Table 1. Correlation between clinicopathologic factors and overall survival in patients with resected hepatic and pulmonary metastases from colorectal cancer

	No.	Median survival (mo)	*P* value
Primary colorectal lesion			
Location			
rectum	13	52.7	0.03
colon	17	38.6	
TNM classification			
I	1	88.9	0.02*
II	10	48.9	
III	15	38.8	
IV	4	14.6	
Lymph node metastasis			
absent	11	54.8	0.64
present	19	32.8	
Histological type of adenocarcinoma			
well or moderately differentiated	28	38.7	0.77
poorly differentiated and others	2	41.7	
Hepatic metastases			
First hepatectomy			
Number of tumors			
1	18	40.8	0.26
≥2	12	36.8	
Maximum size of the tumor (cm)			
<3	14	40.0	0.03
≥3	16	35.8	
Distribution of metastases			
unilobar	20	40.8	0.36
bilobar	10	36.8	
Lymph node of hepatoduodenal ligament			
negative	29	38.8	0.02
positive	1	13.9	
All hepatectomies			
Total number of tumors			
<3	19	38.6	0.79
≥3	11	38.8	
Maximum size of the tumor (cm)			
<3	13	38.8	0.08
≥3	17	38.6	
Distribution of metastases			
unilobar	17	43.0	0.49
bilobar	13	34.8	
Pulmonary metastases			
First pulmonary resection			
Number of tumors			
1	18	47.9	0.31
≥2	12	27.1	
Maximum size of the tumor (cm)			
<3	21	34.8	0.69
≥3	9	38.8	
Distribution of metastases			
unilobar	24	42.1	0.68
bilobar	6	27.1	
Hilar or mediastinal lymph node			
negative	28	36.7	0.89
positive	2	43.6	
All pulmonary resections			
Number of tumors			
<3	22	38.7	0.92
≥3	8	44.8	
Maximum size of the tumor (cm)			
<3	19	34.8	0.93
≥3	11	38.8	
Distribution of metastases			
unilobar	21	41.1	0.97
bilobar	9	30.8	
CEA level at initial recurrence (ng/ml)			
<50	25	38.7	0.34
≥50	5	33.0	
Disease-free interval from resection of primary tumor			
<1 year	19	38.8	0.23
≥1 year	11	38.6	
Simultaneous detection of hepatic and pulmonary recurrences			
yes	11	34.8	0.35
no	19	38.8	
Initial metastasis in the lung			
yes	3	54.8	0.72
no	27	38.6	
Total number of liver and lung resections			
2	13	33.0	0.50
≥3	17	54.3	

CEA, carcinoembryonic antigen.
*Stage I, II or III versus Stage IV.

according to Couinaud's anatomical classification (18)) and 40 pulmonary resections (32 partial resections, seven lobectomies and one pneumonectomy) were performed on the 30 patients. The average number of operations performed for hepatic or pulmonary metastases per patient was 2.8. Three operations were performed on 11 patients, four operations on four patients each and five operations on two patients each.

There was no perioperative mortality. Five complications were observed: two cases of biliary leak and one case each of portal vein thrombosis after hepatectomy, wound infection and air leak after pulmonary resection.

The location of initial metastasis was lung in three patients, liver in 19, and both liver and lung in eight. Eleven patients experienced hepatic and pulmonary metastases detected simultaneously.

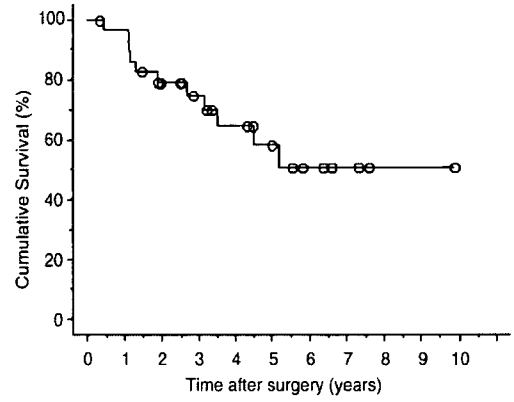

Figure 1. Cumulative survival curves for 30 patients who underwent resections for both hepatic and pulmonary metastases of colorectal cancer.

Recurrence after Surgical Resections for Hepatic and Pulmonary Metastases

Among 30 patients who underwent surgical resections for hepatic and pulmonary metastases, 25 developed recurrences when recurrence was defined as the first recurrent disease after at least one resection each for hepatic and pulmonary metastases. Locations of recurrences were as follows: lung in 11 patients, liver and lymph node in four each, both liver and lung in three, peritoneum, local recurrence and brain in one each. Re-resection could be performed in 15 of the 25 patients. Of the remaining 10 patients, eight received systemic chemotherapy, one each received radiation therapy and best supportive care.

Survival

Survival time was calculated from the date of the first metastasectomy for the second organ metastasized (liver or lung).

Actuarial overall survival was 58% at 5 years with a median survival of 39 months (Fig. 1). Disease-free survival was 56% at 1 year and 8% at 3 years, with a median recurrence-free survival of 13 months. Nine 5-year survivors were observed and eight of the nine patients are still alive without disease. Of the nine 5-year survivors, six had undergone three operations and one had undergone four operations.

When survival time was calculated from the date of the first metastasectomy for the first organ, actuarial overall survival was 70% at 5 years with a median survival of 60 months.

Correlation between Clinicopathologic Factors and Overall Survival

To find prognostic factors for survival after resection of hepatic and pulmonary metastases, clinicopathologic factors and overall survival calculated from the date of the first metastasectomy for the second organ were analyzed in 30 patients (Table 1). Primary colon carcinoma ($P = 0.03$), stage IV in TNM classification ($P = 0.02$), maximum size of hepatic tumor >3 cm at initial hepatectomy ($P = 0.03$), and lymph node metastasis of the hepatoduodenal ligament ($P = 0.02$) were significantly associated with poor overall survival. Whether hepatic and pulmonary metastases were detected simultaneously or sequentially was not correlated with survival ($P = 0.35$). Neither a disease-free interval of less than 1 year from resection of the primary tumor nor initial metastasis in the lung affected survival.

We examined the independent predictive value of the aforementioned factors on overall survival (Table 2). Lymph node metastasis of the hepatoduodenal ligament was excluded from the analysis because only one of the 30 patients had the factor. Primary colon carcinoma (Fig. 2A), stage IV in TNM classification (Fig. 2B), and maximum size of hepatic tumor >3 cm at initial hepatectomy (Fig. 2C) had predictive value for decreased overall survival after resection of hepatic and pulmonary metastases from colorectal cancer.

Comparing clinicopathological factors of patients with primary colon carcinoma and those of patients with primary rectal carcinoma, maximum size of pulmonary tumors (2.6 ± 1.6 cm versus 1.7 ± 0.7 cm) was significantly larger and prethoracotomy CEA level (18.2 ± 23.8 ng/ml versus 5.3 ± 5.4 ng/ml) was significantly higher in patients with primary colon carcinoma. The interval from primary resection to the first pulmonary resection tended to be longer in patients with primary colon carcinoma than in patients with primary rectal carcinoma (25.7 months versus 17.1 months, median).

DISCUSSION

Results of this study indicate that aggressive multiple resections for hepatic and pulmonary metastases of colorectal carcinoma are safe and contribute to long-term survival in some patients.

Hepatic and pulmonary metastases may be detected sequentially or simultaneously in patients with colorectal carcinoma. Although two distant organs are affected by the

Table 2. Multivariate analyses of factors affecting overall survival in patients with resected hepatic and pulmonary metastases from colorectal cancer

	Hazard ratio (95% CI)	*P* value
Location of primary tumor		
Rectum	—	0.01
Colon	8.74 (1.53—49.91)	
TNM classification of primary tumor		
I, II, III	—	0.03
IV	11.37 (1.34—96.53)	
Maximum size of tumor at first hepatectomy (cm)		
<3	—	<0.01
≥3	14.47 (2.33–89.85)	

CI, confidence interval; CEA, carcinoembryonic antigen.

