20 months, excellent local control with an objective response rate of 84%, and has demonstrated the safety of the SP with concurrent radiotherapy (SP-RT) [11] for patients with stage III NSCLC.

In contrast, the role of surgery for LA-NSCLC has been controversial, especially due to the heterogeneity of stage III NSCLC patients who have various numbers, stations, or conditions of mediastinal lymph node metastases, modes of tumor invasion to adjacent thoracic structures, or organs such as the great vessels, mediastinum, vertebral body, carina, esophagus, and so on, which might affect the prognosis of such patients. Such diversity of LA-NSCLC patients has precluded the establishment of an optimal treatment strategy.

We have previously reported the feasibility of SP-RT as an induction therapy that can be followed by curative intent resection for 18 patients with potentially resectable LA-NSCLC [12]. In the present study, we retrospectively analyzed the prognostic benefit for a larger number of patients treated with this strategy.

Patients and Methods

We retrospectively reviewed 42 consecutive patients with potentially resectable stage III LA-NSCLC who underwent preoperative induction concurrent chemoradiotherapy using SP-RT followed by curative-intent surgical resection between June 2005 and February 2011 in the Department of Thoracic Oncology, National Kyushu Cancer Center, Japan. The clinical or pathologic stage of the disease was diagnosed based on the general rules for the TNM Classification of Malignant Tumors (6th edition) [13]. Eligible patients had to have cytologically or histologically confirmed clinical stage III NSCLC that was considered to be potentially resectable. The other eligibility criteria were an age between 20 and 80 years, Eastern Cooperative Oncology Group performance status of 0 to 1, absence of previous chemotherapy or radiotherapy, and adequate hematologic, hepatic, and renal function. Patients with standard laboratory tests results, included the following: a leukocyte count of 3.500/μL or greater; a platelet count of 100,000/μL or greater; serum bilirubin level less than 1.5 mg/dL; serum glutamic oxaloacetic transaminase-glutamic pyruvic transaminase levels 100 IU/mL or less, a creatinine level 1.2 mg/dL or less, or a creatinine clearance level of 60 mL/minute or greater, and a blood gas oxygen tension of 60 Torr or greater, or oxygen saturation as measured by pulse oximetry equal to or greater than 95% in room air were considered to be eligible for this treatment. In addition, pulmonary function tests, chest radiography, computed tomography of the chest and the upper abdomen, computed tomography or magnetic resonance imaging of the brain, bronchoscopy using a flexible optical bronchoscope, and a bone scan or fluorodeoxyglucose-positron emission tomography were routinely performed for all patients. Patients who had malignant pleural effusion, malignant pericardial effusion, or a concomitant malignancy or serious comorbidities such as clinically significant cardiac dysfunction, active infection, or neurologic or psychiatric disorders were excluded.

Treatment Schedule

Chemotherapy With SPS-1 (40 mg/m^2 twice a day [b.i.d.]) in the form of 20 mg and 25 mg capsules containing 20 and 25 mg of tegafur, respectively, were taken orally in 2 separate doses from days 1 to 14 and days 22 to 35 as follows: in a patient with a body surface area (BSA) less than 1.25 m^2, 40 mg b.i.d.; for those with a BSA of at least 1.25 m^2 but less than 1.5 m^2, 50 mg b.i.d.; and for those with a BSA greater than 1.5 m^2, 60 mg b.i.d. was administered. Cisplatin, at a dose of 60 mg/m^2, was administered as a 120-minute infusion on days 1 and 22 while the patients were hydrated with 2,500 mL of saline by infusion. In general, this dose and schedule is equivalent of that of patients without radiotherapy. An antiemetic agent was administered at the discretion of each patient's physician.

Radiotherapy (RT)

All patients were treated with a linear accelerator photon beam of 6 MV or more from day 1. The primary tumor and involved nodes received 40 Gy in 2 Gy fractions over a period of 4 weeks. A three-dimensional treatment planning system was used. Radiation doses were specified at the center of the target volume. The delivered 40 Gy/20 fractions included the primary tumor, ipsilateral hilum, and mediastinal nodal areas from the paratracheal to subcarinal lymph nodes. For the primary tumors and the involved lymph nodes that were 1 cm or larger in the shortest diameter, a margin of at least 0.5 cm was added. The contralateral hilum was not included. The treatment of supraclavicular areas was not mandatory, but they were treated when the supraclavicular nodes were involved.

During the concurrent chemoradiotherapy period, chest X-rays, complete blood cell counts, and blood chemistry studies were repeated once a week, and the treatment was interrupted when a grade 4 hematologic or non-hematologic toxicity, including grade 3 to 4 esophagitis or dermatitis, pyrexia of 38°C or greater, or a decrease in the partial pressure of arterial oxygen of 10 Torr or more, compared with that before radiation therapy, occurred.

Surgical Resection

Immediately after completing the induction SP-RT, the patients were assessed for their response to the induction therapy and were restaged. If disease control, such as a complete response, partial response, or stable disease, was achieved a curative intent resection was planned for 3 to 6 weeks after completion of the concurrent chemoradiotherapy. The principles of resection were en bloc removal of the affected lobe or more lung parenchyma with adjacent structure(s) if necessary, with complete hilar and mediastinal lymph nodal dissection.

Evaluation of the Response and Toxicity

The response was evaluated in accordance with the Response Evaluation Criteria in Solid Tumors version 1.0 guidelines [14]. The histologic analysis of the tumor was based on the World Health Organization classification for cell types [15]. The toxicity for all patients who received any treatment was assessed and graded by using

the National Cancer Institute Common Terminology Criteria for Adverse Event version 3 [16].

Statistical Analysis

To determine the response rate, the exact binomial confidence interval was calculated. Disease-free survival was defined as the time from the starting date of induction concurrent chemoradiotherapy until disease progression or death, and was calculated for the 39 resected patients. Overall survival was defined as the time from the starting date of induction concurrent chemoradiotherapy until death from any cause. The Kaplan-Meier method was used to describe overall survival and disease-free survival curves. All statistical analyses were done with the IBM SPSS Statistics 18 software package (SPSS Japan, an IBM company, Tokyo Japan).

This retrospective analysis was approved by the Institutional Review Board of the National Kyushu Cancer Center. Written informed consent was obtained from all patients before treatment.

Results

Patient Characteristics

As shown in Table 1, there were 34 males (81.0%) and 8 females (19.0%) with the median age of 59 years (range 42 to 77) who were included in this study. Thirty-three (78.6%) patients showed an ECOG performance status of 0. Twenty-one of the 42 patients (50.0%) had adenocarcinoma, while 12 patients had squamous cell carcinoma (28.6%), 8 had non-small cell carcinoma (unclassified), and 1 had large cell carcinoma. The 26 cStage IIIA patients included 24 cases of T1-3N2 and 2 of T3N1, and the 16 cStage IIIB patients included 13 cases of T4N0-2 and 3 of T2-4N3. All N3 patients had ipsilateral supraclavicular lymph node metastasis. The location of the primary tumor was the upper lobe in 38 patients (90.5%) and other lobes in 4 patients (9.5%).

Table 1. Patient Characteristics That Were Eligible for Induction Treatment

Subject	No.	(%)
No. of patients	42	
Age, years		
Median (range)	59 (47–77)	
Gender		
Male to female	34:8	(81.0:19.0)
ECOG PS		
0:1	33:9	(78.6:21.4)
Histology		
Adenocarcinoma	21	(50.0)
Squamous cell carcinoma	12	(28.6)
Large cell carcinoma	1	(2.4)
Unclassified NSCLC	8	(19.0)
cTN[a]		
T3N1	2	
T1-2N2	20	
T3N2	4	
T4N0	2	
T4N1	5	
T4N2	6	
T2-4N3	3	
cStage[a]		
IIIA	26	(61.9)
IIIB	16	(38.1)
Primary site		
Upper lobe	38	(90.5)
Middle/lower lobe	4	(9.5)

[a] TNM Classification of Malignant Tumors (6th edition).

ECOG PS = Eastern Cooperative Oncology Group performance status; NSCLC = non-small cell carcinoma.

Induction Treatment

All patients received the planned dose of radiotherapy, and 41 (97.6%) had 2 cycles of chemotherapy as induction treatment. As shown in Table 2, no grade 4 toxicity was observed during this induction therapy. The most frequently observed adverse event was grade 3 leukopenia, but its incidence was less than 10%; the incidence of the other grade 3 adverse events was 2.4% for neutropenia and febrile neutropenia and 4.8% for thrombocytopenia. One patient received 1 cycle of chemotherapy and another patient required a dose reduction of cisplatin [CDDP] during the second cycle of chemotherapy due to grade 2 serum creatinine level elevation. After receiving the induction treatment, 26 (61.9%) of the 42 patients achieved a partial response (PR), and stable disease (SD) was observed in 16 patients (38.1%). No progressive disease was observed.

Surgical Resection

Among the 42 patients, 39 patients (92.9%) were able to undergo surgical resection. One patient proved to be unresectable after thoracotomy because of the left atrial invasion around the inferior pulmonary vein that could not be detected preoperatively, and 2 patients refused surgical treatment at the end of their induction treatment. Among the 39 patients who received the curative intent resection, 27 patients (69.2%) underwent a lobectomy, including 6 sleeve lobectomies and 12 pneumonectomies (5 in right side and 7 in left side) (30.8%) including 10 intrapericardial pneumonectomies. Sixteen of the 39 patients (41.0%) required combined resection of an adjacent structure or organ: the chest wall with rib(s) in 12 cases; combined partial resection of the vertebra in 3 cases; the internal jugular or brachiocephalic vein that required vascular replacement with a vascular prosthesis each in 1 case; the superior vena cava in 1 case; and the left atrium in 1 case (Table 3). Complete resection was performed in all patients. Of the 3 patients with ipsilateral supraclavicular lymph node metastasis, two underwent a systemic mediastinal and supraclavicular lymph nodal dissection via a median sternotomy, and the other one was confirmed to have no metastasis in his supraclavicular lymph nodes by a pathological examination during surgery, and subsequently underwent systemic

Table 2. Toxicities (n = 42); National Cancer Institute Common Terminology Criteria for Adverse Event Version 3

	Grade		Frequency of 3 or 4 (%)
	3	4	
Hematologic			
Leukopenia	3	0	7.1
Neutropenia	1	0	2.4
Thrombocytopenia	2	0	4.8
Anemia	0	0	/
Non-hematologic			
Febrile neutropenia	1	0	2.4
Nausea	0	0	/
Vomiting	0	0	/
Creatinine	0	0	/
AST to ALT	0	0	/
Diarrhea	0	0	/
Stomatitis	0	0	/
Pneumonitis	0	0	/
Esophagitis	0	0	/

ALT = alanine aminotransferase; AST = aspartate aminotransferase.

mediastinal lymph nodal dissection via posterolateral thoracotomy.

