≥ 5 mm increase since the previous imaging); new appearance of mural nodule(s) in MPD; appearance of pancreatic mass; occurrence of severe symptoms (e.g. acute pancreatitis); or positive cytological findings from pancreatic juice.

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Competing interests: None

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Endoscopic ultrasound-guided fine-needle aspiration for the diagnosis of retroperitoneal schwannoma

Schwannoma is a rare peripheral nerve sheath tumor that is difficult to diagnose by imaging features alone. Endoscopic ultrasound (EUS)-guided fine-needle aspiration (FNA) with specific immunohistochemical (IHC) staining may be the only tool to obtain a diagnostic sample from such lesions. There are only a few case reports describing EUS-FNA diagnosis of retroperitoneal Schwannoma [1,2]. In this report, we describe four cases (three males; mean age: 54.5 ± 16.4 years) with retroperitoneal Schwannoma, in whom the diagnosis was achieved with EUS-FNA and adjunctive IHC staining. All lesions were well demarcated, and had a rounded contour (● Fig. 1 a).

The mean size of the lesions on EUS was 23.7 ± 3.6 mm. EUS-FNA was successfully performed with a 22-gauge needle in all cases (● Fig. 1 b), with a sufficient yield for both cytological and cellblock analysis. The median number of needle passes was 2.5 (range 2–3). The cellblock analysis revealed bland proliferation of spindle cells with a palisading appearance and wavy fibrillar architecture (● Fig. 1 c). Further evaluation with IHC revealed negative staining for actin, CD34, CD-117, and strong positive staining for S-100 antibody in all cases (● Fig. 1 d). Further evaluation of the cellular proliferative activity was studied with Ki-67 staining, and a low proliferation rate (Ki-67 < 5%) was reported in all cases, supporting the benign nature of the lesions.

We recommended conservative follow-up for our patients rather than surgical resection, because all of the patients were asymptomatic and there were no mitotic figures on FNA, with a low Ki-67 index in all the aspirates. It is worth noting that most reports have stressed on complete surgical resection as the appropriate management of retroperitoneal schwannomas [3,4]. Our view is that the morbidity associated with surgical resection is not justified in these benign lesions, and the use of EUS-FNA to establish the diagnosis may help in avoiding unnecessary surgery.

Competing interests: None

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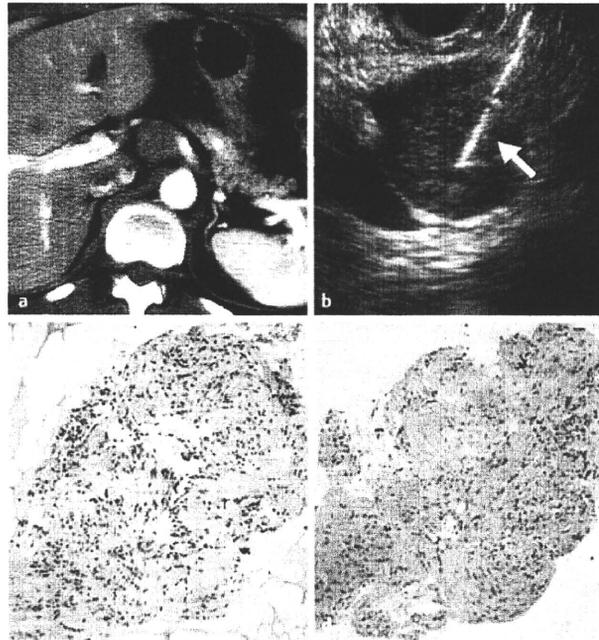


Fig. 1 Imaging findings and histopathological features. **a** Contrast enhanced computed tomography (CT) scan showing well-demarcated low density mass in the retroperitoneal region. **b** Endoscopic ultrasound image showing a fine needle inserted into the mass (arrow). **c** Spindle cells on cellblock sections, without any mitosis (hematoxylin and eosin stain, original magnification $\times 400$). **d** Immunohistochemical S-100 positive staining (magnification $\times 400$).

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Clinical Efficacy and Safety of Sunitinib After Imatinib Failure in Japanese Patients with Gastrointestinal Stromal Tumor

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Background: Imatinib used to be the only effective treatment for advanced gastrointestinal stromal tumor. However, early clinical reports have shown that sunitinib has substantial anti-cancer activity in patients with advanced gastrointestinal stromal tumor after failure of imatinib.

Methods: Eighteen Japanese patients with advanced gastrointestinal stromal tumor who were resistant or intolerant to previous treatment with imatinib were entered into this study. These patients were given sunitinib orally, once daily at a 50-mg starting dose, in 6-week cycles with 4 weeks on and 2 weeks off treatment. Tumor response and drug safety were then evaluated.

Results: Median time-to-treatment failure was 207 days. Overall, 5.6% (1/18) of patients achieved partial response, 38.9% (7/18) had stable disease and 44.4% (8/18) had progressive disease. The common adverse events were hand-foot syndrome, liver dysfunction, fatigue, anorexia and hypertension. Mild anemia, leukocytopenia and neutropenia were also noted. Nine patients required dose reduction or cessation because of adverse events.

Conclusions: This study demonstrates that sunitinib may be an effective agent for advanced gastrointestinal stromal tumor after failure of imatinib in clinical practice.

Key words: gastrointestinal stromal tumor (GIST) – sunitinib – efficacy and safety

INTRODUCTION

Gastrointestinal stromal tumor (GIST) is a form of sarcoma and the most common mesenchymal tumor of the gastrointestinal tract, distinguishable from other soft tissue neoplasms by immunohistochemistry (1). The tumor probably arises from mutations in precursor cells that normally give rise to the interstitial cells of Cajal. Like these cells, most GISTs express the protein product of the *KIT* proto-oncogene, a transmembrane receptor tyrosine kinase, whose activity would normally be regulated by binding of its ligand. Approximately 85–90% of GISTs demonstrate gain-of-function *KIT* gene mutations that lead to constitutive activation of *KIT* kinase (2–4). A much smaller proportion (5%) demonstrates analogous gain-of-function mutations in *PDGFRA*, the gene encoding platelet-derived growth factor receptor α (PDGFR α); less than 10% contain no identified receptor tyrosine kinase mutations (2–4). Activating