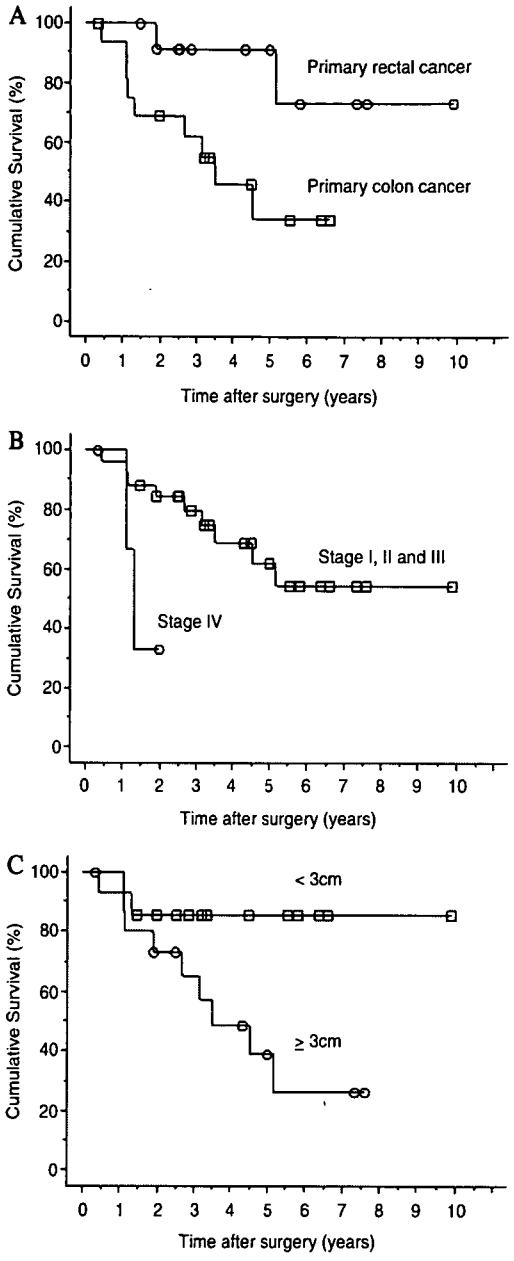

Figure 2. Cumulative survival curves after resections for hepatic and pulmonary metastases of colorectal cancer according to (A) location of primary tumor, (B) stage in TNM classification, and (C) maximum size of hepatic tumor at initial.

disease, several studies have demonstrated the efficacy of resections for both hepatic and pulmonary metastases (2–14). However, because of the frequent recurrences after resections, the best selection criteria for resection have not been established.

Lenhart *et al.* reported a disease-free survival of only 24% at 2 years in patients who underwent sequential hepatic and pulmonary resections for colorectal metastases (9). In the present study, the 2-year disease-free survival rate after the first metastasectomy for the second organ was also 24% with a median disease-free survival of only 13 months. The best treatment strategy for the recurrences after hepatic and pulmonary resections is obscure. However, only surgical removal of metastases offers a chance of cure. Aggressive repeat metastasectomy has been applied for recurrences after hepatic and pulmonary resections in our institution.

For the 30 patients of the present study, 45 hepatectomies and 40 pulmonary resections were performed and 17 patients received three or more resections with a maximum of five resections. Overall survival after the first metastasectomy for the second organ was 58% and nine 5-year survivors were observed. Surprisingly, seven of the nine 5-year survivors had undergone three or more resections. When survival time was calculated from the date of the first metastasectomy for the first metastasized organ, overall survival reached 70% at 5 years with a median survival of 60 months in the present study. Little is available on the result of repeat metastasectomy for recurrences after hepatic and pulmonary resections. Our results of long-term survival after hepatic and pulmonary resections in spite of frequent recurrences support the view that patients who can undergo resections for both hepatic and pulmonary metastases of colorectal cancer are in a selected population but can sometimes survive a long time with multiple metastasectomies. Interestingly, a recent study by Shah *et al.* also reported 74% 5-year survival rate after multidisciplinary surgical metastasectomies for colorectal cancer (19). The strategy and results of Shah *et al.* were similar to ours. However, while a majority of the patients received adjuvant chemotherapy after metastasectomies in Shah's study, no patient underwent adjuvant chemotherapy in the present study. These results indicate that the strategy of aggressive multiple metastasectomies count more than postoperative chemotherapies in the treatment for very restricted population of patients.

We found three factors for poor prognosis: size of hepatic tumor >3 cm at the first hepatectomy, primary colon carcinoma and stage IV tumor.

Maximum size of the hepatic tumor has been reported to be one of the important prognostic factors after hepatic resections for colorectal hepatic metastasis (20,21). This factor could affect prognosis in this population.

The reason for poor prognosis in patients with primary colon cancer is unknown. Patients with primary colon cancer had larger pulmonary tumors, higher CEA levels at the first pulmonary resection and relatively longer intervals from primary resection to the first pulmonary resection than patients with primary rectal cancer. A higher prethoracotomy CEA level was a factor of poor prognosis after hepatic and pulmonary resections in several studies (6,11). However, the reason why patients with primary colon cancer had more advanced pulmonary tumors than those with primary rectal cancer was unclear. A 'cascade' hypothesis based on the anatomy of the draining veins from the colon and rectum suggests that pulmonary metastasis in patients with primary colon carcinoma might come from hepatic metastasis with progressive site-induced change; however, pulmonary metastasis in patients with primary rectal carcinoma might come directly from the primary tumor, which seemed to be compatible with our results (22–24). However, the prognostic power of primary tumor location has not been demonstrated yet in patients with resected colorectal pulmonary metastasis (25–27); further examinations are needed to verify the hypothesis.

Neither the large size of the hepatic tumor nor primary colon carcinoma might influence the selection criteria for hepatic and pulmonary resections, because several long-term survivors were observed, even among patients with those factors.

Patients with stage IV disease had a poorer prognosis and showed no long-term survival. However, stage IV itself should not be considered as a contraindication for resections because the follow-up duration of patients with stage IV was short and the poor prognosis in stage IV was not consistent with the result that the disease-free interval from primary resection showed no correlation with prognosis.

Other factors such as synchronous metastasis (5), bilateral or multiple lung metastases (5,7), multiple liver metastases (8), short disease-free interval (8), simultaneous liver and lung metastases (10), mediastinal nodes involvement (11), primary histology (12) and high levels of both CEA and CA19-9 before metastasectomy (13) have been reported as prognostic factors after hepatic and pulmonary metastasectomy of colorectal cancer. Among those factors, whether the timing of the detection of hepatic and pulmonary metastases influences prognosis after resections has been an issue. In the present study, none of the aforementioned factors, including the timing of the detection of the metastases, showed any prognostic value. Based on our results, no single factor that contraindicated resections for hepatic and pulmonary metastases of colorectal cancer was identified. Thus, surgical resections might be the best option when both hepatic and pulmonary metastases are resectable in colorectal cancer. However, treatment for patients with several poor prognostic factors for multiple resections is still unknown.

The reason for the high survival rate 5 years after resections for hepatic and pulmonary metastases in our study might be partly explained by precise intrathoracic and abdominal examinations using helical computed tomography (28,29). However, it can not be denied that patients who can undergo both hepatic and pulmonary metastasectomy for colon cancer might have unique characteristics in some factors. For example, there may be some unique host-tumor interaction, considering the rare possibility of both hepatic and pulmonary resections for colorectal metastases and the surprisingly high survival rate after the metastasecomies in spite of multiple, multiphase and multi-organ metastases. The aforementioned hypothesis is supported by the fact that excellent survival in the present study was achieved, unexpectedly, without any help of adjuvant chemotherapy, although adjuvant chemotherapy after pulmonary or hepatic metastasectomy is a potential treatment for improving the prognosis of patients with colorectal cancer. Further investigation to clarify the reason for the good prognosis of this population might elucidate the mechanisms of metastases in colorectal cancer.