Surgical Morbidity and Mortality

The postoperative morbidity in this series of patients were the following: 3 cases each of postoperative bleeding and arterial fibrillation; 2 cases of chylothorax; and 1 each of prolonged air leakage, pulmonary edema, empyema, heart failure, and spinal cord injury. Among these cases, 3 patients underwent re-thoracotomy; 2 for postoperative bleeding and 1 for chylothorax. One patient who had undergone a left upper lobectomy experienced postoperative thoracic empyema without a bronchopleural fistula and died on the 65th postoperative day due to massive intrathoracic bleeding.

Table 3. Type of Resection (n = 39)

Subject	No. (%)
Pneumonectomy	12 (30.8)
Intrapericardial pneumonectomy	10
Lobectomy[a]	27 (69.2)
Sleeve lobectomy	6
Combined resection	16 (41.0)
Site of combined resection (redundant)	
Chest wall (ribs)	12
Vertebra	3
Internal jugular or brachiocephalic vein	2
SVC (replacement with graft)	1
Left atrium	1

[a] One patient with a bilobectomy was included.

SVC = superior vena cava.

Pathologic Findings

Concerning the clinical and pathologic response to induction concurrent chemoradiotherapy using SP-RT in the 39 patients who underwent surgical resection, 9 of the 39 (23.1%) patients showed a complete pathologic response in both the primary tumor and involved lymph nodes, while 6 of these 9 presented clinical PR and 3 clinical SD. Among the other 30 patients (76.9%) with partial pathologic response, 18 showed clinical PR and 12 clinical SD.

Adjuvant Chemotherapy

Twenty-five (64.1%) patients received adjuvant chemotherapy, mainly with cisplatin-based regimens. The regimens were determined by the attending surgeon. Ten of these 25 patients received more than 3 cycles of adjuvant chemotherapy.

Survival and Recurrence

The median follow-up time was 32.0 months. One-, 3-, and 5-year disease-free survival rates in all 39 surgically resected patients were 73.8% (95% CI: 59.95% to 87.7%), 52.0% (95% CI: 34.9% to 69.1%), and 44.0% (95% CI: 26.4% to 61.6%), respectively (Fig 1A). One-, 3- and 5-year overall survival rates were 84.3% (95% CI: 72.7% to 95.9%), 77.4% (95% CI: 63.3% to 91.5%), and 61.7% (95% CI: 42.1% to 81.3%), respectively (Fig 1B). When patients were stratified into those with cStage IIIA versus cStage IIIB, pN0 versus pN1-3, clinical response (ie, PR versus SD and lobectomy versus pneumonectomy), there were no statistically significant differences in either disease-free survival or overall survival (data not shown). However, when patients were stratified by their pathologic response, 3-year disease-free survival rates in the 9 patients with pathologic complete response were 76.2% (95% CI: 47.2% to 100%), while those of the other 30 patients with any pathologic response were 44.5% (95% CI: 24.7% to 64.3%) (Fig 2A). Three-year overall survival rates were 88.9% (95% CI: 68.3% to 100%) in the 9 patients with pathologic complete response, whereas those of the other 30 patients were 74.0% (95% CI: 56.9% to 91.1%) (Fig 2B).

Of the 39 resected patients, recurrence developed in 18 patients. The first site of recurrence in 16 patients was a distant region. The most common first recurrence site was the brain (7 cases) and the lungs (6 cases). Two patients had recurrence in the contralateral mediastinal lymph nodes that was out of the irradiated field during induction treatment. One of the 9 patients who achieved a pathologic complete response experienced recurrence in the contralateral lung.

Comment

The data presented here imply that treatment with concurrent chemoradiotherapy using SP-RT followed by surgery might provide better local disease control and better survival in patients with potentially resectable LA-NSCLC. Because LA-NSCLC is associated with a high risk of local and systemic recurrence of approximately 80% and 60%, respectively [17], combined local and systemic treatments are warranted. In this regard, the

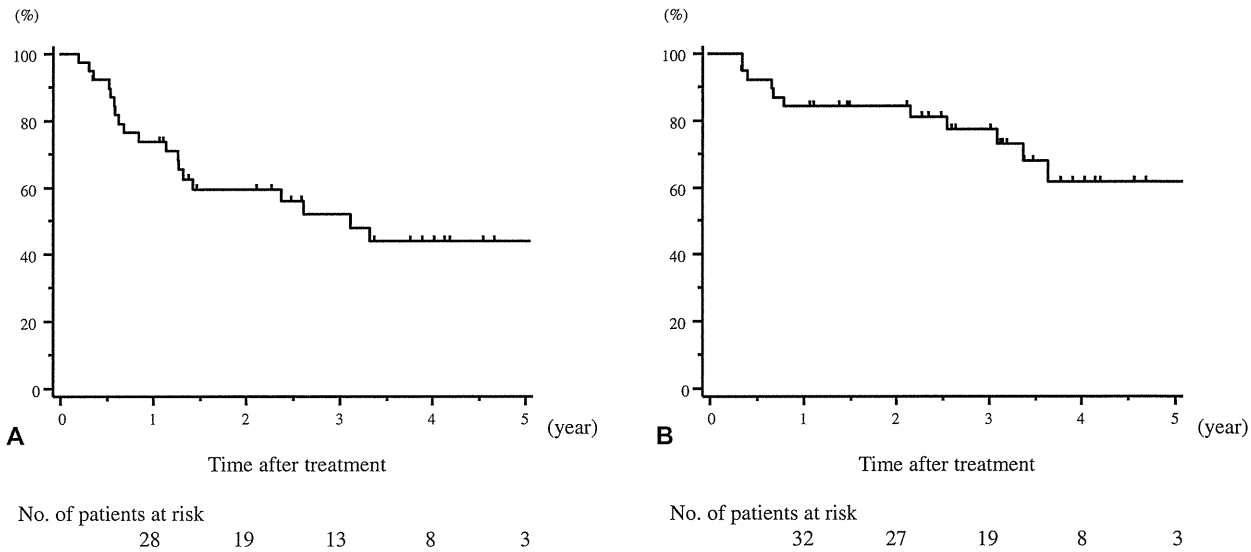

Fig 1. The survival curve of all 39 resected patients. (A) Disease-free survival and (B) overall survival.

optimal treatment strategy for LA- NSCLC is generally considered to be concurrent chemoradiotherapy [18]; however, the most frequent relapse site after concurrent chemoradiotherapy is at a distant region. A possible reason for this type of relapse is that the full-dose chemotherapeutic regimens developed for metastatic-NSCLC in the 1990s cannot be used at the full doses concurrently with radiotherapy due to the associated acute toxicities. Recently, Ichinose and colleagues [11] showed that the combination of full dose SP and concurrent radiotherapy of 60 Gy could be administered with acceptable toxicity, and the treatment with this regimen demonstrated a favorable survival, with a median progression-free survival of 20 months and an ORR of 84%.

Some phase III trials of concurrent chemoradiotherapy with radiation doses ranging from 56 to 66 Gy have shown good response rates of approximately 55% to 80% [19, 20]. In the present study, we observed that 59.5% of patients had a partial response and 40.5% had stable

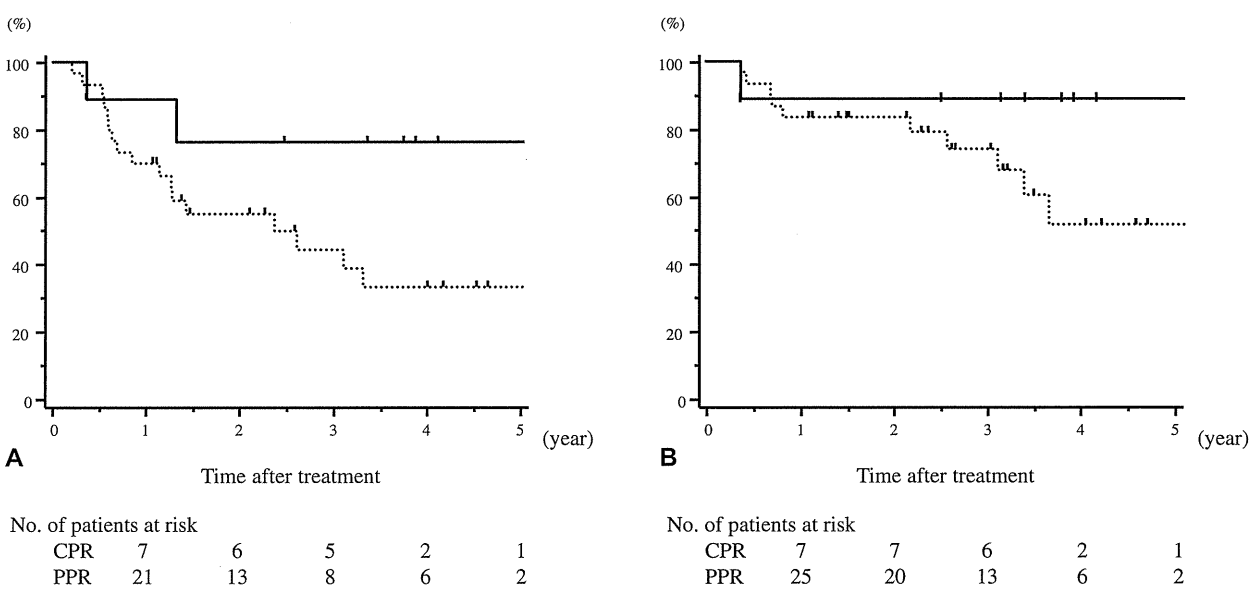

Fig 2. The prognosis of patients stratified by the pathologic response of the resected specimen. Solid line represents patients with complete pathologic response (CPR), while dashed line represents the patients with partial pathologic response (PPR). (A) Disease-free survival; (B) overall survival.

disease after using SP, even with 40 Gy of concurrent radiation therapy. The toxicity of our SP-RT induction treatment was also excellent, without any grade 4 events, which allowed patients to safely undergo the subsequent surgical resection.