mutations of *KIT* and *PDGFRA* have been defined as the driving force behind development and maintenance of the malignant phenotype in most cases of GIST. Understanding the molecular pathophysiology of this condition has allowed rational development of agents that target these signaling aberrations in the cancer cell. Imatinib mesylate, a selective inhibitor of the kinase activities of *KIT* and *PDGFRA*, has substantially improved clinical outcomes for patients with advanced disease (5–7). However, in a pivotal study of imatinib in advanced GIST, 5% of patients showed primary resistance to imatinib and another 14% developed early resistance (8). Secondary or acquired resistance develops after a median of about 2 years of treatment with imatinib (9). Such resistance can develop through various mechanisms, the most common being secondary *KIT* mutations in clonally expanded cancer cells (5,10,11). Effective alternative treatments for use after failure of imatinib therapy became an important unmet medical need, justifying the

development of alternative agents. Sunitinib malate is an oral multitargeted receptor tyrosine kinase inhibitor, which has shown antiangiogenic and antitumor activities in several *in vitro* and *in vivo* tumor models (6,7,12–15). Sunitinib inhibits the VEGFR kinases, which are important in tumor-related angiogenesis, a property not shared by imatinib. Results from a phase III study (16) showed that sunitinib had promising clinical activity in patients with imatinib-resistant disease. Although Shirao *et al.* (17) also reported the efficacy and safety of sunitinib in Japanese patients, there are no Japanese data about the clinical potential of sunitinib in clinical practice. The objectives of this retrospective study were to assess the efficacy and safety of sunitinib in Japanese patients with advanced GIST after failure of imatinib.

PATIENTS AND METHODS

PATIENTS

Eighteen Japanese patients with advanced GIST who were resistant or intolerant to previous treatment with imatinib were recruited to this retrospective study. Inclusion criteria were: pathological evidence of GIST; unidimensionally measurable with computed tomography (CT) or magnetic resonance imaging (MRI); failure of treatment with imatinib, based either on progression of disease according to Response Evaluation Criteria in Solid Tumors [RECIST1.0] (18) or on unacceptably severe toxic effects that precluded further treatment; imatinib last administered at least 2 weeks before starting sunitinib; resolution of all toxic effects of imatinib or other therapy to Grade 1 or less; adequate hepatic, renal and cardiac function; absolute neutrophil count of at least 1500 per microliter; platelet count of at least 100,000 per microliter; and an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. The study was approved by the Ethics and Scientific Committee of our institution.

CRITERIA OF EFFICACY AND SAFETY

Tumor responses were assessed by CT or MRI. We used RECIST (19) to determine the best overall response. For patients with multiple metastases, the five largest lesions were followed and measured for response evaluations. Radiographic responses were confirmed by an independent radiologist. Best response was defined as the most complete response achieved by a patient (thus, each patient had a single best response: complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD) and the date of best response was the date that response was first detected. The rate of clinical benefit was defined as the proportion of patients who achieved a confirmed objective response (CR or PR) or who had stabilization or no change lasting for at least 24 weeks. These follow-up evaluations were used to determine the duration of response and the date that disease progression was first detected. Patients

were assessed for toxicity using the National Cancer Institute Common Toxicity Criteria (NCI-CTC2.0) (20). According to NCI-CTC2.0, each toxicity incident was categorized on a scale of 1–5, with 5 being the most severe.

SUNITINIB TREATMENT

Patients received sunitinib daily for 4 consecutive weeks, followed by a 2-week period without treatment, comprising a 6-week cycle. Sunitinib was given at a starting dose of 50 mg daily; it was given orally in the morning with water and without regard to meals, beginning on day 1 of sunitinib. Treatment was continued until the disease progressed, unacceptable toxic effects occurred, or the patient refused to continue. Dose reductions of sunitinib were required in the case of clinically relevant Grade 3 or 4 toxic effects (to 37.5 mg per day and, if additional reduction was warranted, to 25 mg per day), provided that criteria for withdrawal from sunitinib were not met. Dose reduction was also performed if patients were determined to be at the risk of Grade 3 toxic effects. If cardiac toxicity classified as Grade 2 or 3 occurred, we reduced sunitinib to 12.5 mg (1 capsule) per day. However, when patient suffered from symptomatic toxicities of Grade 2 or less, we stopped sunitinib and reduced the dose to 12.5 mg (1 capsule) per day or administration duration of sunitinib like 3 weeks on and 2 weeks off schedule on the next cycle. Patients had regular physical examinations and evaluations of performance status, body weight, complete blood count and serum chemistry. Thyroid function, chest X-ray, electrocardiography and echocardiography were essentially performed every two courses. The administration of each dose and any adverse events were recorded in a diary for each patient.

EVALUATION

The primary endpoint was time-to-treatment failure (TTF). Secondary endpoints included overall confirmed objective response rate as defined using RECIST. Safety and tolerability were assessed by analysis of adverse events, physical examinations, vital signs, electrocardiography, ECOG performance status and laboratory assessments (such as complete blood count with differential count and serum electrolyte measurements). Cardiac function was assessed using electrocardiograms and echocardiography. Severity of adverse events was rated by each investigator using the NCI-CTC.

STATISTICAL ANALYSIS

TTF was defined as the interval between the commencement of and cessation of sunitinib using the Kaplan–Meier method. Statistical analysis was performed using SPSS version 12 (SPSS, Chicago, IL, USA) statistical software.

RESULTS

PATIENT CHARACTERISTICS

From June 2008 to December 2009, 18 patients with malignant GIST were enrolled into the study. In all patients, GIST was diagnosed on the basis of immunohistochemical reactivity to KIT. Patients' baseline demographic and clinical characteristics are summarized in Table 1. Sixteen (88.9%) patients were resistant and two (11.1%) were intolerant to previous treatment with imatinib. The median patient age was 58.7 years (range, 26–77 years). All patients had a performance status of 0–1, and the most common primary tumor site was the small intestine, including the duodenum. Median total dose of administered sunitinib was 3806 mg and the median number of treatment cycles was 3.5. Median dose intensity of sunitinib was 71.3%. All patients were assessed for response to sunitinib treatment in terms of toxicity and efficacy.

RESPONSE TO TREATMENT

Data on the antitumor response to sunitinib are shown in Table 2. No patients had a CR, PR was observed in 1 patient (5.6%) and SD occurred in 7 patients (38.9%). The patient with PR had a small intestinal GIST of spindle-cell type with liver and peritoneal metastases, and had initially responded to imatinib with PR as assessed by RECIST. After failure with imatinib, this patient was changed to sunitinib. He has had a good response to sunitinib for 15 months (Fig. 1).