A limitation of our study is the relatively small population, because patients who can undergo resections for both hepatic and pulmonary metastases of colorectal carcinoma are rare. There is some possibility that correlations between several clinicopathological factors such as positive lymph nodes of the hepatoduodenal ligament, hilus pulmonis, or mediastinum and survival after resections could not be sufficiently validated because of the small cohort. A large multi-institutional study is recommended to verify the correlation.

In conclusion, multiple resections for hepatic and pulmonary metastases of colorectal cancer are safe and effective. Surgical resections could be the best option for resectable hepatic and pulmonary metastases in colorectal cancer.

Acknowledgments

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Conflict of interest statement

None declared.

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Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma

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Sorafenib is an orally active multikinase inhibitor that targets serine and threonine, and tyrosine kinases that are involved in tumor-cell signal transduction and tumor angiogenesis. This phase I trial was conducted to evaluate the pharmacokinetics (PK), safety, and preliminary efficacy of sorafenib in Japanese patients with hepatocellular carcinoma (HCC) with underlying liver dysfunction. Patients with unresectable HCC, Child–Pugh status A or B, and adequate organ functions were treated. A single dose of sorafenib was administered, followed by a 7-day wash-out period, after which patients received either sorafenib 200 mg (cohort 1) or 400 mg (cohort 2) twice daily. The PK were investigated after a single dose and during steady state. The efficacy was evaluated using the Response Evaluation Criteria in Solid Tumors. A total of 27 patients were evaluated for PK, safety, and efficacy. Although both area under the concentration–time curve for 0–12 h and maximal concentration at steady state were slightly lower in Child–Pugh B patients than in Child–Pugh A patients, the difference was not considered to be clinically relevant. Common adverse drug events included elevated lipase, amylase, rash or desquamation, diarrhea, and hand–foot skin reaction. A dose-limiting toxicity of hand–foot skin reaction was observed in one patient (cohort 2). Among the 24 patients evaluable for tumor response, one patient (4%) achieved a partial response, 20 (83%) had stable disease, and three (13%) had progressive disease. Sorafenib demonstrated a favorable tolerability and safety profile in Japanese HCC patients. Moreover, promising preliminary antitumor activity has been observed. Finally, there were no clinically relevant differences in PK between Child–Pugh A and B patients. (*Cancer Sci* 2008; 99: 159–165)

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Surgery and local ablation therapy, including radiofrequency, are considered curative treatment for HCC.[1–3] Transcatheter arterial chemoembolization (TACE) has been applied to patients with advanced incurable HCC.[3–5] The majority of patients, however, have recurrence or metastasis after these treatments. Although systemic therapy, including chemotherapeutic agents, is available for metastatic or TACE-refractory advanced HCC, the prognosis remains poor. No standard systemic therapy that prolongs survival has been identified.

Sorafenib (BAY 43-9006; Bayer HealthCare Pharmaceuticals, West Haven, CT, USA) was discovered based on its potent activity against Raf kinase in a battery of biochemical, cellular, and *in vivo* assays.[6,7] Extensive mechanism of action studies have shown that sorafenib may inhibit tumor growth through multiple mechanisms: by inhibiting tumor-cell proliferation that is dependent on activation of the mitogen-activated protein kinase (MAPK) pathway, and by inhibiting tumor angiogenesis through inhibition of vascular endothelial growth factor receptor (VEGFR)-2 and platelet-derived growth factor receptor (PDGFR)-β. Some evidence points to the MAPK signal-transduction pathway as playing an important role in tumor growth and progression in HCC.[8] Published data suggest that vascular endothelial growth factor (VEGF) also plays a critical role in angiogenesis of HCC, which is important for the growth and progression of HCC.[9] Sorafenib has been investigated in various solid tumors in clinical studies[10–15] and has been approved in many countries for the treatment of renal cell carcinoma. Promising results with sorafenib were recently observed in a phase II study in HCC patients.[15]

Various factors, such as liver function or disease extension, influence treatment selection and prognosis for HCC.[2,3,16] Etiology, underlying condition, and treatment for HCC vary across countries or regions.[2,3,17] Most HCC patients in Japan have hepatitis or cirrhosis due to hepatitis B or C virus[2] and suffer from complications of liver dysfunction, with potential changes in the activity of metabolic enzymes, a reduction in blood flow in the liver, or protein-binding ability due to low serum albumin. However, the degree of influence of these factors on the pharmacokinetics (PK) and tolerability of sorafenib in Japanese patients with HCC is unknown. A phase I study in Japanese patients with advanced solid tumors was conducted before the present study,[18] and found that sorafenib at 400 mg b.i.d. was well tolerable and recommended for phase II studies based on safety and efficacy data. To investigate the effect of liver dysfunction and its complications on the PK, safety, and tolerability of sorafenib in Japanese patients with HCC, a phase I study was conducted. The primary objective of the present study was to evaluate the PK of sorafenib, and the secondary objectives were to evaluate the safety and tolerability of sorafenib, tumor response, time to progression (TTP), and overall survival in Japanese patients with HCC.

Materials and Methods

Patient eligibility. The eligibility criteria for enrolment in the study were: (1) histologically confirmed HCC; (2) unresectable and incurable with ablation therapy or TACE; (3) age ≥ 20 years; (4) Eastern Cooperative Oncology Group performance status of 0 or 1; (5) adequate bone marrow (absolute neutrophil count ≥ 1500 cells/mm^3, platelet count ≥ 75 000 cells/mm^3, and hemoglobin ≥ 8.0 g/dL), coagulation (prothrombin time ≤ 1.5 × upper limit of normal [ULN] and activated partial thromboplastin time ≤ 1.5 × ULN), renal function (serum creatinine concentration ≤ 1.5 × ULN), and hepatic function (serum total bilirubin level ≤ 3.0 mg/dL, serum aspartate and alanine transaminase levels ≤ 5.0 × ULN); (6) cirrhotic status of Child–Pugh A or B; (7) life expectancy of at least 12 weeks; and (8) written informed consent from the patient.

Exclusion criteria included clinically evident congestive heart failure, serious cardiac arrhythmias, active or symptomatic coronary artery disease or ischemia, active clinically serious infections, seizure disorder requiring medication, history of organ allograft, prior malignancy (any cancer treated curatively

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>3 years prior to entry was not excluded), metastatic brain or meningeal tumors, anticancer therapy within 3 months of study entry, and pregnancy or lactation for women. This protocol was approved by the National Cancer Center's institutional review board for clinical investigation with the provisions of the Declaration of Helsinki, Good Clinical Practice guidelines, and local laws and regulations.

Treatment methods. The dose for the first cohort was 200 mg bid sorafenib, and the dose for the second cohort was escalated to 400 mg bid. To investigate the PK profile of sorafenib, including its elimination phase, a single dose was given as one-time administration followed by a 7-day wash-out period. Subsequently, the drug was given twice daily for 28 days without a resting period (cycle 1). Either 200 mg or 400 mg sorafenib was given to all patients orally twice daily, in the morning and in the evening (every 12 h as far as possible). Patients were allowed to continue on sorafenib after cycle 1 if they consented to continue, and no intolerable adverse event was experienced, as assessed by investigators. Treatment was continued until disease progression, intolerable adverse event, or consent of withdrawal.

Examination and observation for safety was conducted every 2 weeks, and administration of the drug was to be terminated immediately when the patient met the criteria for removal from the study, described in this protocol with due consideration for the patient's safety.