Concerning the survival benefit of concurrent chemoradiotherapy for LA-NSCLC, Segawa and colleagues (OLCSG 0007) [19] compared docetaxel plus cisplatin to mitomycin, and vindesine plus cisplatin with concurrent radiotherapy in their phase III study, and reported better 1- and 3-year progression-free survival rates of 53.4% and 24.9%, respectively, and 1- and 3-year overall survival rates of 82.8% and 38.1%, respectively, in the docetaxel plus cisplatin group. Yamamoto and colleagues [20] (WJOG 0105) also compared mitomycin, vindesine plus cisplatin, irinotecan plus carboplatin and paclitaxel plus carboplatin, and demonstrated that a median progression-free survival rate was 9.5 months and a median overall survival was 22.0 months in their docetaxel plus cisplatin group. Focusing on induction concurrent chemoradiotherapy followed by surgery for LA-NSCLC, some phase I and II studies demonstrated promising results in their surgery arm; Friedel and colleagues [21] showed a better median overall survival of 39 months in the subset analysis of their phase II study, and an improved 5-year overall survival rate of 43.1% in patients who underwent surgical resection after induction chemoradiotherapy with carboplatin and paclitaxel with 45 Gy of concurrent radiotherapy for stage III NSCLC compared with those treated without surgical resection, which were 29.6 months and 0%, respectively. Edelman and colleagues [22] reported a good median overall survival of 55.8 months in their series of stage III NSCLC patients with negative mediastinal nodes after induction concurrent chemoradiotherapy using carboplatin and vinorelbine in their phase I/II study. We also previously showed the impact of induction concurrent chemoradiotherapy with cisplatin and UFT on the survival of stage IIIB NSCLC patients who underwent surgical resection, with 1- and 3-year overall survival rates of 82% and 67%, respectively [23].

In their recent report, Albain and colleagues (INT 0139) [24] reported no significant overall survival difference between patients who received induction concurrent chemoradiotherapy with or without surgery; however, the patients who underwent lobectomy showed significant better survival. Additionally, in their resected pT0N0 patients, an excellent median survival of 39 · 8 months was observed. In the present study, a considerably better prognosis was observed; 1-, 3-, and 5-year disease-free survival rates were 73.8%, 52.0%, and 44.0%, respectively (Fig 1A), and 1-, 3-, and 5-year overall survival rates were 84.3%, 77.4%, and 61.7%, respectively (Fig 1B). Our study also indicated that pathologic good responders (ie, patients with complete pathologic response) showed a 3-year disease-free survival rate of 76.2% and 3-year overall survival of 88.9%. We did not evaluate the relationship between the pre-induction and post-induction treatment TNM stage because we believe that one of the important predictive factors for postoperative survival is the pathologic response. That is the reason why we focused on this issue and did not show the correlation between pre-induction and post-induction staging. These results seem to indicate that SP-RT can provide a sufficient systemic dose to prevent occult distant metastasis. In addition, 5-FU is known to have a radiosensitizing effect [25] and S-1 was orally administered for 14 consecutive days twice during the radiotherapy in the present study.

The limitations of the present study are the retrospective nature of the analysis and the relatively small number of patients. We are currently performing a single institutional phase II study of SP-RT as an induction concurrent chemoradiotherapy, followed by surgical resection, for LA-NSCLC patients.

In conclusion, SP-RT followed by surgery may provide a better prognosis for LA-NSCLC patients. Further clinical investigations are warranted.

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INVITED COMMENTARY

To date, 5-fluorouracil (5-FU) has not been utilized in the treatment of non-small cell lung cancer (NSCLC) because of its bioavailability profile, providing lower levels outside the gastrointestinal system. S-1 is an oral fluoropyrimidine drug that combines tegafur, a prodrug of 5-FU, with gimeracil (CHDP) and potassium oxonate (OXO), to increase serum 5-FU levels and minimize gastrointestinal toxicity, respectively. Usually, approximately 80% to 90% of 5-FU administered intravenously is rapidly catabolized by liver dihydropyrimidine dehydrogenase (DPD), and others have also shown high levels of DPD may exist in lung tumors. With S-1, CHDP inhibits both liver and tumor DPD more than 150 times more effectively than uracil and OXO inhibits 5-FU phosphorylation by gastrointestinal mucosal cells. Capecitabine is another oral 5-FU prodrug, but its metabolism is different from that of than tegafur, relying on a final step requiring the enzyme thymidine phosphorylase, which is expressed variably in NSCLC tumors [1].

Although early reports of S-1 in the treatment of NSCLC are now more than a decade old, the clinical use of S-1 has not gained significant traction worldwide yet. Although S-1, in combination with platinum, exhibits antitumor effects in NSCLC as shown in a recent multicenter phase II study (overall response rate 20%, median time to progression 4 months), these results were comparable but not superior to those of other current platinum doublets [2]. This North American study [3], however, used lower doses of S-1 (25 mg/m^2) than in previous studies from Japan in combination with cisplatin at 75 mg/m^2. Yet, S-1 plus platinum demonstrated 50% fewer grade 4 toxicities as compared with other standard platinum doublets for NSCLC. In the present study by Yamaguchi and colleagues [3], S-1 given at 40 mg/m^2 with cisplatin (60 mg/m^2) resulted in a very favorable toxicity profile. The radiosensitizing effects of 5-FU are well known and the higher S-1 dose in the present study likely contributes to the overall results. However, the cohort size here is small, and additional studies will be needed to further explore the optimum dose levels and most effective drug combinations with S-1 for NSCLC.

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Dramatic Response to Crizotinib in an *ALK*-Positive Adenocarcinoma Patient with Disseminated Intravascular Coagulation

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CASE REPORT

A 43-year-old female nonsmoker was diagnosed with an advanced lung adenocarcinoma with bone, liver, and brain metastases approximately 1.5 years before the administration of crizotinib, and had received four previous regimens of chemotherapy, including cisplatin plus pemetrexed, docetaxel, carboplatin plus gemcitabine, and an investigational drug. The *anaplastic lymphoma kinase* (*ALK*) rearrangement was detected by fluorescence in-situ hybridization at the time of third relapse. After the four regimens of chemotherapy, fatigue, purpura, and jaundice were observed. No progression of the primary lung lesion was identified; however, the multiple liver metastases were found to have rapidly progressed (Fig. 1*A* and *B*, arrows). Laboratory tests showed abnormalities in the coagulation and fibrinolytic systems, with evidence of fragmented red blood cells and elevation of the bilirubin level, with a predominance of indirect bilirubin (prothrombin: 54%, fibrinogen: 56 μg/ml, fibrin degradation products: 62.96 μg/ml, platelet count: 137,000/μl, total bilirubin: 4.2 mg/dl, indirect bilirubin: 3.3 mg/dl), suggesting a disseminated intravascular coagulation (DIC) (Fig. 2*A* and *B*; Table 1). Therefore, the patient was urgently admitted to our department with an Eastern Cooperative Oncology Group performance status (PS) of 3. With no other causes assumed to have induced the DIC, acute progression of the hepatic lesions was thought to be the primary cause. After admission, a total of 26 units of fresh frozen plasma were transfused (Fig. 2, arrowheads). However, the patient remained in critical condition despite the transfusions, and crizotinib was administered 2 days after admission, after obtaining the patient's consent. Surprisingly, the symptoms, including fatigue, purpura, and jaundice, as well as the abnormalities in laboratory test results, dramatically improved after the administration of crizotinib (Fig. 2 and Table 1). A radiological response was observed 12 days after the administration of the drug (Fig. 1*C*) with an improvement in the PS (from 3 to 0), and the patient was discharged without any complications 17 days after admission.

DISCUSSION

ALK gene rearrangement has been identified as playing a critical role in the oncogenesis of non–small-cell lung cancer (NSCLC), particularly adenocarcinoma,[1] and is detected in 4% to 6% of patients with lung adenocarcinoma.[2] Importantly, these patients with *ALK* gene rearrangement can be successfully treated with ALK inhibitors. For instance, a phase I study showed that crizotinib is highly effective in *ALK*-positive NSCLC patients (even in poor PS patients), with the median time to the first documented objective response being as short as 7.9 weeks, and with an objective response rate of 60.8%. In addition, the antitumor activity of the drug was proven to be independent of the line of therapy.[3] Although controversial, the efficacy of crizotinib was also reported in an *ALK*-positive patient with brain metastases.[4] With regard to the treatment of *ALK*-positive NSCLC patients in critical conditions, Ahn et al.[5] reported three cases of NSCLC in which the patients were completely weaned from mechanical ventilation after treatment with crizotinib. Similarly, although the current patient was in a critical condition because of DIC, a rapid clinical and radiological improvement was achieved in spite of crizotinib being the fifth regimen. To our knowledge, there have been no reports of the administration of crizotinib in NSCLC patients with DIC who have exhibited such dramatic responses as that observed in the present case. Our experience indicates that molecular-targeted therapy should be considered for NSCLC patients with driver mutations, such as the *ALK* rearrangement, even when the patient is in critical condition because of disorders such as DIC.

In summary, we herein reported the case of an *ALK*-positive lung adenocarcinoma patient with DIC who was successfully treated with crizotinib. Although this report highlighted the case of a single patient, it can serve as a reference for the treatment of *ALK*-positive NSCLC patients with DIC.