Table 1. Patient characteristics (*n* = 18)

Median age (range)	58.7 (26–77) years
Gender	
Male	13
Female	5
ECOG performance status	
0	13
1	5
Primary tumor site	
Stomach	3
Small intestine	11
Colon	1
Others	3
Median duration of imatinib (range)	39 (1–65) months
Reason of imatinib cessation	
Resistance	16
Intolerance	2
Median administered dose of sunitinib (range)	3806 (100–15 400) mg
Median treatment cycle (range)	3.5 (1–14) cycle
Median dose intensity of sunitinib	71.3 (7.1–100)%

Table 2. Tumor response (*n* = 18)

	<i>n</i> (%)
Complete response	0
Partial response	1 (5.6)
Stable disease	7 (38.9)
Progressive disease	8 (44.4)
Not evaluable	2 (11.2)

Values are represented by *n* (%).

Eight patients (44.4%) had PD; these patients showed rapid progression of the disease within 1 month after entry into the study. Two patients (11.1%) were intolerant of sunitinib. After a median follow-up period of 8.0 months (range, 1.8–11.1 months), 15 patients were still alive; the overall median survival time has not yet been reached. Median TTF was 207 days (Fig. 2). Patients who were given a total sunitinib dose \geq 4000 mg showed a longer TTF (243.8 days) than those receiving a smaller total dose (90.0 days).

ADVERSE EVENTS

The main adverse effects were hand-foot syndrome, liver dysfunction, fatigue, anorexia and hypertension (Table 3). Hand-foot syndrome was observed in 16 patients, liver dysfunction in 16, fatigue in 12, anorexia in 9 and hypertension in 8. Mild anemia, leukocytopenia and neutropenia were also noted (Table 4). Nine patients required dose reduction or drug cessation because of adverse events. Hyperammonemia was observed in one patient, who was given seven courses of sunitinib.

CASE OF HYPERAMMONEMIA

The patient, who was a 56-year-old man, attended our hospital irregularly because his wife noted the developed somnolence during seven courses of sunitinib treatment. He presented with a flapping tremor and smell of ammonia. He had no past history of liver disorder or viral hepatitis. Investigations revealed an increased serum level of ammonia, which was irreversible after 2 months' discontinuation of sunitinib. Celiac and superior mesenteric artery angiography was performed to examine the cause of hyperammonemia; CT with arterial portography showed a portovenous shunt (Fig. 3).

DISCUSSION

Based on TTF and tumor response, sunitinib demonstrated similar efficacy to that previously reported in a phase III trial involving predominantly western patients with failure of imatinib (16) and in a Japanese phase I/II trial (17). The present TTF was 207 days (29.6 weeks), while time to

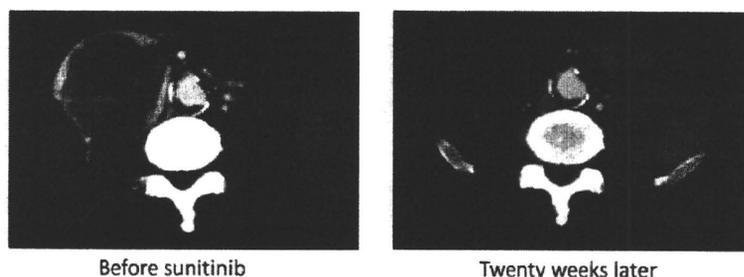


Figure 1. Partial response case. Well-enhanced peritoneal metastases shrank to resemble cyst-like masses.

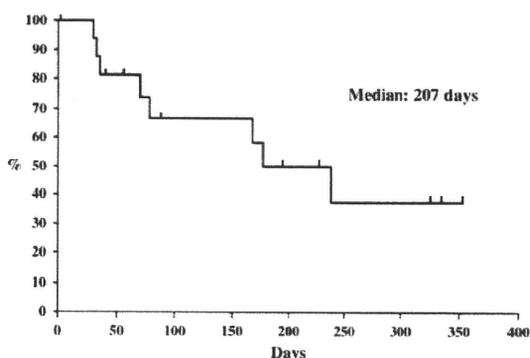


Figure 2. Kaplan–Meier estimates of time-to-treatment failure.

Table 3. Adverse events that occurred with a frequency at least 5% greater (non-hematological)

	Grade 1/2	Grade 3	Grade 4
Nausea vomiting	6 (33.3)	0 (0)	0 (0)
Diarrhea	5 (27.8)	0 (0)	0 (0)
Anorexia	9 (50.0)	0 (0)	0 (0)
Fatigue	10 (55.6)	2 (11.1)	0 (0)
Rash	3 (16.7)	0 (0)	0 (0)
Hypertension	8 (44.4)	0 (0)	0 (0)
Stomatitis	7 (38.9)	0 (0)	0 (0)
Liver dysfunction	13 (72.2)	3 (16.7)	1 (5.6)
Hand-foot syndrome	16 (88.9)	0 (0)	0 (0)
Bleeding	2 (11.1)	1 (5.6)	0 (0)
Hypothyroidism	4 (22.2)	0 (0)	0 (0)
QT delay syndrome	1 (5.6%)	0 (0)	0 (0)

Values are represented by *n* (%).

progression (TTP) in the above-mentioned phase III and phase I/II trials was 27.3 and 27.9 weeks, respectively. The present study was retrospective, and the date of disease progression could not be determined because of the lack of radiological examinations. We were not able to compare TTF with TTP directly, but these durations are considered to

Table 4. Adverse events that occurred with a frequency at least 5% greater (hematological)

	Grade 1/2	Grade 3	Grade 4
Leucopenia	11 (61.1)	1 (5.6)	0 (0)
Neutropenia	10 (55.6)	2 (11.1)	0 (0)
Anemia	10 (55.6)	2 (11.1)	0 (0)
Thrombocytopenia	9 (50.0)	4 (22.2)	0 (0)

Values are represented by *n* (%).

show a similar tendency. In the current study, 5.6% of patients showed objective response (CR + PR) and 44.5% demonstrated clinical benefit response (CR + PR + SD). On the other hand, objective response and clinical benefit response of the phase III study were 7 and 24%, and those of the Japanese phase I/II study were 13 and 40%, respectively. The benefit of sunitinib in clinical practice is very important because sunitinib is the only drug approved for second-line treatment of imatinib-resistant/intolerant GIST.

Toxicities of sunitinib, which were predominantly mild to moderate, were manageable and reversible. Fatigue was the most common adverse event in the phase III trial, but hand-foot syndrome was the most common adverse event in the present study, as in the Japanese phase I/II trial. The difference in the incidences of toxicities may be caused by racial differences, as well as by the small number of patients and insufficient follow-up period in the present study. Long-term follow-up of these patients will be important to fully define the tolerability of multitargeted kinase inhibition. In the Japanese phase I/II trial, the incidence of severe adverse events was 25%. In the present study, although two severe adverse events (11.1%) associated with sunitinib led to treatment discontinuation, these were reversible through dose interruption and standard supportive medical treatment.