Study design. The present study was a non-randomized, uncontrolled, non-blinded, single-center phase I study to investigate the PK, safety, and tolerability of sorafenib in Japanese patients with HCC. The dose level investigated in this study was 200 mg bid for the first cohort and 400 mg bid for the second cohort. Twelve patients, including six with Child–Pugh A and six with Child–Pugh B, were to be enrolled in each cohort. Tolerability was evaluated at the end of cycle 1 by Child–Pugh classification. If less than two out of six patients experienced dose-limiting toxicity (DLT) in the 200-mg bid cohort, the study would proceed to the 400-mg bid cohort. DLT that needed dose modification was defined as: (1) grade 3 and grade 4 non-hematological toxicity, except for pancreatic enzyme abnormality and hand–foot skin reaction; (2) grade 4 pancreatic enzyme elevation with values that persisted on two consecutive determinations with a 3-day interval, or clinical and/or imaging findings of pancreatitis, or pancreatic adverse event considered to be life threatening, or having a high risk of serious or chronic disorders; (3) severe hand–foot skin reaction, moist desquamation, ulceration, blistering, or severe pain of the hands or feet, or severe discomfort that caused the patient to be unable to work or carry out the activities of daily living; (4) grade 4 neutropenia (absolute neutrophil count less than 500/μL) for 7 days duration; (5) grade 4 neutropenia of any duration with fever of 38.5°C and above; and (6) platelet count < 25 000 cells/mm^3. Toxicity was graded according to the National Cancer Institute common toxicity criteria version 2.0. The independent safety committee for this study gave advice on the evaluation of tolerability of the dose level and the cohort transition.

Pharmacokinetics. All patients who received at least one dose of study medication were included in the PK analysis. Blood samples for the determination of plasma concentrations of sorafenib (and its metabolites) were collected prior to drug administration, as well as 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, and 120 h after single-dose administration. For the first cycle, blood was sampled prior to the first dosing on days 1, 4, 7, 10, 14, 21, and 28, along with 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after the first dose on days 14 and 28. Urine voided up to 48 h after single administration was collected.

Concentrations of sorafenib and its metabolites in plasma and urine were determined using validated liquid chromatography and tandem mass spectrometry methods. Plasma PK parameters were calculated by non-compartment analysis by the KINCALC program (Bayer HealthCare Pharmaceuticals).[10] Primary plasma PK parameters were area under the concentration–time curve (AUC), AUC for 0–12 h (AUC_{0-12}), and maximal concentration (C_{max}). Plasma concentrations and PK parameters were analyzed by dose and Child–Pugh classification.

Clinical assessments. Physical examination, complete blood cell counts, serum chemistries, and urinalysis were carried out at baseline and at least twice monthly after initiating treatment with sorafenib. Patients underwent dynamic computed tomography (CT) to evaluate tumor response at baseline, the end of cycle 1, and every two cycles thereafter. CT was carried out by obtaining contiguous transverse sections with the helical scanning method at a section thickness of 5 mm. Tumor evaluation was assessed using the Response Evaluation Criteria in Solid Tumours (RECIST).[19]

Statistical analysis. The data were analyzed using SAS (SAS Institute, Cary, NC, USA). The safety and efficacy were evaluated on an intention-to-treat basis. Progression-free survival was calculated from the first day of treatment until evidence of tumor progression, clinical progression, or death due to any cause. Overall survival was calculated from the first day of treatment until death due to any cause. Survival data were analyzed using the Kaplan–Meier method.

Results

Patient characteristics and treatments. From April 2004 through January 2005, a total of 27 patients were enrolled in the present study. Thirteen patients were enrolled at the treatment level of 200 mg (cohort 1) and 14 at the treatment level of 400 mg (cohort 2) twice daily (b.i.d.) for 28 days (cycle 1). One out of 13 patients in cohort 1 discontinued the study due to consent withdrawal after single-dose administration. One out of 14 patients in cohort 2 dropped out of this study due to adverse events during cycle 1. Patient characteristics are shown in Table 1. The median number of cycles administered per patient was five (range, 1–13 cycles). None of the patients from the 200-mg group reduced the dose of sorafenib, whereas two patients required dose reduction in the 400-mg group.

Evaluation of PK. Plasma drug concentrations were analyzed in 27 patients in the PK analysis. Plasma PK parameters of patients in the 200 and 400 mg bid groups are shown in Tables 2 and 3. There was a large interpatient variability in the PK of sorafenib. Geometric means of AUC, AUC_{0-12}, and C_{max} on day 1 of single-dose administration were not statistically different between 200 and 400 mg bid or between Child–Pugh A and B. Dose-dependent increases in AUC_{0-12} and C_{max} were observed at steady state (day 14) in the 200-mg bid and 400-mg bid patients; however, these increases were not dose proportional. Geometric means of AUC_{0-12} and C_{max} were slightly lower in the Child–Pugh B patients compared with the Child–Pugh A patients at steady state. The $t_{1/2}$ after single dose was similar between the Child–Pugh A and B groups for both dose levels.

Dose-dependent increases in the AUC_{0-12} and C_{max} of metabolites M-2 (*N*-oxide), M-4 (*N*-demethyl), and M-5 (*N*-oxide, desmethyl derivative) were observed. M-2 was the main metabolite in plasma. Ratios of each metabolite to the sum of all analytes were similar between the 200-mg bid and 400-mg bid patients and for baseline Child–Pugh class (Tables 2,3). M-7 (glucuronide of sorafenib) and M-8 (glucuronide of M-2) were detected in urine though no unchanged substance or M-2 was detected. There was no difference between the Child–Pugh A (1.21% for M-7 and 0.02% for M-8 at 400 mg) and B (1.18% and 0.02%, respectively, at 400 mg) groups in the urinary excretion rate of compounds at steady state. Interestingly, these PK results were similar to those obtained from the Japanese phase I study in non-HCC tumors.[18]

Adverse events. Adverse events of all 27 patients are shown in Table 4. Twenty-six out of 27 patients (96.3%) experienced an adverse event: 12 out of 13 patients (92.3%) in the 200-mg

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Table 1. Patient characteristics

Characteristic	200 mg bid (n = 13)	400 mg bid (n = 14)	Total (n = 27)
Sex (n)			
Male	12	13	25
Female	1	1	2
Median age (years)	69 (range 48–77)	70 (range 63–79)	70 (range 48–79)
Eastern Cooperative Oncology Group performance status			
0	13	14	27
Child–Pugh classification			
A	7	6	13
B	6	8	14
Viral markers			
HB antigen$^+$, HCV antibody$^-$	3	1	4
HB antigen$^-$, HCV antibody$^+$	9	11	20
HB antigen$^-$, HCV antibody$^-$	1	2	3
Previous treatment			
–	1	3	4
+	12	11	23
Tumor stage			
II	1	2	3
III	7	8	15
IVa	1	1	2
IVb	4	3	7
Portal vein tumor thrombus			
–	12	13	25
+	1	1	2
Metastasis			
–	9	11	20
+	4	3	7
Lung	3	1	4
Lung + lymph node	1	1	2
Lymph node	0	1	1

HB, hepatitis B; HCV, hepatitis C virus.

Table 2. Pharmacokinetic parameters of sorafenib and metabolites M-2, M-4, and M-5: sorafenib following single dose and multiple dose of 200 mg and 400 mg geometric mean (coefficient of variation)

Sorafenib	Parameter	Unit	200 mg bid				400 mg bid			
			Child–Pugh A		Child–Pugh B		Child–Pugh A		Child–Pugh B	
Single	n		7		6		6		8	
Dose	AUC	mg*h/L	28.29	190.29[‡]	18.64	74.1	20.33	90.31	26.87	96.97
Day 1	AUC_{0-12}	mg*h/L	5.02	190.36	2.75	61.06	3.82	86.06	3.11	88.16
	C_{max}	mg/L	0.81	195.96[‡]	0.49	67.85	0.55	83.75	0.53	86.68
	T_{max}	h[†]	7	3–12[‡]	18	4–24	8	6–24	24	4–24
	$T_{1/2}$	H	25.14	30.13[‡]	30.44	35.67	22.28	12.49	27.2	45.19
Cycle 1	N		6		6		6		6	
Day 14	AUC_{0-12}	mg*h/L	25.52	75.04	15.28	55.26	33.47	60.13	29.45	59.44[§]
	C_{max}	mg/L	3.36	87.29	1.89	62.14	4.66	66.12	3.04	94.39
Cycle 1	N		6		6		6		5	
Day 28	AUC_{0-12}	mg*h/L	31.63	101.64	20	73.4	28.91	86.79	20.71	72.06
	C_{max}	mg/L	4.22	92.32	3.32	78.65	3.32	113.47	4.01	79.12

[†]Median (range), [‡]n = 6, [§]n = 5. AUC_{0-12}, area under the concentration–time curve for 0–12 h.

group and 14 out of 14 patients (100%) in the 400-mg group. The most common drug-related adverse events were elevated lipase or amylase (88.9%), dermatological events (81.5%), and gastrointestinal events (70.4%). Common dermatological events were rash or desquamation (55.6%), and hand–foot skin reaction (44.4%). The incidence of adverse events in the 400-mg dose level was higher than that in the 200-mg dose level by ≥20%. These events fell under the categories of dermatology/skin (100.0 *vs* 61.5%), general cardiovascular (35.7 *vs* 7.7%), and renal/genitourinary (21.4 *vs* 0%).