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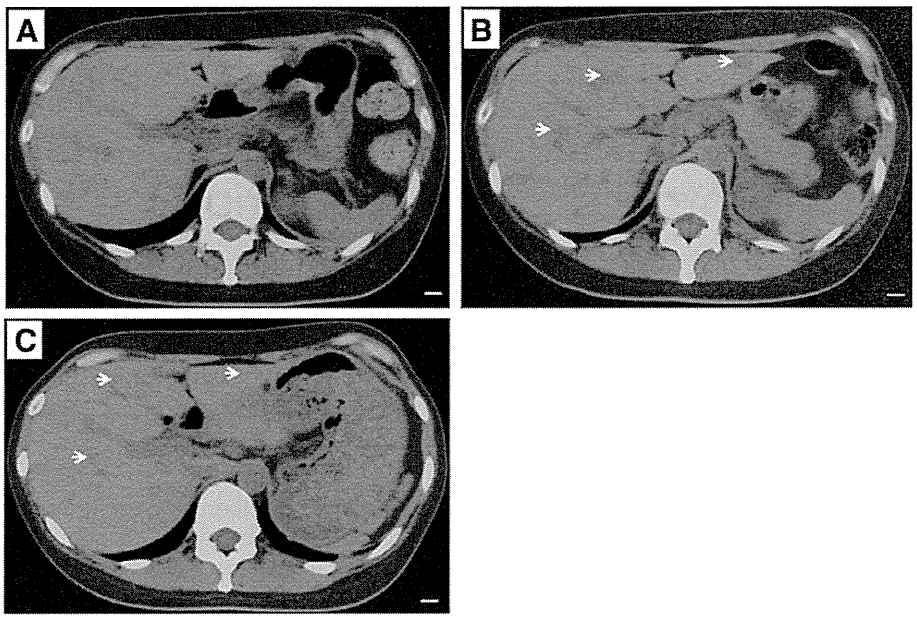

FIGURE 1. Computed tomography was performed approximately 2 months (*A*) and 10 days before (*B*), and 12 days after (*C*) the administration of crizotinib. The arrows show the multiple liver metastases. Scale bar: 1 cm.

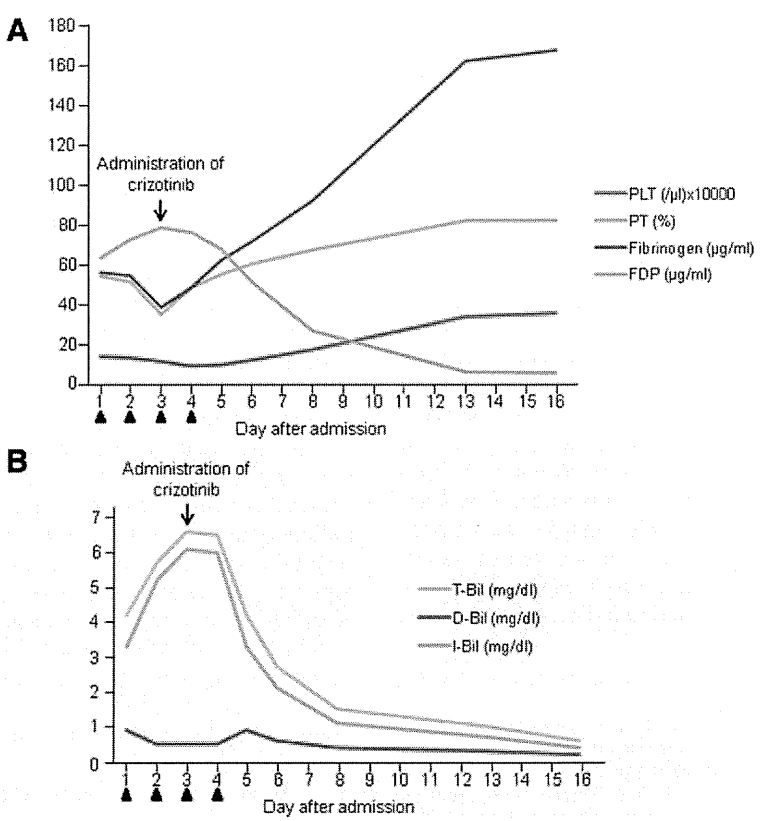

FIGURE 2. Changes in laboratory test results, including the prothrombin, fibrinogen, and FDP levels, the platelet count (*A*) and the bilirubin level (*B*), before and after the start of crizotinib therapy. The arrows show the days on which fresh frozen plasma was transfused (26 units in total). BIL, bilirubin; FDP, fibrin degradation products; PLT, platelet count; PT, prothrombin.

TABLE 1. Changes in Laboratory Test Results Associated with Disseminated Intravascular Coagulation

Day after admission	1	2	3	4	5	6	8	13	16
Total bilirubin (mg/dl)	4.2	5.7	6.6	6.5	4.2	2.7	1.5	1	0.6
Direct bilirubin (mg/dl)	0.9	0.5	0.5	0.5	0.9	0.6	0.4	0.3	0.2
Indirect bilirubin (mg/dl)	3.3	5.2	6.1	6	3.3	2.1	1.1	0.7	0.4
Platelet count (/μl) × 10000	13.7	13	11.4	8.8	9.6	11.9	17	33.3	35.2
Prothrombin (%)	54	51	35	48	55	60	67	82	82
Fibrinogen (μg/ml)	56	54	38	48	62	71	92	162	167
Fibrin degradation products (μg/ml)	62.96	72.66	78.09	75.81	67.88	51.21	26.53	6.12	5.29
D-dimer (μg/ml)	24.91	42.8	45.47	43.07	27.4	16.99	8.5	2.66	2.74

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The effect of CYP2C19 polymorphism on the safety, tolerability, and pharmacokinetics of tivantinib (ARQ 197): results from a phase I trial in advanced solid tumors

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Background: Tivantinib (formerly ARQ 197) is a selective inhibitor of c-Met mainly metabolized by CYP2C19. CYP2C19 is known for genetic polymorphisms, and ~20% of Asians are poor metabolizers (PMs), while others are extensive metabolizers (EMs). In this study, we examined the safety, pharmacokinetics (PK), and preliminary efficacy of tivantinib as a single agent to determine recommended phase II doses (RPIIDs).

Patients and methods: Forty-seven patients (EMs, 33; PMs, 14) with solid tumors were orally treated with tivantinib, from 70 to 360 mg bid in a 3 + 3 dose-escalation scheme. EMs and PMs were separately enrolled at the doses >120 mg bid.

Results: Tivantinib was well tolerated up to 360 mg bid for EMs and 240 mg bid for PMs. Neutropenia, leukopenia, anemia, fatigue, and anorexia were the frequent adverse events related to tivantinib and were commonly observed in both EMs and PMs. PMs had 1.9-fold higher AUC_{0-12} compared with EMs at 240 mg bid. Regardless of CYP2C19 phenotype, Gr.4 neutropenia occurred in patients with relatively high exposure to tivantinib. A confirmed partial response was achieved in two non-small-cell lung cancer (NSCLC) patients.

Conclusion: Two different settings of RPIIDs, 360 mg bid for EMs and 240 mg bid for PMs, were determined.

Key words: c-Met inhibitor, CYP2C19 polymorphism, pharmacokinetics, phase I study, tivantinib

introduction

c-Met and its ligand hepatocyte growth factor (HGF) play important roles in oncogenesis [1, 2]. Aberrant activation of the HGF/c-Met signaling pathway may lead to increased tumor cell proliferation, resistance to apoptosis, invasive growth, and tumor angiogenesis. In view of critical effects of HGF/c-Met signaling on cancer progression, several biologics, and low-molecular-weight compounds are currently under clinical investigation as HGF/c-Met pathway inhibitors [3–5].

Tivantinib (also known as ARQ 197) is a low-molecular-weight compound, and is the first in class orally available selective inhibitor of c-Met [6]. Tivantinib disrupts c-Met phosphorylation in a non-adenosine triphosphate competitive manner, distinguishing it from other c-Met inhibitors in clinical trials [7]. Tivantinib has been studied in several clinical trials across multiple tumor types [8–11]. So far, all of these studies were conducted in Western countries, including the United States and Europe [12–14].

An *in vitro* study showed that recombinant cytochrome P450 2C19 (CYP2C19) most rapidly degraded tivantinib when compared with other recombinant cytochrome P450 family enzymes, thus suggesting that CYP2C19 should play a key role in drug metabolism in humans [12]. CYP2C19 is known for the genetic polymorphisms that can affect the pharmacokinetics (PK) of the drugs which are the substrates of CYP2C19. Three major single-nucleotide polymorphisms (SNPs) have been identified in the CYP2C19 gene, including the wild-type CYP2C19*1 and two functionally deficient variants, CYP2C19*2 and CYP2C19*3 [15, 16]. The prevalence of the functionally deficient variants varies among races; Asians (30%–80%) showed much higher prevalence than White and Black (12%–19%) [17]. Therefore, by taking into account the CYP2C19 polymorphism variability, this study

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provided a more careful safety evaluation of tivantinib in an Asian patient population.

This is an open-label phase I dose-escalation study among Japanese patients with metastatic solid tumors. The primary objectives were safety, tolerability, and recommended phase II doses (RPIIDs) using the continuous twice-daily dosing schedules. The secondary objectives were preliminary antitumor activity and PKs, and the exploratory objectives were pharmacodynamics and predictive biomarkers. Because of the higher incidence of CYP2C19 SNPs in Japanese, all subjects were prospectively tested for their genetic background of CYP2C19 SNPs and divided into two subgroups: (i) extensive metabolizers (EMs) who possess at least one allele of wild-type CYP2C19*1; (ii) poor metabolizers (PMs) whose two alleles consist of either CYP2C19*2 or CYP2C19*3, but not any wild-type.

patients and methods

study design

This was a multicenter (eight institutes in Japan), open-label, dose-escalating phase I study. Patients were enrolled into sequential dose-escalation cohorts starting from 70 mg bid. Tivantinib was supplied by the sponsor as capsules containing formula A of tivantinib [12], and was orally administered twice a day at fasted condition. Dose escalation or cohort expansion followed a standard 3 + 3 design; cohort expansion to six patients took place if only one dose-limiting toxicity (DLT) was reported within the first 28 days of tivantinib treatment for each patient, and dose escalation stopped if two DLTs in a cohort were observed during that period. DLT was a drug-related adverse event, and was defined as Gr. ≥ 3 non-hematological toxicity, except for controllable Gr. ≥ 3 nausea, vomiting and diarrhea, or Gr. ≥ 4 hematological toxicity. Toxic effects were graded according to the Common Terminology Criteria for Adverse Events v3.0 [18]. A genetic test for CYP2C19 [Invader assay, measured by BML Inc. (Tokyo, Japan, a commercial laboratory)] was conducted for all enrolled patients. Patients were allowed to continue tivantinib treatments, as long as there was no evidence of disease progress or safety concerns.