Hand-foot syndrome was generally observed 2 weeks after the start of sunitinib. If this syndrome occurs within 1 week and is categorized as Grade 2, we suspend sunitinib therapy. The next course is then started at a reduced dose (12.5 mg; a one-capsule reduction). Although this reduces the dose intensity of sunitinib, it allows a long duration of therapy. In fact,

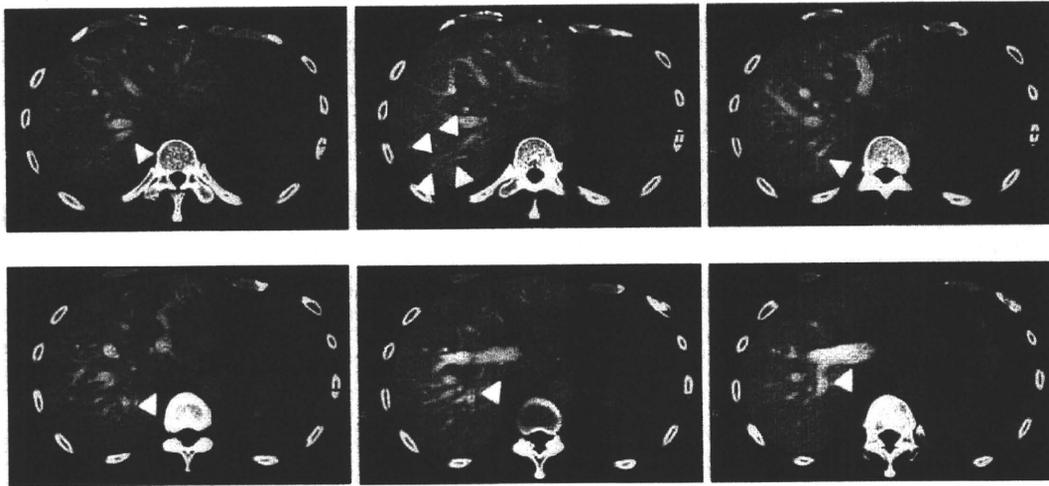


Figure 3. Computed tomography with arterial portography. Six consecutive images of computed tomography show the well-enhanced hepatic vein and clearly reveal the connection between the portal vein (P7) and right hepatic vein (see arrow heads on images).

median dose intensity of sunitinib was 71.3% and TTF was long at 29.6 weeks, similar to the TTP of the Japanese phase I/II study. Long-term administration should be the first priority of sunitinib treatment. However, in the present study, the follow-up period and number of patients given sunitinib were inadequate to provide direct evidence that it can prolong survival. Hence, we consider that the most important purpose of sunitinib treatment is to maintain previous activities of daily living. Maximum efficacy of antitumor therapy relates to sufficient dose and time. Sunitinib is a well-tolerated medication but has slightly greater toxicity than first-line imatinib. Therefore, we emphasized medication duration rather than dose of sunitinib, but despite this strategy, dose intensity was adequate (median relative dose intensity: 71.3%) and TTF was satisfactory at 29.6 weeks.

It is very important to manage symptomatic as well as non-symptomatic toxicities. Although the incidence of hyperammonemia is reported to be very low, we encountered one case. This adverse event may in part be caused by a vascular disorder related to the antiangiogenic properties of sunitinib. Sunitinib is multitargeted agent and might cause different toxicities from conventional cytotoxic agents. Although common toxicities have been reported by the global phase III trial and Japanese phase I/II trial, rare toxicities are not well demonstrated in such clinical trials and further data collection is therefore essential for the appropriate management of sunitinib. We intend to focus on the best management of toxicities so that long-term exposure and high dose intensity of sunitinib are acquired. Further investigations should be considered to maximize the benefits of sunitinib in patients with GIST.

Conflict of interest statement

None declared.

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RESEARCH COMMUNICATION

Effects of Genetic Polymorphisms in the ABCB1 Gene on Clinical Outcomes in Patients with Gastric Cancer Treated by Second-line Chemotherapy

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Abstract

Objective: Tumor cells that overexpress P-glycoprotein (Pgp) may be resistant to several anticancer agents due to altered pharmacokinetics and reduced intracellular concentrations of the anticancer agents. Pgp is encoded by the ATP binding cassette gene B1 (ABCB1). To our knowledge, only one previous report has evaluated the effect of ABCB1 gene polymorphisms on clinical outcomes of gastric cancer. The purpose of this analysis was to evaluate the impact of genetic polymorphisms of the ABCB1 gene on clinical outcomes in patients with advanced gastric cancer (AGC) treated with second-line chemotherapy. **Methods:** We retrospectively analyzed the impact of ABCB1 gene polymorphisms (ABCB1 3435C>T) on clinical outcomes in 100 patients with AGC who received second-line chemotherapy. **Results:** Median overall survival (OS) since the initiation of second-line chemotherapy was 6.0 months (95% confidence interval [CI], 4.8 to 8.0 months), and median progression-free survival (PFS) was 2.7 months (95% CI, 2.1 to 3.4 months). In a multivariate analysis of PFS, a 3435 CC polymorphism (n = 45) was significantly associated with longer PFS compared with the CT/TT type polymorphism (n = 55), with borderline significance (PFS of 3.2 months vs. 2.2 months, respectively; HR 1.50; 95% CI, 0.98-2.30; P = 0.061). ABCB1 3435 C>T polymorphisms were not associated with OS. No interaction was seen between ABCB1 polymorphisms and treatment regimens. **Conclusion:** Genetic polymorphisms of ABCB1 3435C>T might have a possible impact on clinical outcomes of second-line chemotherapy in AGC. Further prospective evaluation using a larger sample size is required.

Keywords: gastric cancer - ABCB1 polymorphisms - chemotherapy

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Introduction

P-glycoprotein (Pgp) is a plasma membrane protein that works as an efflux pump to transport natural products or toxins (Fromm et al., 2004). Pgp is expressed in normal cells, such as peripheral blood mononuclear cells, intestinal cells, renal cells, hepatic cells, and cells of the blood-brain barrier. Pgp protects cells and organs from toxic substrates. Pgp is comprised of two hydrophobic transmembrane domains that dimerize and form a pore. Several hydrophobic chemotherapeutic agents, such as paclitaxel, docetaxel and irinotecan, are known to be substrates of Pgp (Kolesar et al., 2009). Therefore, tumor cells that overexpress Pgp may be resistant to these anticancer agents due to altered pharmacokinetics and

reduced intracellular concentrations.