Elevation of lipase and amylase was transient in most of the cases, and decreased gradually in all patients without treatment. One patient on 400 mg bid experienced acute pancreatitis that necessitated sorafenib withdrawal. The patient experienced abdominal pain 6 months after beginning treatment (cycle 6). Moreover, high lipase and amylase, as assessed by blood test,

Table 3. Pharmacokinetic parameters of sorafenib and metabolites M-2, M-4, and M-5: metabolites following multiple dose of 200 mg and 400 mg, measured at steady state (cycle 1, day 14) geometric mean (% coefficient of variation)

Parameter	200 mg bid			400 mg bid		
	M-2	M-4	M-5	M-2	M-4	M-5
Child–Pugh A						
n	6	6	6	6	6	6
AUC_{0-12} (mg × h/L)	4.18 (126)	0.92 (158)	0.79 (167)	6.18 (127)	1.68 (159)	1.22 (193)
Ratio† (%)	13.08 (30)	2.89 (60)	2.48 (81)	14.16 (39)	3.85 (55)	2.79 (85)
Child–Pugh B						
n	6	5	4	5	5	5
AUC_{0-12} (mg × h/L)	1.62 (173)	0.36 (131)	0.44 (351)	5.67 (90)	2.13 (142)	1.25 (117)
Ratio† (%)	9.05 (67)	1.85 (42)	1.95 (157)	14.46 (36)	5.44 (56)	3.19 (47)

†Median ratio of each metabolite to sum of all analytes. BAY 43-9006: M-2, BAY 67-3472; M-4, BAY 43-9007; and M-5, BAY 68-7769. AUC_{0-12}, area under the concentration–time curve for 0–12 h.

Table 4. Adverse events

Child–Pugh	Grade 3/4				All grades			
	200 mg bid		400 mg bid		200 mg bid		400 mg bid	
	A (*n* = 7)	B (*n* = 6)	A (*n* = 6)	B (*n* = 8)	A (*n* = 7)	B (*n* = 6)	A (*n* = 6)	B (*n* = 8)
Hematological								
Leukocytopenia	0	0	0	0	2 (29%)	0	1 (17%)	0
Lymphopenia	2 (29%)	1 (17%)	1 (17%)	1 (13%)	2 (29%)	1 (17%)	1 (17%)	2 (25%)
Platelets	0	0	1 (17%)	1 (13%)	0	1 (17%)	2 (33%)	3 (38%)
Non-hematological								
Hypertension	0	1 (17%)	1 (17%)	3 (38%)	0	1 (17%)	1 (17%)	3 (38%)
Fatigue	0	0	0	0	0	1 (17%)	0	0
Fever	0	0	0	0	1 (14%)	2 (33%)	0	1 (13%)
Weight loss	0	0	0	0	2 (29%)	1 (17%)	1 (17%)	4 (50%)
Hand–foot skin reaction	0	0	0	2 (27%)	2 (29%)	2 (33%)	5 (83%)	3 (38%)
Rash	0	0	0	2 (27%)	2 (29%)	3 (50%)	4 (67%)	6 (75%)
Alopecia	0	0	0	0	2 (29%)	1 (17%)	2 (33%)	0
Dry skin	0	0	0	0	0	0	0	3 (38%)
Pruritus	0	0	0	0	0	1 (17%)	4 (67%)	3 (38%)
Anorexia	0	0	0	0	2 (29%)	1 (17%)	1 (17%)	2 (25%)
Diarrhea	0	0	1 (17%)	0	4 (57%)	4 (67%)	2 (33%)	5 (63%)
Stomatitis	0	0	0	0	0	0	1 (17%)	2 (25%)
Lipase	3 (43%)	4 (67%)	4 (67%)	6 (75%)	6 (86%)	6 (100%)	6 (100%)	6 (75%)
Amylase	1 (14%)	1 (17%)	1 (17%)	1 (13%)	4 (57%)	3 (50%)	4 (67%)	5 (63%)

and swelling of the pancreas were observed. The patient's abdominal pain resolved 1 day after stopping sorafenib, and lipase and amylase normalized 2 days later. Sorafenib was restarted 20 days after resolution and continued over 122 days, without recurrence of pancreatitis.

Grade 3 or worse drug-related adverse events were observed in 23 patients (85.2%), the majority of which were related to laboratory abnormalities: 10 patients in the 200-mg group and 13 in the 400-mg group. One patient with Child–Pugh B in the 400-mg bid group experienced DLT of hand–foot skin reaction at the end of cycle 1. There were no drug-related deaths in either of the groups.

There was no major difference in the incidence and grade of drug-related adverse events between the Child–Pugh A and B groups. At the dose level of 200 mg, the drug-related adverse event whose incidence was at least 20% higher in the Child–Pugh B group than in the Child–Pugh A group was rash or desquamation (50.0 *vs* 28.6%). The differences at the 400-mg dose level were diarrhea (62.5 *vs* 33.3%), weight loss (50.0 *vs* 16.7%), hypertension (37.5 *vs* 16.7%), dry skin (37.5 *vs* 0%), and fatigue (25.0 *vs* 0%).

Table 5. Tumor response

Response	200 mg bid (*n* = 13)	400 mg bid (*n* = 14)	Total (*n* = 27)
Partial response	1	0	1 (3.7%)
Stable disease	10	11	21 (77.8%)
Progressive disease	1	2	3 (11.1%)
NA	1	1	2 (7.4%)

NA, not assessed because these patients did not complete cycle 1.

Tumor response and survival. Partial response was achieved in one of the 27 patients. No complete response was observed (Table 5; Fig. 1). The overall response rate was 3.7% (95% confidence interval, 0.1–14.0%). Stable disease was noted in 21 patients (77.8%) and the disease control rate (partial response + stable disease rate) was 81.5% in 27 patients. Progressive disease was noted in three patients (11.1%).