Two major protocol amendments were implemented during the study. The first amendment was required due to the updated clinical study result: a clinical pharmacological study in healthy volunteers showed much higher plasma exposure of tivantinib in PMs than EMs at the same dose. Accordingly, the protocol was amended to determine the recommended doses of tivantinib separately for EMs and PMs. As a consequence, the first three cohorts included both EMs and PMs, and thereafter, separate registrations for EMs and PMs began with the cohorts testing 150 mg bid and 120 mg bid, respectively. The second amendment was implemented when two DLTs were observed in the cohort testing 300 mg bid in EMs. One of the two DLTs was Gr.4 neutropenia which returned to Gr.3 within 1 day with neither tivantinib interruption nor granulocyte-colony stimulating factor (G-CSF) treatment. The Safety Review Committee (SRC) concluded that the transient Gr.4 neutropenia was clinically acceptable and did not pose a grave safety concern. Accordingly, in the amended protocol, it was adopted as an alternative DLT definition that Gr.4 neutropenia lasting for <7 days was NOT defined as a DLT. Thus, dose escalation was carried out up to 360 mg bid.

This study was sponsored by Kyowa Hakko Kirin Co., Ltd., and was conducted in accordance with institutional guidelines, Good Clinical Practice guidelines and the Declaration of Helsinki. Documented approvals from the Institutional Review Boards were obtained. All patients provided written informed consent. This trial was registered in ClinicalTrials.gov as ID: NCT00609921.

eligibility criteria

Patients with cytologically or histologically confirmed solid malignancy for which no standard therapy was available were candidates for this study. The patients also met the inclusion criteria: ≥20 years of age; an Eastern Cooperative Oncology Group performance status (ECOG PS [19]) of ≤1 or Karnofsky performance status of >70% [20]; a life expectancy of ≥3 months; adequate organ functions [serum alanine aminotransferase (ALT) and aspartate aminotransferase ≤2.5 times ULN (or ≤5 times ULN in the case of liver metastases), hemoglobin concentration ≥10.0 g/dl (or ≥8.5 g/dl in case of gastric cancer), serum total bilirubin ≤1.5 times ULN, serum creatinine ≤1.5 mg/dl, neutrophil count ≥1500/μl, and platelet count ≥100 000/μl); and contraception for a designated period. Patients were excluded if they had prior anti-cancer therapies within 4 weeks, blood transfusion and/or colony stimulating factor therapy within 2 weeks, previous tivantinib treatment, familial history of QTc-prolongation syndrome, digestive organ dysfunction affecting tivantinib absorption, symptomatic CNS metastasis, and an uncontrollable complication. Pregnant or lactating women were also excluded.

patient evaluation

Baseline evaluation before the first administration of tivantinib included vital signs, blood count, and serum biochemistry, as well as genotyping and tumor evaluation. Adverse events were assessed continuously throughout the study. Vital signs, blood counts, and serum biochemistry were measured on days 8, 15, 22, 29, and thereafter, every 2 weeks. In addition, electrocardiograms were taken every 2 weeks, and tumor response was evaluated at 4 weeks after the beginning of treatment and every 6 weeks thereafter according to the Response Evaluation Criteria in Solid Tumors version 1.0 [21].

pharmacokinetics analysis

Pharmacokinetic (PK) blood samples were obtained on days 1 and 22 (pre, 1, 2, 4, 8, and 12 h after the first dose of the day), and at trough on days 15 and 29. PK evaluation was conducted in all patients during the DLT observation period [13]. Plasma samples were stored at −70°C until analysis by liquid chromatography/tandem mass spectrometry. Noncompartmental PK parameters were calculated using WinNonlin (Pharsight, Mountain View, CA).

exploratory biomarker studies

HGF, TGF-alpha and amphiregulin ELISA

Plasma samples were obtained on days 1 (baseline), 15, and 29 from 21 patients (16 EMs and 5 PMs) who consented to the exploratory biomarker study. Plasma HGF, TGF-alpha, and amphiregulin concentrations were determined using a HGF, TGF-alpha, and amphiregulin ELISA Development kit (R&D Systems) according to the manufacturer's instructions, respectively. A 50-μl aliquot of plasma was used for the analysis in duplicate and the absorbance of the samples was measured at 450 nm by a 96-well microplate reader (model 680 Microplate Reader, Bio-Rad Laboratories). Statistical analyses were carried out using JMP version 9.0 for Windows (SAS Institute Inc., Cary, NC).

antibody suspension bead array system

The plasma concentrations of angiogenesis-related factors were measured using an antibody suspension bead array, Bio-Plex Pro™ Assays (Bio-Rad Laboratories, Hercules, CA). Human Angiogenesis 9-Plex Panel consisting of angiopoietin-2, follistatin, G-CSF, HGF, interleukin-8, leptin, platelet-

derived growth factor beta polypeptide, platelet endothelial cell adhesion molecule-1 and vascular endothelial growth factor was used [22]. Data were obtained using a Bio-Plex suspension array system* (Bio-Rad Laboratories, Hercules, CA). The assay was carried out according to the manufacturer's instructions. The samples were tested in duplicate and the averages were used for analysis.

results

patient characteristics

A total of 176 Japanese patients with written consent were screened for CYP2C19 genotyping assessment, and the PM phenotype was found in 32 patients (18.2%). A total of 47 patients (EMs: $n = 33$, PMs: $n = 14$) were enrolled into this study from February 2008 to August 2010. After filling up of all cohorts for EMs, the genetic test for CYP2C19 had continued to screen the PMs, to an extent of a number of patients more than the registered 47 patients. There were no notable differences in patient characteristics between the EMs and the PMs who were administered with tivantinib (Table 1). As a result of dose escalation, EMs and PMs were finally assigned to each dose level as described in Table 2.

safety and tolerability

Table 3 presents the list of drug-related adverse events occurring <10% throughout the study, in either or both in EMs and PMs. Tivantinib was generally well tolerated. Leukopenia, neutropenia, anemia, lymphopenia, fatigue, and anorexia were commonly observed events in both EMs and PMs, experienced in >10% of overall patients. Hematologic toxic effects (leukopenia, neutropenia, and anemia) were the major Gr. ≥ 3 drug-related adverse events observed throughout the study for both EMs and PMs. The details of the toxicity, such as occurrence of AEs per dosing cohort or per grading, are provided in Supplementary Table S3, available at *Annals of Oncology* online.

Three patients experienced DLTs which occurred during the first 28 days of tivantinib treatment, to lead to the cohort expansion (Table 2). In cohort 7 of EMs, a colon cancer patient (54 years, F) developed Gr.3 γ-GTP elevation and Gr.4 neutropenia but recovered following G-CSF treatment after tivantinib discontinuation. In the same cohort, a non-small-cell lung cancer (NSCLC) patient (65 years, F) developed Gr.4 neutropenia that returned to Gr.3 within a day with neither tivantinib interruption nor G-CSF treatment. This transient Gr.4 neutropenia was regarded as clinically acceptable by the SRC, which led to the amendment of the protocol. One PM patient (63 years, F, NSCLC) treated with 240 mg bid developed Gr.4 leukopenia and Gr.4 neutropenia, and recovered following G-CSF treatment after tivantinib interruption. Finally, dose escalation was stopped at 360 mg bid for EMs and at 240 mg bid for PMs, respectively, according to the agreement with the SRC, based on the results of safety and PK analysis.

No death was observed throughout the study. A total of six drug-related serious adverse events (SAEs), which prolonged hospitalization, were observed. Two SAEs were Gr.3 febrile neutropenia and Gr.3 pneumonia, which were observed in one EM patient (62 years, M, colon cancer) treated with 360 mg bid, on days 71 and 79, respectively. Those two SAEs were ameliorated with G-CSF treatment during tivantinib interruption. The other four SAEs were the DLTs mentioned above in the EM patient (54 years, F, colon cancer) and the PM patient (63 years, F, NSCLC).

pharmacokinetics and neutropenia

PK parameters of tivantinib were highly variable in each cohort, and therefore, a relationship between the dose level and plasma exposure was not clearly demonstrated (Supplementary Table S1, available at *Annals of Oncology* online). Nonetheless, there were

Table 1. Patient characteristics

	EM	PM	Overall
Patient No.	33	14	47
Age (years; median)	61.0	59.5	61.0
Gender			
Male	22	8	30
Female	11	6	17
Primary cancer			
Non-small-cell lung cancer (NSCLC)	17	8	25
Colon	7	4	11
Gastric	3	1	4
Other	6	1	7
Eastern Cooperative Oncology Group performance status (ECOG PS)			
0	15	6	21
1	18	8	26
No. of prior chemotherapy			
1	1	0	1
2	6	1	7
3	9	5	14
≥4	17	8	25

Table 2. Patient enrollment

Cohort	Dose (mg bid)	No. of patients	
		EM	PM
1	70	3 (including 0 PM)	
2	90	3 (including 1 PM)	
3	120	5 (including 3 PMs)[a]	
4	150	3	3
5	180	4[b]	Not tested
6	240	7[b,c]	7[b,d]
7	300	6[d]	Not tested
8	360	6	Not tested
Overall	33	14	

[a]Protocol amendment required a total of three PMs at least in this cohort.
[b]One patient was replaced due to tivantinib-unrelated adverse events that led to an insufficient compliance to evaluate the safety of the dose level.
[c]Cohort 6 was expanded after the development of two DLTs in cohort 7. Thereafter, amendment of DLT definition allowed the dose escalation up to cohort 8.
[d]Cohort expansion due to DLT.