Pgp is encoded by the ATP binding cassette gene B1 (ABCB1). More than 50 single nucleotide polymorphisms (SNPs) are identified for ABCB1, and their effects on Pgp expression and substrate distribution have been evaluated (Fromm, 2002; Kolesar et al., 2009). Although several reports have investigated the clinical impact of these polymorphisms on clinical outcomes after chemotherapy in several types of cancer (Green et al., 2006; Marsh et al., 2007; Johnatty et al., 2008; Pan et al., 2008; Sissung et al., 2008; Chang et al., 2009), to our knowledge, there is only one report that analyzed small number of patients with advanced gastric cancer (AGC) treated with paclitaxel (Chang et al., 2010).

Key chemotherapeutic agents for gastric cancer

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are fluorouracil/fluoropyrimidine, platinum, taxanes (paclitaxel and docetaxel) and irinotecan (NCCN Clinical Practice Guidelines in Oncology). Among them, the combination of fluorouracil/fluoropyrimidine and platinum is commonly used as first-line chemotherapy in Japan (Koizumi et al., 2008), while taxanes or irinotecan are frequently used in the second-line setting. Since taxanes and irinotecan are known to be substrates of Pgp, we hypothesized that genetic polymorphisms in ABCB1 may have an effect on clinical outcomes in patients with gastric cancer who received second-line chemotherapy in Japan.

To address this issue, we carried out a retrospective cohort study using data from the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), Japan.

Methods

Patients

Cases were selected from the database of the HERPACC, conducted at Aichi Cancer Center Hospital (ACCH). Details of the HERPACC have been described elsewhere (Tajima et al., 2000; Hamajima et al., 2001). In brief, 23,408 HERPACC-enrolled, first-visit outpatients treated between January 2001 and November 2005 were asked to provide blood samples in addition to information on lifestyle factors. Of those who participated, 22,727 (97.1%) completed the questionnaire satisfactorily and were enrolled in the HERPACC. The study was approved by the Institutional Ethical Committee of ACCH.

In the present study, cases of patients with newly diagnosed AGC who participated in the HERPACC with the following criteria were included: (1) presence of histologically or cytologically proven, inoperable gastric cancer (2) performance status according to the Eastern Cooperative Oncology Group criteria of 0-2 (3) treatment with second-line chemotherapy after failure of first-line chemotherapy (4) available blood samples.

A total of 100 patients with AGC were included in this study. Detailed patient characteristics prior to initiation of second-line chemotherapy were acquired from the hospital's patient records.

Evaluation of genetic polymorphisms

DNA of each subject was extracted from the buffy coat fraction with the DNA Blood mini Kit (Qiagen K.K., Tokyo, Japan). Genotyping for the ABCB1 ABCB1 3435T>C (dbSNP ID: rs1045642) was based upon TaqMan Assays (Applied Biosystems, Foster City, CA). Five percent of the samples were examined in duplicate to ensure consistency.

Evaluation of treatment and statistical methods

The primary purpose of this study was to evaluate the association between genetic polymorphisms of ABCB1 and progression-free survival (PFS) or overall survival (OS). PFS associated with second-line chemotherapy was measured from the beginning of treatment to the date of disease progression, which was evaluated by each physician. PFS with first-line chemotherapy was also

measured from the beginning of treatment to the date of disease progression. OS was defined as the interval between the date of initiation of second-line chemotherapy to the date of death or last follow-up using the Kaplan-Meier method. Vital status or disease status was confirmed by checking of medical record at the last date of follow-up visit. In the case of lost to follow-up, vital status was confirmed by census registration conducted annually.

To evaluate the effect of genetic polymorphisms on PFS and OS, univariate and multivariate Cox proportional hazards modeling was applied. Therefore, a measure of association in this study was the hazard ratio (HR) along with a 95% confidence interval (95% CI). Forward and backward stepwise methods were used for model building using threshold P values of 0.10 for inclusion and 0.20 for exclusion. Distribution of subject characteristics was assessed by the chi-square test or the Fisher exact test, as appropriate. Statistical analyses were performed using STATA ver. 10 (StataCorp LP, College Station, TX, USA). All tests were 2-sided, and P values less than 0.05 were considered statistically significant.

Results

Patient characteristics and survival

Detailed characteristics of the 100 patients analyzed are shown in Table 1. All patients received fluorouracil/fluoropyrimidine with or without cisplatin as first-line chemotherapy, with a median PFS of 5.6 months (95% CI: 4.5-6.6 months). Among the 100 patients, 61 patients (61%) received taxane-based, second-line chemotherapy (paclitaxel monotherapy in 46 patients and docetaxel

Table 1. Patient Characteristics and Genetic Polymorphisms

Characteristics		All n=100(%) n=61(%)	Taxane based n=39(%)	Irinotecan based
Age	Median (range)	60 (31-80)	58 (31-80)	62(33-74)
Gender	Male	66 (66)	39(64)	27 (69)
	Female	34 (34)	22(36)	12 (31)
EOCG PS	0 or 1	65(65)	36(59)	29(74)
	2	35 (35)	25(41)	10(26)
Disease status	Advanced	70(70)	40(66)	30(76)
	Recurrent	30 (30)	21(34)	9(24)
Pathological type	Diffuse	78 (78)	51(83)	27(69)
	Intestinal	22 (22)	10(17)	12(31)
Prior gastrectomy	Yes	49 (49)	34(55)	15(38)
	No	51 (51)	27(45)	24(62)
Adjuvant	Yes	9 (9)	5(8)	4(10)
	No	91 (91)	56(92)	35(90)
Metastatic places	1	56 (56)	37(43)	19(49)
	≥2	44 (44)	24(57)	20(51)
Ascites	Yes	25 (25)	18(30)	7(18)
	No	75 (75)	43(70)	32(82)
PFS (median) of 1st-line	<5.6 months	50 (50)	30(49)	20(51)
	>5.5 months	50 (50)	31(51)	19(49)
ABCB1 C3435T	C/C	45 (45)	28(46)	17(44)
	C/T	38 (38)	24(39)	14(36)
	T/T	17 (17)	9(15)	8(20)

EOCG PS, Eastern Cooperative Oncology Group performance status; PFS, progression-free survival

Table 2. Univariate and Multivariate Analysis of PFS

Variant	Classification	n	Univariate analysis			Multivariate analysis		
			HR	95% CI	P value	HR	95% CI	P value
ABCB1 C3435T	C/C	45	1.00			1.00		
	C/T or T/T	55	1.41	0.93-2.13	0.10	1.50	0.98-2.30	0.061
ECOG PS	0 or 1	65	1.00			1.00		
	2	35	2.89	1.86-4.48	<0.001	2.95	1.86-4.67	<0.001
Metastatic place	1	56	1.00			1.00		
	2 or more	44	1.34	0.89-2.01	0.16	1.45	0.93-2.24	0.098
PFS of 1stline	<5.6 months	50	1.00			1.00		
	>5.6 months	50	0.58	0.39-0.88	<0.001	0.67	0.44-1.02	0.067

Adjusted by age, gender, disease status, pathology, prior gastrectomy, adjuvant, ascites, regimens; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; PFS, progression-free survival.