Disease progression or death was observed in all patients. Sixteen of the 27 patients died of disease progression, and two

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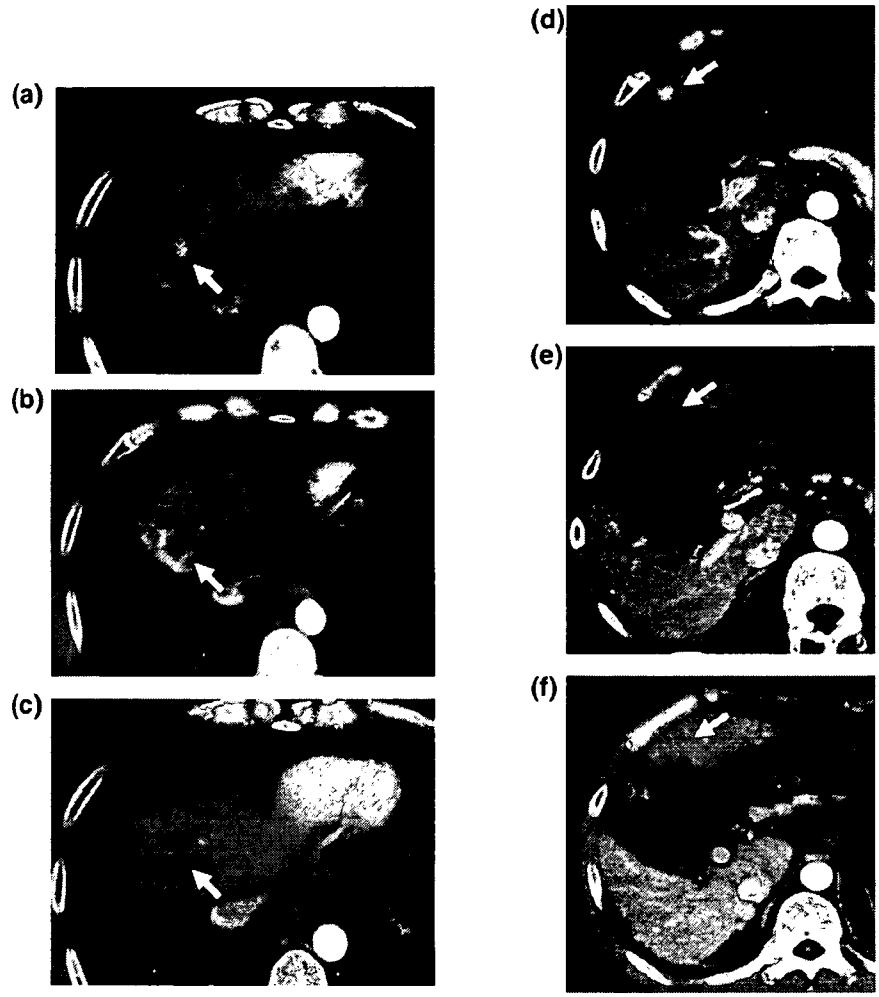

Fig. 1. A 48-year-old man with multiple tumors of hepatocellular carcinoma (HCC) after hepatectomy, percutaneous ethanol injection, and transcatheter arterial embolization. (a) Hypervascular HCC lesion, 1 cm in diameter, was revealed at the early phase of dynamic computed tomography (CT) before administration of sorafenib at the anterior superior segment of the liver (arrow). (b) The vascularity of this tumor disappeared 1 month after the administration of sorafenib. (c) The tumor was reduced 3 months after the administration of sorafenib. (d) Another hypervascular HCC lesion, 1 cm in diameter, was revealed at the early phase of dynamic CT before administration of sorafenib in the left lobe of the liver (arrow). (e) The vascularity of this tumor disappeared 8 months after the administration of sorafenib. (f) The tumor almost completely disappeared 10 months after the administration of sorafenib.

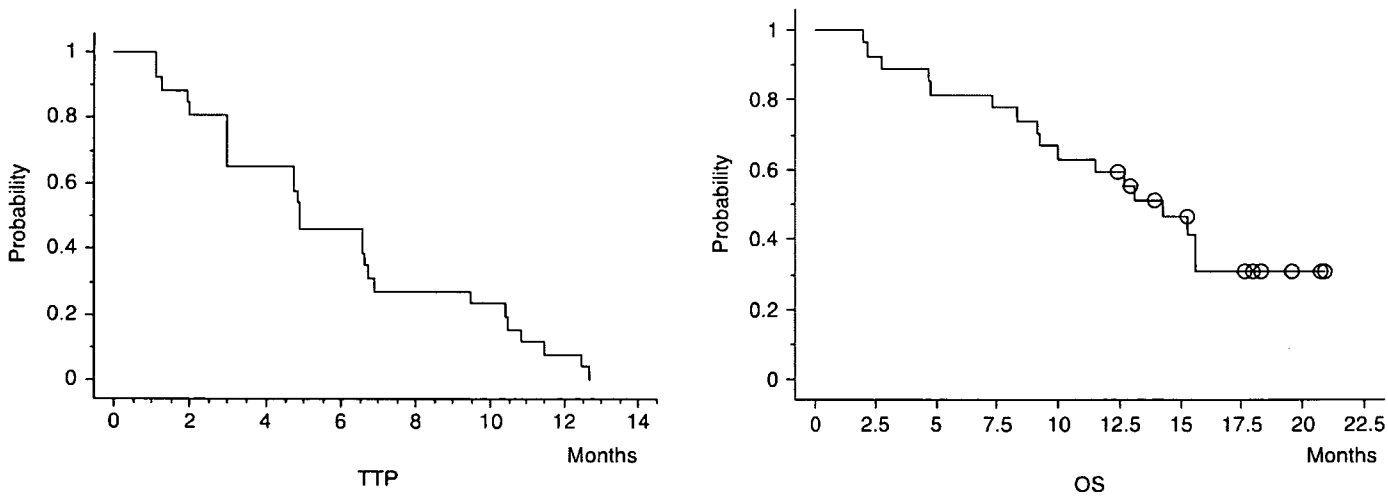

Fig. 2. Time to progression (TTP) in all 27 patients treated with sorafenib. The median TTP was 4.9 months, and the 6-month survival rate was 46.2%. Overall survival (OS) in the 27 patients treated with sorafenib. The median OS period was 15.6 months, and the 1-year survival rate was 59.3%.

died of cerebral infarction or myocardial infarction. Of the 27 patients, the median TTP was 4.9 months, and the median overall survival (OS) was 15.6 months (Fig. 2). The 6-month progression-free rate based on TTP was 46.2%, and 1- and 2-year OS were 59.3 and 30.9%, respectively.

Discussion

The PK, safety, and tolerability of sorafenib were investigated in Japanese patients with HCC treated with doses of 200 mg bid or 400 mg bid.

Most of the HCC patients had hepatitis or cirrhosis with underlying liver disorder and a reduction in hepatic blood flow to various degrees. Liver dysfunction in patients with HCC may affect the PK of sorafenib. When comparing the PK by Child–Pugh classification, geometric means of AUC_{0-12} and C_{max} at steady state were lower in the Child–Pugh B group than in the Child–Pugh A group, whereas after multiple doses of sorafenib, the mean plasma concentrations were highly variable and showed no clear dose dependency. Although the numerical differences in geometric means for PK parameters such as AUC, C_{max}, and $t_{1/2}$ were observed between Child–Pugh classifications, these differences were considered not to be clinically relevant in consideration of their large intersubject variability. No significant difference in clinical findings between these two groups was observed. There was also no major difference (i.e. over 20%) in the incidence of adverse events between Child–Pugh A and B groups. However, geometric means of AUC_{0-12} and C_{max} at steady state were slightly lower in the Child–Pugh B patients compared with the Child–Pugh A patients.

There were no remarkable differences in the overall incidence of adverse events for each dose level (92% for the 200-mg group and 100% for the 400-mg group). For a few drug-related adverse events, the incidences were at least 20% higher in the 400-mg group than in the 200-mg group, including rash or desquamation (71.4 *vs* 38.5%), hand–foot skin reaction (57.1 *vs* 30.8%), pruritus (50.0 *vs* 7.7%), decrease of platelets (35.7 *vs* 7.7%), hypertension (28.6 *vs* 7.7%), dry skin (21.4 *vs* 0%), and stomatitis or pharyngitis (21.4 *vs* 0%). DLT of hand–foot skin reaction was observed in a patient with Child–Pugh B at the end of cycle 1 with 400 mg bid, whereas no DLT was observed in the 200-mg bid group.

The most common drug-related adverse events were elevated lipase (88.9%) and amylase (59.3%). Twenty-four (88.9%) of the 27 patients showed high values of grade 3 or worse. Most of the patients were asymptomatic and only one patient had abdominal pain with findings to indicate pancreatitis on ultrasonography during cycle 6. His pancreatitis resolved shortly after discontinuation of sorafenib, and the patient restarted and continued with a reduced dose of sorafenib after recovery.