Table 3. Drug-related adverse events occurring >10% of either or both of EM and PM, through the study

Drug-related adverse events	EM ($n = 33$)		PM ($n = 14$)		Overall ($n = 47$)	
	All grade	≥Gr.3	All grade	≥Gr.3	All grade	≥Gr.3
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
Hematological						
Leukocytopenia	13 (39.4)	4 (12.1)	9 (64.3)	4 (28.6)	22 (46.8)	8 (17.0)
Neutropenia	7 (21.2)	4 (12.1)	8 (57.1)	5 (35.7)	15 (31.9)	9 (19.1)
Anemia	8 (24.2)	3 (9.1)	4 (28.6)	2 (14.3)	12 (25.5)	5 (10.6)
Lymphopenia	5 (15.2)	1 (3.0)	2 (14.3)	0 (0.0)	7 (14.9)	1 (2.1)
Non-hematological						
Fatigue	16 (48.5)	1 (3.0)	3 (21.4)	0 (0.0)	19 (40.4)	1 (2.1)
Anorexia	6 (18.2)	1 (3.0)	2 (14.3)	1 (7.1)	8 (17.0)	2 (4.3)
Nausea	4 (12.1)	0 (0.0)	0 (0.0)	0 (0.0)	4 (8.5)	0 (0.0)
Alopecia	1 (3.0)	0 (0.0)	3 (21.4)	0 (0.0)	4 (8.5)	0 (0.0)
Prolonged QTc	1 (3.0)	0 (0.0)	2 (14.3)	0 (0.0)	3 (6.4)	0 (0.0)
Alanine aminotransferase (ALT)	1 (3.0)	0 (0.0)	2 (14.3)	0 (0.0)	3 (6.4)	0 (0.0)
Rash	1 (3.0)	0 (0.0)	2 (14.3)	0 (0.0)	3 (6.4)	0 (0.0)
Sinus bradycardia	0 (0.0)	0 (0.0)	2 (14.3)	0 (0.0)	2 (4.3)	0 (0.0)

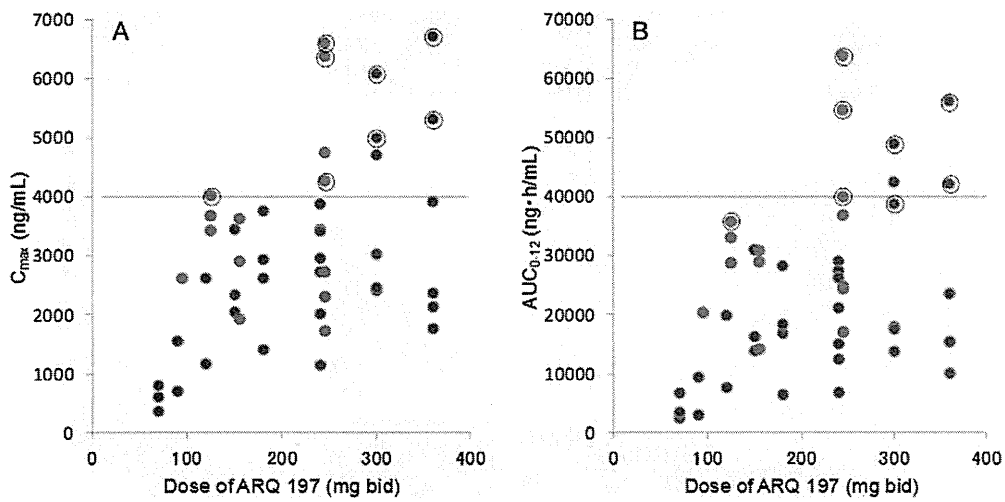

Figure 1. Plasma exposures to tivantinib on day 1. C_{max} (A) or AUC_{0-12} (B) is dotted for each individual (blue dots: EMs, red dots: PMs) treated with the indicated doses. Some circle-surrounded dots indicate a subject who developed Gr. ≥ 4 neutropenia or febrile neutropenia. The lines on the dot blots were putative threshold implying the possible occurrence of neutropenia above the line.

some important findings in the PK analysis. In EMs, mean exposures of tivantinib (AUC_{0-12}) on day 1 were likely to increase dose-dependently up to 300–360 mg. On the other hand, PMs received 240 mg had the mean AUC_{0-12} approximately twofold higher than the AUC_{0-12} in EMs receiving the same dose, which was comparable with or slightly higher than that in EMs at the plateau dose of 300–360 mg bid (Figure 1).

In Figure 1, the patients who experienced Gr.4 neutropenia or febrile neutropenia throughout the study are presented as a surrounded symbol. Most of these subjects were found to distribute above the certain level of tivantinib exposure on day 1, ~>4000 ng/ml of C_{max} (Figure 1A) or >40 000 ng h/ml of AUC_{0-12} (Figure 1B), regardless of CYP2C19 phenotypes.

efficacy

The best overall response included: SD:25 (76%) and PD:8 (24%) in 33 EMs; PR:2 (14%), SD:7 (50%) and PD:5 (36%) in 14 PMs; PR:2 (4%), SD:32 (68%) and PD:13 (28%) in overall patients. A total of eight NSCLC patients experienced a benefit, including two patients with confirmed PR (Figure 2A), and six with long-term SD (>28 weeks, Figure 2B). One of the PR patients possessed wild-type EGFR, and the other possessed mutated EGFR (Supplementary Table S2, available at *Annals of Oncology* online).

PD assessment

We measured the plasma concentrations of HGF from each patient on days 1 (baseline), 15, and 29. Statistical analysis

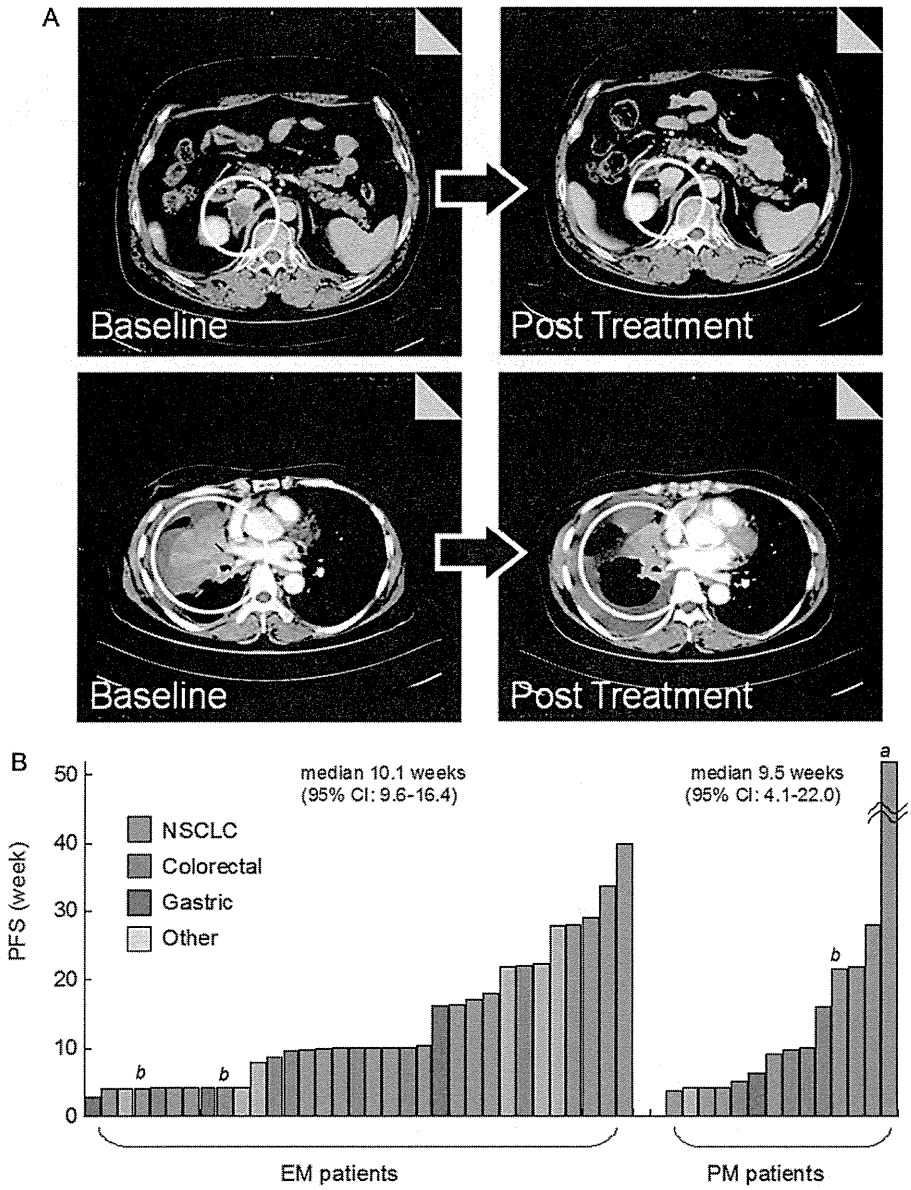

Figure 2. (A) CT scan images of the patients who experienced a confirmed PR. A circle in the images indicates a target lesion. (B) Progression-free survival for individual patient. EM and PM are, respectively, sorted for low and high. Color of the bars indicates his/her cancer type. '*a*' means a patient who was censored for data cut-off at 71.0 weeks with PFS, '*b*' means patients who was censored for treatment discontinuation due to adverse events.

showed that the plasma concentration of HGF after the treatment with tivantinib was significantly lower than that of HGF before the treatment (ANOVA, $P = 0.0339$) (Figure 3). We did not find any significant difference between EMs and PMs regarding the change of plasma HGF level (Supplementary Figure S4, available at *Annals of Oncology* online). On the other hand, there were no significant correlations between the measured biomarkers and tivantinib treatments.

discussion

This is the first clinical trial of tivantinib in Asia. At the commencement of this phase I trial, patients were enrolled without distinguishing between EMs and PMs, similar to the previous clinical trials in Western countries [12, 13]. However, during the course of this trial, we were informed that a clinical pharmacological study in healthy volunteers demonstrated much higher plasma exposure of tivantinib in PMs than EMs at the same dose [12]. Accordingly, considering the fact that ~20% of Asians are PMs [17], the protocol in this Japanese trial was amended to determine the recommended doses of tivantinib separately for EMs and PMs. As a result, tivantinib treatment in Japanese EMs was well tolerated with manageable toxic effects up to 360 mg bid, which is the same dose used in phase II/III studies in Western countries where EMs comprise