Table 3. Univariate and Multivariate Analysis of OS

Variant	Classification	n	Univariate analysis			Multivariate analysis		
			HR	95% CI	P value	HR	95% CI	P value
ABCB1 C3435T	C/C	45	1.00			1.00		
	C/T or T/T	55	1.15	0.81-1.63	0.43	0.93	0.60-1.43	0.72
ECOG PS	0 or 1	65	1.00			1.00		
	2	35	3.91	1.80-4.05	<0.001	3.94	2.34-6.05	<0.001
Metastatic place	1	56	1.00			1.00		
	2 or more	44	1.64	1.16-2.31	0.01	1.58	1.11-2.32	0.01
Pathology	Diffuse	78	1.00			1.00		
	Intestinal	22	0.63	0.41-0.96	0.035	0.61	0.37-1.03	0.063
PFS of 1stline	<5.6 months	50	1.00			1.00		
	>5.6 months	50	0.48	0.34-0.68	<0.001	0.43	0.26-0.69	<0.001

Adjusted by age, gender, disease status, prior gastrectomy, adjuvant, ascites, regimens; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status; HR, hazard ratio; OS, overall survival; PFS, progression-free survival

monotherapy in 15 patients), while the other 39 patients (39%) received irinotecan-based chemotherapy (irinotecan monotherapy in 26 patients and irinotecan plus cisplatin in 13 patients). The treatment schedules were similar to those of previous Japanese phase II trials (Futatsuki et al., 1994; Taguchi et al., 1998; Sato et al., 2001; Koderu et al., 2007). Median OS since initiation of second-line chemotherapy was 6.0 months (95% CI, 4.8 to 8.0 months), and median PFS was 2.7 months (95% CI, 2.1 to 3.3 months). Among the 100 patients, 48 patients (48%) received third-line chemotherapy. The frequencies of ABCB1 polymorphisms were as follows: C/C in 45 patients, C/T in 38 patients and T/T in 17 patients.

PFS according to genetic polymorphisms of ABCB1

Table 2 demonstrates univariate and multivariate analyses of ABCB1 polymorphisms and other clinical factors as prognostic factors for better PFS. In the multivariate analysis, CC type polymorphisms (n = 45) were associated with longer PFS than C/T or TT type polymorphisms, with borderline statistical significance (n = 55; 3.2 vs. 2.2 months; HR 1.50; 95% CI 0.98-2.30; P = 0.061, Figure 1a). Only a performance status of 0-1 was significant (P < 0.05); other predictive factors with borderline significance were PFS with first-line chemotherapy and number of metastatic sites.

OS according to genomic polymorphisms of ABCB1

Table 3 shows univariate and multivariate analyses of OS. In the multivariate analysis, no significant difference was observed between ABCB1 3435 CC type vs. C/T

or TT type polymorphisms (OS 5.6 vs. 6.1 months, respectively; HR 0.93; 95% CI 0.60-1.43; p = 0.72, Figure 1b). Significant predictive factors for OS were

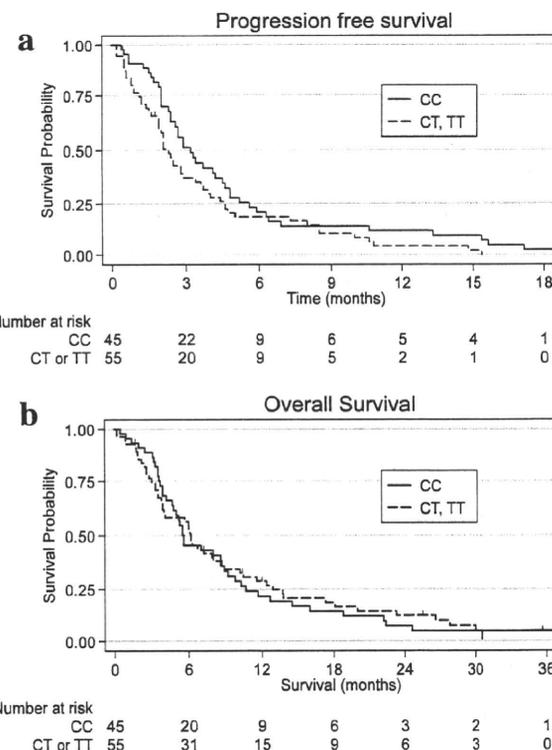


Figure 1. Kaplan-Meier Survival Curves for Progression Free (a) and Overall (b) Survival

Table 4. Association of Genotype and Survival Stratification by Regimens

	Genotype		Analysis for PFS			Analysis for OS		
	ABCB1 C3435T	n	HR	95% CI	P*	HR	95% CI	P*
Taxane based (n = 61)	C/C	28	1.00			1.00		
	C/T or T/T	33	1.56	0.91-2.69		0.83	0.47-1.48	
Irinotecan based (n = 39)	C/C	17	1.00		0.63	1.00		0.74
	C/T or T/T	22	1.02	0.43-2.22		0.60	0.22-1.68	

Adjusted by age, gender, PS, pathological type, disease status, prior gastrectomy, adjuvant, metastatic site, ascites, PFS with first-line therapy; *P for interaction; CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival

performance status, number of metastatic sites, and PFS with first-line chemotherapy.

Interaction of genetic polymorphisms and treatment regimens

The interaction of ABCB1 genotypes and treatment regimens (taxanes or irinotecan) with PFS or OS is shown in Table 4. The impact of this polymorphism on PFS seemed to be stronger in taxane-treated patients than in irinotecan-treated patients, although no significant interaction between genotype and regimens was observed either PFS or OS.

Discussion

In this study, we observed that genetic polymorphisms in ABCB1 3435C>T were associated with better PFS with borderline significance in patients with AGC treated with second-line chemotherapy. This observation was similar to the past report by Chang et al, which also showed longer PFS in patients with ABCB1 3435CC type (Chang et al., 2010). These results suggest that genetic polymorphisms of ABCB1 have some predictive value for second-line chemotherapy in AGC.