A separate phase I clinical study was carried out to evaluate the safety of sorafenib in patients with solid tumor, excluding HCC, at doses of 100, 200, 400, and 600 mg bid.[18] In that study, the most common type of adverse events included skin reaction, elevation of pancreatic enzyme, and gastrointestinal (GI) toxicity such as diarrhea. In the current study, a similar pattern of adverse events was observed. These results suggest that 'gastrointestinal' and 'dermatology/skin' are common adverse events regardless of cancer type and liver function status. One finding to note is that the incidence of elevation (grade 3/4) of lipase (63.0%) or amylase (14.8%) in the present study in HCC patients was higher than that observed in non-HCC patients (lipase 23% and amylase 10%).[18]

In summary, the present study showed no clinically significant difference in PK, safety, tolerability, or efficacy by Child–Pugh status or between HCC patients and non-HCC patients, whereas some dose dependency in adverse events was observed.

Investigations into cytotoxic agents for HCC have been conducted.[20,21] However, no standard chemotherapy has been established. Recently, a number of agents targeting growth factors were investigated in HCC. Through these investigations, it was indicated that epidermal growth factor receptor/human epidermal growth factor receptor 1 (EGFR/HER1) is actively expressed in human hepatoma cells.[22,23] Erlotinib, which is an EGFR/HER1 tyrosine kinase inhibitor, and lapatinib, which is an EGFR/HER1 and ErbB-2 (Her2/neu) dual tyrosine kinase inhibitor, have been investigated in phase II studies in HCC patients.[24–26] For erlotinib, the response rate was 4–9%, the median TTP was 2.1–3.2 months, and the OS was 5.8–13 months,[24,25] whereas for lapatinib, the response rate was 0%, and the median progression-free survival time was 1.8 months.[26]

Hepatocellular carcinoma, given its hypervascular characteristics, may be sensitive to antiangiogenic agents.[9] It is known that VEGF augments the development and metastasis of HCC. Bevacizumab, a monoclonal antibody against VEGF, has been investigated in phase II studies.[27] The response rate with bevacizumab was 10% and the disease control rate was 80%. A combination of gemox (gemcitabine plus oxaliplatin) and bevacizumab showed a better response rate of 20%.[28]

Sorafenib, an orally active multikinase inhibitor, blocks tumor-cell proliferation by targeting Raf/MEK/ERK signaling at the level of Raf kinase, and exerts an antiangiogenic effect by targeting VEGFR-2, VEGFR-3, and PDGFR-β tyrosine kinases. In phase II studies in non-Japanese and Japanese HCC patients, comparable median TTP of 4.2 and 4.9 months, respectively, and response rates of 2 and 4%, respectively, were shown.[15] However, OS in the two studies were different: 9.2 months in the non-Japanese study and 15.6 months in the Japanese study. Difference in backgrounds such as liver function or treatment after progression may play a role in this discrepancy in survival time.

In the current study, one patient achieved partial response (Fig. 1). The patient had several small viable HCC lesions after hepatectomy, percutaneous ethanol injection, and TACE. Following administration of sorafenib, tumor vascularity decreased dramatically preceding a gradual tumor reduction. Time to tumor shrinking varied across lesions, ranging from 1 to 8 months after initiation of treatment with sorafenib. It is likely that, with anti-VEGF agents such as sorafenib, it may take time to achieve tumor reduction to meet partial response by RECIST, whereas the duration of stable disease may persist due to its tumor stabilization activity.

With the relatively long TTP of VEGF pathway-targeting agents such as bevacizumab or sorafenib, these agents may have antitumor effects on HCC and prolong survival. With its profile of tumor stabilization and tolerability, sorafenib may be applicable not only for advanced HCC but also for the adjuvant setting after curative treatment, such as surgery or radiofrequency ablation therapy.

In conclusion, in the present phase I study, sorafenib demonstrated favorable safety and tolerability, and promising preliminary antitumor activity in Japanese HCC patients. Considering that DLT was observed in one of 14 patients treated with 400 mg bid, 400 mg bid could also be recommended for future studies in Japanese HCC patients, as well as non-HCC Japanese and Caucasian patients. However, as the number of patients was limited in this phase I study, a confirmatory study will be required with a larger number of patients.

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話　題

進行肝細胞癌の化学療法—Sorafenib placebo-control randomized study (SHARP trial)を中心に*

古瀬純司**

Key Words : hepatocellular carcinoma, sorafenib, molecular-targeted therapy, systemic chemotherapy, placebo-control randomized study

はじめに

肝細胞癌は世界では5番目に多い癌であり，年間約626,000名の新規患者が診断されている[1]．地域別にみると，東アジア37,000名，日本40,000名，ヨーロッパ32,000名，米国19,000名の年間発症数が報告されている[2)~4)]．とくに米国，ヨーロッパではC型肝炎の増加に伴い，肝細胞癌の発症数が増加している．肝細胞癌の病因はB型，C型肝炎ウイルス感染，アルコール性肝硬変，アフラトキシン，非アルコール性脂肪性肝炎(non-alcoholic steatohepatitis：NASH)など多彩であり，東アジア諸国，アフリカ諸国ではB型肝炎，日本ではC型肝炎が主な病因であるなど地域による差が大きいのも特徴である[5)6)]．

肝細胞癌の治療は一般に癌進行度と肝障害度に応じて治療選択が行われ，肝切除などの局所療法や動脈塞栓療法から化学療法までその治療法は多岐にわたる．肝細胞癌に対する治療選択については日本では肝癌診療ガイドラインによる肝細胞癌治療アルゴリズムが公表されている[7)]．また，今回sorafenibによる大規模な第III相試験でも引用されたBarcelona groupによるBarcelona Clinic Liver Cancer (BCLC) staging classification[8)]がヨーロッパ中心に適応されている．これらの治療選択のガイドラインにおいて，肝切除やラジオ波(RFA)など局所壊死療法，肝移植，動脈塞栓化学療法(TACE)は適切な症例選択の下に標準治療として確立している．一方，化学療法はこれまで多くのレジメンが臨床試験として試みられてきたが，生存期間の改善が確認された標準治療もその位置づけも確立していない．

肝細胞癌に対する化学療法

肝細胞癌に対する化学療法は，肝動脈から注入する経動脈性化学療法(動注化学療法)と経静脈あるいは経口による全身化学療法に分けられる．肝細胞癌に対する化学療法は，肝切除，局所壊死療法，動脈塞栓(化学塞栓)療法の局所治療が無効あるいは適応困難な例(高度門脈腫瘍栓など)および遠隔転移例が適応となる．また肝細胞癌では肝硬変など慢性肝障害を背景にもつ例が多いことから，肝障害を助長するリスクも大きく，肝障害度C(Child-Pugh C)の肝機能不良例では化学療法は禁忌である．

わが国では肝動脈からの動注化学療法が盛んに行われている．動注化学療法剤としてepirubicin, mitomicin C, 5-FUが主に用いられてきたが，2004年7月，cisplatin(アイエーコール®)の保険適応が承認された．最近では5-FU＋cisplatinや5-FU＋interferon(IFN)で高い奏効率が報告されているが，いずれも前向きな臨床試験による検証は行われていない[9)10)]．

全身化学療法では，これまで肝細胞癌における無作為化比較試験としてdoxorubicin(DXR), tamoxifen, interferonなどいくつか行われてき

* Systemic chemotherapy of growth factor inhibitors for advanced hepatocellular carcinoma from the results of sorafenib placebo-control randomized study.