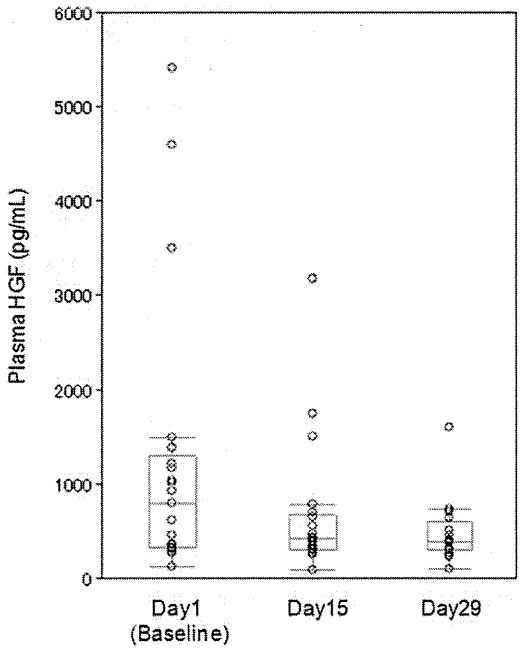

Figure 3. Each dot represents a plasma hepatocyte growth factor (HGF) level on days 1 (baseline), 15, and 29 for individual patients. The box plot shows the median ± confident interval in each day [$n = 21$, except for day 15 ($n = 20$)]. The difference in plasma HGF levels among indicated sampling points was assessed by ANOVA ($P = 0.0339$, 0.1003, respectively).

the large majority of patients. Tivantinib was also well tolerated with manageable toxicit effects in Japanese PMs, up to 240 mg bid. PK analysis demonstrated that not only in EMs at 360 mg but also in PMs at 240 mg, the AUC_{0-12} values were almost the same as or slightly higher than that at 360 mg in Western countries [12, 13], suggesting that 360 mg for EMs and 240 mg for PMs would be sufficient dosages to achieve the plasma exposure level seen in the clinical trials in Western countries [8–11].

Although plasma exposures of tivantinib among Japanese patients were quite variable, it was suggested that CYP2C19 polymorphism may have an impact on the pharmacokinetics of tivantinib. Roughly 1.3–1.8 fold higher AUC_{0-12} was found in PMs than EMs, when administered with tivantinib at the same dose. The increased exposure of tivantinib in PMs was relatively small when compared with other CYP2C19 substrates such as omeprazole, where four- to eightfold higher AUC was found in omeprazole-treated PMs as compared withEMs [23, 24]. The reason for the difference in the fold increase between tivantinib and omeprazole is still unclear, but one possibility would be a change of metabolic conditions in cancer patients. A pharmacokinetics study comparing the omeprazole exposure between cancer patients and non-cancer patients has demonstrated that the CYP2C19 activity was severely compromised in advanced cancer patients with EM genotypes [25]. Another possible explanation for the difference would be involvement of other cytochrome P450 family and/or other nonenzymatical degradation mechanisms in metabolizing CYP2C19 substrates [25]. On the other hand, no clear difference in plasma exposure of tivantinib was found between wild-type CYP2C19 homozygote and wild-type/functionally deficient CYP2C19 homozygote (data not shown).

A notable toxicity related to tivantinib in this Japanese phase I trial was hematologic toxic effects. This finding is consistent with the previous phase I studies conducted in Western countries [12, 13]. Hematologic toxic were commonly found in both EMs and PMs. Our findings demonstrated a good accordance between the occurrence of severe neutropenia and the high plasma level of the parental tivantinib, regardless of CYP2C19 phenotypes. This possible correlation led us to conclude that tivantinib could be administered to both EMs and PMs with a similar profile of adverse events by properly modifying the dose level of tivantinib.

To our knowledge, this is the first report showing that tivantinib administered as a single agent resulted in partial responses in NSCLC patients. It was interesting that PRs were found in NSCLC with both mutated and wild-type EGFR, and this result may suggest that the contribution of c-Met in aggravating NSCLC would be independent of EGFR signaling. In fact, it has been reported that tivantinib in combination with erlotinib showed higher efficacy than erlotinib alone in a phase II trial in NSCLC conducted in Western countries [8]. These results strongly suggested an additive or synergistic effect in NSCLC patients resulting from simultaneous inhibition of EGFR and c-Met. In Asia, a phase III study was recently initiated to evaluate the efficacy of tivantinib in combination with erlotinib in NSCLC patients. In this study, the dose of tivantinib was established following genetic testing for CYP2C19.

Despite the small number of patients, a significant decrease in plasma HGF from baseline was observed in tivantinib-treated patients. Our result indicated a possibility that the decrease of HGF would be a pharmacodynamic biomarker for c-Met inhibitors including tivantinib. In addition, the effect on the plasma HGF level would potentiate the tivantinib-induced inhibition on HGF/c-Met signaling by both inhibiting the kinase activity and reducing the plasma level of HGF. It is reported that higher level of plasma HGF is associated with poor prognosis [26, 27].

In conclusion, the RPIID of tivantinib was similar in both Western countries and Asian countries, when the Asian patient shows an EM phenotype based on CYP2C19 SNPs assessment. However, a lesser dose was recommended for the Asian patient with a PM phenotype of CYP2C19. Hematological toxicity was the most prevalent toxicity for tivantinib in both EMs and PMs, and was well manageable. In further Asian trials, the dose of tivantinib should be individually determined on the basis of pre-treatment testing for CYP2C19 SNPs.

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disclosure

SA is an employee of Kyowa Hakko Kirin Co., Ltd. Other authors have declared no conflicts of interest.

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ORIGINAL ARTICLE

A rare point mutation in the Ras oncogene in hepatocellular carcinoma

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Abstract
Purpose The Ras gene is one of the oncogenes most frequently detected in human cancers, and codes for three proteins (K-, N-, and H-Ras). The aim of this study was to examine the mutations in codons 12, 13 and 61 of the three Ras genes in cases of human hepatocellular carcinoma (HCC).
Methods Paired samples of HCC and corresponding non-malignant liver tissue were collected from 61 patients who underwent hepatectomy. A dot-blot analysis was used to analyze the products of the polymerase chain reaction (PCR) amplification of codons 12, 13, and 61 of K-, N- and H-Ras for mutations.
Results Only one mutation (K-Ras codon 13; Gly to Asp) was detected among the 61 patients. Interestingly, this patient had a medical history of surgery for both gastric cancer and right lung cancer. No mutations were found in codons 12 and 61 of K-Ras or codons 12, 13 and 61 of the N-Ras and H-Ras genes in any of the HCCs or corresponding non-malignant tissues.
Conclusions These findings indicated that the activation of Ras proto-oncogenes by mutations in codons 12, 13, and 61 does not play a major role in hepatocellular carcinogenesis.

Keywords Ras · Mutation · Hepatocellular carcinoma · Sorafenib

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Abbreviations

Asp	Asparagine
Glu	Glutamate
Gly	Glycine
HCC	Hepatocellular carcinoma
Lys	Lysine
PCR	Polymerase chain reaction
TTP	Time to progression
Val	Valine

Introduction

Hepatocellular carcinoma (HCC) is a global health problem, accounting for more than 80 % of all primary liver cancers, and is one of the most common malignancies worldwide [1]. Most patients with HCC also present with concomitant cirrhosis, which is the major clinical risk factor for hepatic cancer, and results from alcoholism or infection with the hepatitis B or hepatitis C virus. Primary liver malignancies (95 % of which are HCC) are the third and fifth leading causes of cancer death among males and females, respectively, in Japan [2]. Both liver resection and liver transplantation are potentially curative treatments for HCC [3–5]. Although other treatment options, including percutaneous radiofrequency ablation or chemolipiodolization are also available, there is no standard systemic therapy for advanced cases.

Sorafenib (BAY 43-9006, Nexavar) is a novel oral kinase inhibitor that targets multiple tyrosine kinases in vivo and in vitro, and is widely used for HCC [6]. The main targets of sorafenib are the receptor tyrosine kinase pathways which are frequently deregulated in cancer, such as the Ras pathway. The Ras pathway represents a dominant signaling network promoting cell proliferation and

survival. The binding of different growth factors (e.g. epidermal growth factor: EGF) to their receptors (e.g. epidermal growth factor receptor: EGFR) induces the activation of Ras, which in turn activates c-raf, MEK and ERK. Phosphorylated ERK in the nucleus activates transcription factors that regulate the expression of genes involved in cell proliferation and survival.

A phase II trial involving 137 patients with advanced HCC showed that sorafenib induced partial responses in less than 5 % of patients, but the observed median survival of 9.2 months with a median time to progression of 5.5 months was classified as evidence of potential clinical benefit, since the expected median survival of these patients is 6 months [7]. Consequently, a large phase III clinical trial (SHARP) was conducted in 602 patients with advanced HCC. The results showed a 31 % decrease in the risk of death, with a median survival of 10.6 months in the sorafenib arm versus 7.9 months for placebo [8]. In addition, sorafenib showed a significant benefit in terms of the time to progression (TTP) as assessed by independent radiological review, with a median TTP of 5.5 months for the sorafenib and 2.8 months for the placebo arm.

Because Ras is one of the targets of sorafenib, it is important to determine whether mutations in the Ras gene result in the activation of the Ras/MAPK pathway in human HCCs. However, the relationship between Ras mutations and human HCC has not been fully evaluated. The present study was designed to investigate K-, N- and H-Ras (*KRAS, NRAS, HRAS*) somatic mutations in human HCC.

Materials and methods

Patients and tumor samples

Tumor tissue samples were obtained from 61 Japanese patients who underwent surgical resection for HCC during the period between December 1989 and April 1992 in the Department of Surgery and Science, Kyushu University Hospital, Fukuoka, Japan. Surgically resected tissue samples were frozen at −80 °C immediately after resection and were stored until use in this study. Written informed consent was obtained from all patients examined, and the current study was approved by the Kyushu University ethics committee.