The influence of ABCB1 polymorphisms has been evaluated in patients with several types of cancers treated with chemotherapy. Among the chemotherapeutic agents used, taxanes have been most commonly studied (Green et al., 2006; Marsh et al., 2007; Johnatty et al., 2008; Sissung et al., 2008; Chang et al., 2009). Although taxanes are commonly used as chemotherapeutic agent for AGC, only one report (Chang et al., 2010) evaluated ABCB1 polymorphisms with small number of patients (n=43), and no reports was seen from Japan. Additionally, an in vitro study suggested that ethnic differences influenced paclitaxel sensitivity in cancer cells, based on different gene expression patterns in patients of Asian and Western ethnicity (Kwon et al., 2009). Therefore we conduct this study to evaluate Japanese patients with gastric cancer, for whom taxanes are commonly used as second-line chemotherapy.

The ABCB1 3435 polymorphism is one of the most common polymorphisms of the ABCB1 gene. In vitro, the homozygous CC genotype in C3435T was reported to be associated with twofold higher Pgp protein expression levels compared with the TT genotype (Hoffmeyer et al., 2000; Cascorbi et al., 2001). Therefore, patients with the CC genotype would be expected to respond worse to chemotherapy, with higher drug efflux rates and lower tissue concentrations (Kolesar et al., 2009). However, in

our study, the CC type tended to have better PFS than the TT or TC types with the borderline significance, which was compatible to the another report in patients with AGC (Chang et al., 2010). Other studies in breast cancer and non-small cell lung cancer also showed a better clinical outcome (disease control rate or response rate) in patients with the CC type (Pan et al., 2008; Chang et al., 2009). Although some studies showed no impact of this polymorphism (Green H et al., 2006; Marsh et al., 2007; Johnatty et al., 2008), no reports have shown a worse outcome with the CC type. To support our result, Nakamura et al. showed higher MDR1 mRNA levels in healthy Japanese subjects carrying the ABCB1 3435T allele compared with subjects with the 3435C allele (Nakamura et al., 2001). Considering these controversial preclinical and clinical data, further study is necessary to investigate genetic polymorphisms, Pgp expression of normal organs and tumor cells and clinical outcomes of patients comprehensively.

In the subset analysis of each treatment group, the impact of this polymorphism on PFS seemed to be stronger in taxane-treated patients than in irinotecan-treated patients, although no significant interaction was observed. Some studies have evaluated the influence of ABCB1 polymorphisms on irinotecan pharmacokinetics (Kaniwa et al., 2003; Mathijssen et al., 2003; Han et al., 2007). In a study of Japanese patients with colorectal cancer, an ABCB1 polymorphism of 3435TT was associated with reduced renal clearance of irinotecan and its metabolites (Kaniwa et al., 2003). In contrast, in patients with non-small cell lung cancer treated with irinotecan and cisplatin, 3435TT carriers showed a lower plasma AUC of SN-38G compared with 2677GG/3435CC carriers (Han et al., 2007). These controversial results were suggested to be based on the complex metabolic pathway of irinotecan, where ABCB1 makes a relatively smaller contribution than other enzymes, such as uridine diphosphate glucuronosyltransferase (UGT).

In our study, the ABCB1 3435 polymorphism had no impact on OS against the borderline significance on PFS. This observation was also similar to another report in AGC. The cause of this inconsistency is unknown, but one possibility is that other factors such as PS or PFS of first-line chemotherapy were too strong for ABCB1 polymorphisms to show an impact on OS. Another possibility was that ABCB1 polymorphisms are related to patient prognosis itself independently of treatment effect since ABCB1 is reported to play a significant survival role in normal and cancer cells during tumor progression, and metastasis (Tahara et al., 2007; Chang et al., 2010).

In this point of view, further study might be important to comprehensively investigate genetic polymorphisms in both patients with cancers treated with or without chemotherapy and healthy subjects.

There are several methodological issues in this study. This study did not evaluate other ABCB1 polymorphisms, such as 2677G>T/A. There is some controversy regarding the effect of this polymorphism (2677G>T/A) on ABCB1 transport activity (Kaniwa et al., 2003; Yi et al., 2004; Lee et al., 2005; Han et al., 2007; Tahara et al., 2007). In addition, the minor 2677A allele, as well as the major 2677G allele, is strongly linked to the major 3435C allele (Cascorbi et al., 2001; Johne et al., 2002; Yi et al., 2004; Lee et al., 2005; Song et al., 2006). Therefore, we did not evaluate this polymorphism in this study, although it might contribute to the inconclusive results.

Additionally, the small sample size used may be a study limitation, which may contribute to lack of statistical power to show the statistically significant difference in PFS or significant interaction between outcome with each treatment regimens and genetic polymorphisms. Therefore further similar studies or meta-analysis study is required to duplicate this work in larger cohort.

In conclusion, our findings indicate that the genetic polymorphisms of ABCB1 have some predictive value for clinical outcome of AGC patients treated with second-line chemotherapy. Further prospective evaluation is required.

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Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial



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Summary

Background Trastuzumab, a monoclonal antibody against human epidermal growth factor receptor 2 (HER2; also known as ERBB2), was investigated in combination with chemotherapy for first-line treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer.

Methods ToGA (Trastuzumab for Gastric Cancer) was an open-label, international, phase 3, randomised controlled trial undertaken in 122 centres in 24 countries. Patients with gastric or gastro-oesophageal junction cancer were eligible for inclusion if their tumours showed overexpression of HER2 protein by immunohistochemistry or gene amplification by fluorescence in-situ hybridisation. Participants were randomly assigned in a 1:1 ratio to receive a chemotherapy regimen consisting of capecitabine plus cisplatin or fluorouracil plus cisplatin given every 3 weeks for six cycles or chemotherapy in combination with intravenous trastuzumab. Allocation was by block randomisation stratified by Eastern Cooperative Oncology Group performance status, chemotherapy regimen, extent of disease, primary cancer site, and measurability of disease, implemented with a central interactive voice recognition system. The primary endpoint was overall survival in all randomised patients who received study medication at least once. This trial is registered with ClinicalTrials.gov, number NCT01041404.

Findings 594 patients were randomly assigned to study treatment (trastuzumab plus chemotherapy, n=298; chemotherapy alone, n=296), of whom 584 were included in the primary analysis (n=294; n=290). Median follow-up was 18.6 months (IQR 11–25) in the trastuzumab plus chemotherapy group and 17.1 months (9–25) in the chemotherapy alone group. Median overall survival was 13.8 months (95% CI 12–16) in those assigned to trastuzumab plus chemotherapy compared with 11.1 months (10–13) in those assigned to chemotherapy alone (hazard ratio 0.74; 95% CI 0.60–0.91; p=0.0046). The most common adverse events in both groups were nausea (trastuzumab plus chemotherapy, 197 [67%] vs chemotherapy alone, 184 [63%]), vomiting (147 [50%] vs 134 [46%]), and neutropenia (157 [53%] vs 165 [57%]). Rates of overall grade 3 or 4 adverse events (201 [68%] vs 198 [68%]) and cardiac adverse events (17 [6%] vs 18 [6%]) did not differ between groups.

Interpretation Trastuzumab in combination with chemotherapy can be considered as a new standard option for patients with HER2-positive advanced gastric or gastro-oesophageal junction cancer.

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Introduction

Gastric cancer is the fourth most commonly diagnosed cancer and the second most common cause of cancer-related deaths worldwide.^{1,2} Most patients present with inoperable advanced or metastatic disease requiring palliative treatment, although early detection is more common in Asia than in other regions. In the UK MAGIC study,³ 5-year survival was 36% in patients with operable disease who were assigned to perioperative chemotherapy. However, 5-year survival for advanced or metastatic gastric cancer is around 5–20%, with median overall survival being less than 1 year.^{2,4,5} A meta-analysis of phase 2 and 3 randomised gastric cancer trials has shown that combination chemotherapy results in substantially improved overall survival compared with single-agent

chemotherapy or best supportive care.⁶ Typically, a fluoropyrimidine and a platinum compound form the backbone of chemotherapy for patients with advanced gastric cancer. There is currently no single well established standard of care, but fluoropyrimidine-based and platinum-based combinations with or without a third drug (usually docetaxel or epirubicin) are the most widely used combinations in Europe and the USA. The oral fluoropyrimidine capecitabine was shown to be non-inferior to fluorouracil in terms of progression-free survival and overall survival in clinical trials.^{7,8}

Despite the recently reported benefits of combination therapies, the prognosis of advanced gastric or gastro-oesophageal cancer remains poor, and new treatments showing acceptable toxicity profiles are urgently needed.

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Surgical specimen staining pattern	Biopsy specimen staining pattern	HER2 overexpression assessment
0 No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any tumour cell	Negative
1+ Faint or barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster with a faint or barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2+ Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal
3+ Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Positive

HER2=human epidermal growth factor receptor 2 (also known as ERBB2).

Table 1: Immunohistochemistry scoring for HER2 in gastric and gastro-oesophageal junction cancer, by type of diagnostic specimen

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One well established target is human epidermal growth factor receptor 2 (HER2; also known as ERBB2), a member of a family of receptors associated with tumour cell proliferation, apoptosis, adhesion, migration, and differentiation.⁹ There is growing evidence that HER2 is an important biomarker and key driver of tumorigenesis in gastric cancer, with studies showing amplification or overexpression in 7–34% of tumours.^{9–11} Although reports

are conflicting, some studies have suggested that HER2-positive status in gastric cancer is associated with poor outcomes and aggressive disease.^{9,11}

Trastuzumab, a monoclonal antibody that targets HER2, induces antibody-dependent cellular cytotoxicity, inhibits HER2-mediated signalling, and prevents cleavage of the extracellular domain of HER2.¹² In HER2-positive breast cancer, trastuzumab has shown a survival advantage in early and metastatic disease and is now the standard of care.^{13–15} In patients with metastatic breast cancer, high levels of HER2-protein expression and amplification predict for better outcomes with trastuzumab.¹⁴ However, this relation is less clear in patients with early breast cancer¹⁶ and has not been established in other tumour types with HER2 overexpression. In preclinical models of gastric cancer, trastuzumab showed at least additive antitumour effects when combined with capecitabine or cisplatin, or both.¹⁷ In view of the high unmet medical need in gastric cancer, a HER2 positivity rate similar to breast cancer,^{18–20} and the good tolerability profile of trastuzumab in patients with breast cancer,^{13,15} investigation of trastuzumab in patients with gastric cancer was warranted.

The objective of the Trastuzumab for Gastric Cancer (ToGA) study was to assess the clinical efficacy and safety of trastuzumab added to chemotherapy for first-line treatment of advanced gastric or gastro-oesophageal junction cancers with overexpression of HER2.

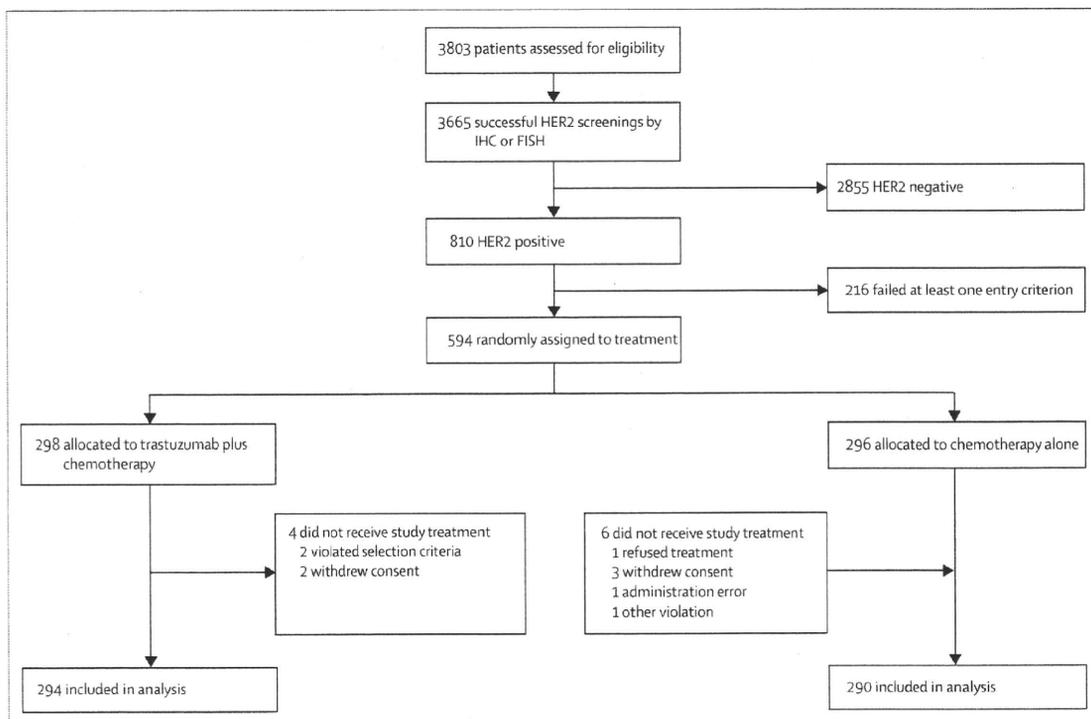


Figure 1: Trial profile
HER2=human epidermal growth factor receptor 2 (also known as ERBB2). IHC=immunohistochemistry. FISH=fluorescence in-situ hybridisation.