DNA preparation and detection of Ras point mutations

High molecular weight DNA was isolated from frozen tumor samples, as described elsewhere [9]. Selective amplification of the Ras gene sequence was done using a PCR technique. The nucleotide sequences of the primers used are listed in Table 1. The PCR was performed at

Table 1 Ras gene primers used in this study

Gene/codon	Length (bp)	Sequence	
KRAS/12, 13	108	Forward	GACTGAATATAAACTTGTGG
		Reverse	CTATTGTTGGATCATATTCG
KRAS/61	128	Forward	TTCCTACAGGAAGCAAGTAG
		Reverse	CACAAAGAAAGCCCTCCCCA
HRAS/12, 13	63	Forward	GACGGAATATAAGCTGGTGG
		Reverse	TGGATGGTCAGCGCACTCTT
HRAS/61	73	Forward	AGACGTGCCTGTTGGACATC
		Reverse	CGCATGTACTGGTCCCGCAT
NRAS/12, 13	109	Forward	GACTGAGTACAAACTGGTGG
		Reverse	CTCTATGGTGGGATCATATT
NRAS/61	103	Forward	GGTGAAACCTGTTTGTTGGA
		Reverse	ATACACAGAGGAAGCCTTCG

bp base pairs

96 °C to denature the DNA (1 min), at 55 °C (*NRAS*), 57 °C (*KRAS*), 62 °C (*HRAS*) to anneal the primer (30 s), and at 72 °C to synthesize DNA (10 s to 1 min) using Taq DNA polymerase for 35–40 cycles in a DNA thermal cycler (Perkin-Elmer-Cetus). Amplified DNA samples were spotted onto nylon membranes (Hybond N+) for the hybridization analysis. All of the DNA isolated from the 61 tumor samples and the corresponding non-malignant liver tissues were screened for activated point mutations in codons 12, 13, and 61 of all three Ras genes using an oligonucleotide specific for the different sequences. The filters were prehybridized for 1 h at 55 °C in solution A (3.0 M tetramethylammonium chloride, 50 mM Tris-HCI, 2 HIMEDTA, 0.1 % SDS, 5× Denhardt's solution, 100 fg/ml denatured herring sperm DNA), and hybridized for l h at 55 °C in the same solution with 5 pmol ^{32}P-labeled probe. These filters were washed twice in 0.3 M NaCl, 0.02 M NaH2PO4, 2 mM EDTA and 0.1 % SDS at room temperature for 5 min, and in solution A without Denhardt's solution and herring sperm DNA, once for 5 min at room temperature and twice for 10 min at 60 °C. These filters were then exposed to Kodak XAR5 film. Human cancer cell lines carrying Ras genes mutations were used as positive controls. The colon cancer cell lines: SW620 (*KRAS* codon 12 GTT:Val), LSI80 (*KRAS* codon 12 GAT:Asp), and LOVO (*KRAS* codon 13 GAC:Asp) were obtained from the Japanese Cancer Research Resources Bank, and KMS4 (*KRASs* codon 12 TGT:Cys) was provided by Dr. Sugio (Institution?).

Results

The age of the 61 patients ranged from 43 to 79 years (average, 64.1 years), and 46 were males and 15 were

females. The positive rate of hepatitis surface B antigen was 12.9 %, and the positive rate of anti-hepatitis C virus antibody was 72.7 %. The mean tumor size was 4.47 cm.

One of the 61 HCCs (1.6 %) carried a point mutation, which was a G to A transition at codon 13 of the *KRAS* gene (Fig. 1). DNA extracted from the corresponding non-malignant liver tissue had the normal codon, suggesting that mutational activation of K-ras was involved in the malignant transformation in this case. This patient was positive for anti-hepatitis C virus antibodies, and was classified to have Child-Pugh A disease. The diameter of this patient's tumor was 12 cm, and the tumor was composed of well to moderately differentiated hepatocellular carcinoma. Interestingly, this patient had undergone surgery for gastric cancer 18 years before and lung cancer 12 years before the surgery for HCC.

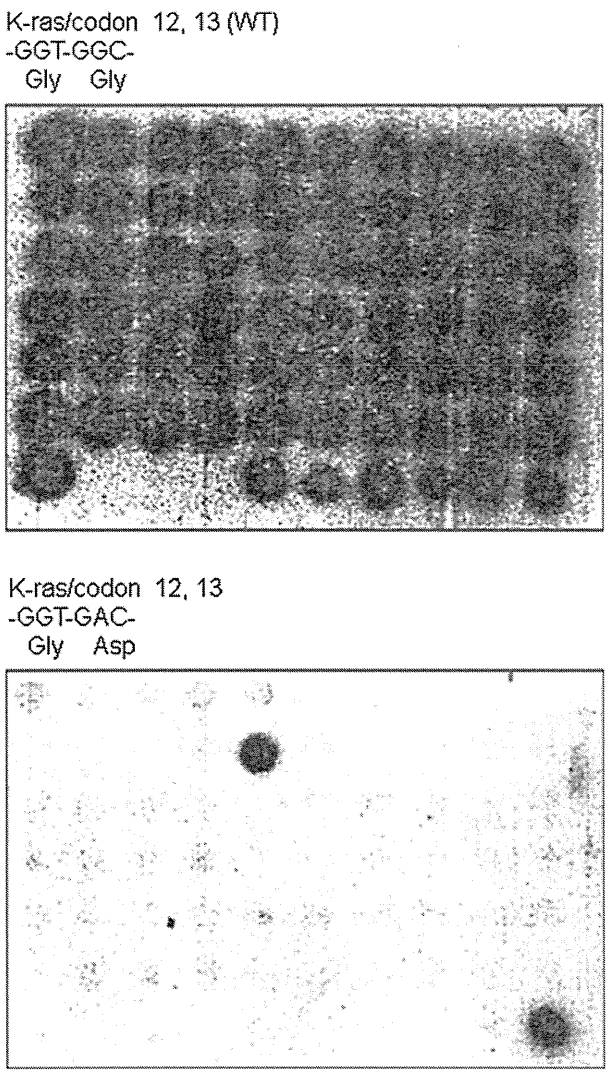

Fig. 1 Detection of a *KRAS* gene mutation in a patient with hepatocellular carcinoma. PCR-amplified DNA from 61 tumor samples was dotted onto nylon membranes and hybridized to a ^{32}P-labeled oligonucleotide probe. *WT* wild type *KRAS*

No mutational activation was found in codons 12 and 61 of *KRAS* or codons 12, 13 and 61 of the *NRAS* and *HRAS* genes in any of the HCCs or corresponding non-malignant tissue samples.

Discussion

This study examined 61 HCC tissues and their corresponding non-malignant liver tissues for a somatic mutation in codons 12, 13, and 61 of the *KRAS*, *HRAS*, or *NRAS* genes, which are known hot spots in various malignancies. However, the study showed the only one of the 61 HCCs (1.6 %) had a somatic mutation in codon 13 of the *KRAS* gene, indicating that Ras gene mutations do not appear to be related to the pathogenesis of most HCCs.

There have been several reports with small sample sizes regarding Ras gene mutations in HCC (Table 2). Most have reported that somatic mutations of the Ras gene in HCCs are uncommon, similar to the current study. Tsuda et al. [10] found only two tumors with Ras point mutations in surgically resected specimens from 30 HCC patients. In their patients, codon 12 of *KRAS* was altered from GGT, coding for Gly, to GTT, coding for Val in one case, and codon 61 of *NRAS* was altered from CAA, coding for Glu, to AAA, coding for Lys, in the other case. Tada et al. analyzed the mutations of the three Ras genes in 23 primary hepatic malignant tumors (12 hepatocellular carcinomas, nine cholangiocarcinomas, and two hepatoblastomas). Point mutations in *KRAS* codon 12 or *KRAS* codon 61 were found in 6 of the 9 cholangiocarcinomas. In contrast, there were no point mutations in any of 12 HCCs or two hepatoblastomas in codons 12, 13, or 61 of the Ras genes. The authors concluded that Ras gene mutations are not related to the pathogenesis of HCC, but play an important role in pathogenesis of cholangiocarcinoma.

Sorafenib is the first molecule with specific targets involved in the pathogenesis of HCC that has become available for routine clinical use. It is an orally applicable

Table 2 Reported Ras gene mutations in HCC patients

Author [references]	No. of patients	Ras gene mutation		
		KRAS	*NRAS*	*HRAS*
Tsuda et al. [10]	30	1 (codon 12)	1 (codon 61)	0
Tada et al. [14]	12	0	0	0
Ogata et al. [15]	19			2
Challen et al. [16]	19	1 (codon 61)	3 (codon 61)	0
Leon et al. [17]	12	1 (codon 61)	0	0
This study	61	1 (codon 13)	0	0

multi-kinase inhibitor that acts by blocking tumor cell proliferation and angiogenesis through the inhibition of serine/threonine kinases [11]. Sorafenib can increase survival by up to 3 months in patients with advanced HCC and acceptable liver function [8]. On the other hand, severe side effects have been reported with sorafenib, including hand-foot skin reactions or liver dysfunction [7, 8]. Therefore, it is important to identify prognostic markers and to establish the proper selection criteria for using sorafenib. Mutations of the Ras genes in cases of HCCs were systemically evaluated in this study because the Ras signaling pathway is the main target of sorafenib. The results indicated that mutational activation of Ras genes is uncommon in the pathogenesis of HCCs. Caraglia et al. [12] reported that the presence of phosphorylated ERK activity in peripheral blood mononuclear cells is valuable for predicting the response to sorafenib therapy in HCC patients. An in vitro study confirmed that phosphorylated ERK was a potential biomarker predicting the sensitivity of HCC to sorafenib [13]. Therefore, a mutation in the RAF/MEK/ERK pathway may be involved in the drug resistance to sorafenib, rather than a Ras mutation.

In summary, only one of 61 HCCs (1.6 %) in the present study carried a point mutation, which was a G to A transition in codon 13 of the *KRAS* gene. No mutational activation was found in codons 12 and 61 of *KRAS* or in codons 12, 13 and 61 of the *NRAS* or *HRAS* genes in any of the HCCs or corresponding non-malignant tissue samples. These findings suggested that Ras gene mutations are not related to the pathogenesis of most HCCs. The signaling pathways downstream of Ras should be examined to identify markers to predict a response to sorafenib.

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Conflict of interest None of the authors has any conflict of interest.

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