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表1　切除不能肝細胞癌におけるドキソルビシンと無治療との無作為化比較試験

	Doxorubicin	Best supportive care	
n	60	46	
Response	2 (3.3%)	−	
Median OS	10.6 weeks	7.5 weeks	$P=0.036$
Fetal complication	15 (25%)		
Cause of death			
Tumor progression with cachexia	60.0%	76.1%	
Sede effects of therapy	25.0%	0	
GI bleeding	6.6%	8.7%	
Ruputure of tumor	3.3%	6.5%	
Hypoglycemia	5.0%	4.3%	
Subarachnoid hemorrhage	0	2.3%	
Sicide	0	2.3%	

（文献[13]より引用）

表2　切除不能肝細胞癌におけるドキソルビシンとシスプラチン/インターフェロン/ドキソルビシン/フルオロウラシル併用療法(PIAF)との無作為化第III相試験

	Doxorubicin	PIAF	*P*-value
n	94	94	
Response	10.5%	20.9%	0.058
Median overall survival	6.83 months	8.67 months	0.83
Treatment-related mortality	3 %	9 %	0.194
Major toxicity*			
Neutropenia	63%	82%	0.003
Trhrombocytopenia	24%	57%	＜0.001
Vomiting	4 %	12%	0.058
Hypokalemia	0 %	7 %	0.007
Hyponatremia	1 %	6 %	0.054

* grade 3 or above　　（文献[18]より引用）

た[11)~15)]．DXRでは無治療群に比べ有意に生存期間の延長が得られたが，25%の症例で致命的な合併症が認められている(表1)[11)]．TamoxifenやIFN-αでは生存期間の改善は認められておらず[12)~15)]，標準的治療法は確立していない．最近では多剤併用療法が試みられ，5-FU/mitoxantrone/cisplatin (FMP)，cisplatin/doxorubicin/5-FU/IFN-α (PIAF)などで25%を超える高い奏効率が報告されたが[16)17)]．しかし，DXRをcontrol armとしたPIAF regimenの第III相試験が行われたが，有意な生存期間の改善は示せず(表2)[18)]，既存の抗癌剤による化学療法は悲観的にとらえられている．

Sorafenibによる第III相試験 (SHARP trial)

SorafenibはRAFキナーゼ，VEGFR-1-3，PDGFR-βなどを標的とするマルチキナーゼ阻害薬である．肝細胞癌においてもRafキナーゼの高発現が認められ，RAF/MEK/ERKシグナル伝達経路が肝細胞癌発症に関与しているとの報告がある[19)]．またsorafenibの第I相試験では肝細胞癌例でpartial response(PR)が得られていた[20)]．以上の背景から，米国やヨーロッパなどでsorafenib 400mg, 1日2回経口投与量により進行肝細胞癌に対する有効性と安全性を確認する第II相試験が行われた[21)]．その結果，奏効率は2%と低率であったが，十分な忍容性が確認され，無増悪期間中央値(median TTP)4.2か月，生存期間中央値(median OS)9.2か月と有効性も期待される結果であった(表3)．わが国では日本人肝細胞癌患者での薬物動態，安全性，推奨用量などを明らかにする目的で第I相試験が行われた[22)]．

表3 肝細胞癌に対するSorafenibの臨床第Ⅰ相，第Ⅱ相試験

Study	Phase II study	Phase I study
n	137	25
Dose	400 mg bid	200, 400 mg bid
Response	2 %	4 %
Stable disease	39%	76%
Disease control rate	42%	80%
Median time-to progression	4.2 mo	4.9 mo
Median overall survival	9.2 mo	15.6 mo
Author	Abou-Alfa (JCO 2006)[21]	Furuse (EORTC 2006)[22]

表4 進行肝細胞癌患者におけるsorafenibとplaceboの無作為化第III相試験(SHARP Trial)：試験デザイン

主要評価項目	Overall survival Time to symptomatic progression
副次評価項目	Time to progression
デザイン	国際多施設共同 二重盲検化プラセボ対照ランダム化第III相試験(Sorafenib群 vs. プラセボ群)
割付因子	門脈腫瘍栓 and/or 肝外転移 ECOG PS 地域
仮　説	Median survival timeを7か月から9.7か月(40%)に改善 検出力90%，$\alpha=0.02$(片側)，予定症例数560例，死亡数424例

その結果，他癌種，米国・ヨーロッパと同様の薬物動態および忍容性が確認され，推奨用量も400mg，1日2回と決定された(表3)．同試験では症例数は少ないものの，有効性も同等であった．

以上，sorafenibの肝細胞癌に対する前臨床データおよび第I，II相試験の結果をもとに今回のプラセボコントロールによる無作為化比較試験SHARP(Sorafenib HCC Assessment Randomized Protocol) trialが実施された[23]．本試験の試験デザインを表4にまとめた．主な患者選択基準は，組織学的な肝細胞癌の確認，進行肝細胞癌，ECOG PS 0-2，Child-Pugh A Class，全身化学療法歴なし，などである．

2005年3月から2006年4月までにSorafenib群299例，プラセボ群303例が登録された．治療はsorafenib 400mg/回，1日2回内服，あるいはplacebo 1日2回内服に割り振られ，両群の患者背景に有意な差はみられなかった(表5)．

表5 進行肝細胞癌患者におけるsorafenibとplaceboの無作為化第III相試験(SHARP Trial)：患者背景

	Sorafenib	Placebo
n	299	303
Median age	67歳	68歳
Male	87%	87%
Region Europe	88%	87%
Etiology HCV/HBV /Alcohol/other	29/19 /26/26%	27/18 /26/29%
ECOG PS 0	54%	54%
Child-Pugh A	95%	98%
BCLC stage C	82%	83%

BCLC stage：Barcelona Clinic Liver Cancer staging classification

主要評価項目である全生存期間はsorafenib群10.7か月，placebo群7.9か月であり，ハザード比0.69(95%CI：0.55-0.87；$P=0.0006$)と両者間に明らかな統計学的有意差を認めた(表6)．もう一つの主要評価項目である症状増悪までの期間(time to symptomatic progression)では差は認められなかった．副次評価項目である無増悪期間(time to progression)はsorafenib群5.5か月，placebo群2.8か月であり，ハザード比0.0.58(95%CI：0.45-0.74；$P=0.000007$)と全生存期間と同様両者間に明らかな統計学的有意差を認めた．有害事象については両群に差はなく，主なGrade 3/4の有害事象は下痢(sorafenib vs. placebo：11% vs. 2 %)，手足皮膚反応(8 % vs. 1 %)，疲労感(10 % vs. 15%)，出血(6 % vs. 9 %)であった．Sorafenibは十分な忍容性があり，進行肝細胞癌患者の生存期間を延長した初めての全身治療である．臨床的に大きな意義のある結果であり，sorafenibはこれらの患者に対する第一選択の治療法として確立すると報告された．

解　説

肝細胞癌は比較的遠隔転移が少なく，肝機能低下による肝不全が主な死因となることが多いことから，肝内病変への局所治療が主な治療法として行われる．しかし，再発がきわめて多く，局所治療が抵抗性になった病態や肝外転移を有する場合，有効な全身治療がないのが現状であっ

表6　進行肝細胞癌患者におけるsorafenibとplaceboの無作為化第III相試験(SHARP Trial)：結果

	Sorafenib	Placebo	HR (sorafenib/placebo)	*P*-value
n	299	303		
Median overall survival	10.7 mo	7.9 mo	0.69	0.0006
Time to progression	5.5 mo	2.8 mo	0.58	0.000007
Overall response				
Partial response(PR)	2.3%	0.7%		
Stable disease(SD)	71%	67%		
Progressive disease(PD)	18%	24%		
Progression-free rate at 4 month	62%	42%		
Serious adverse event (SAE)	52%	54%		
Drug-related treatment emergent SAE	13%	9 %		

た．これまでいくつかの無治療と全身治療の無作為化比較試験が行われてきたが，確立した標準治療は認められていない．そのような状況で，sorafenibによるplacebo-control無作為化比較試験が行われた．本試験では，placebo群のmedian OSを7か月に設定し，40%の改善を見込むとの仮説が立てられたことは臨床的に妥当であり，本試験により仮説通りの結果が得られたことは肝細胞癌治療にとって画期的なことと考えられる．

本試験の解釈において，患者背景でヨーロッパからの登録が90%近くと偏っていること，対象をChild-Pugh Aのみに限ったことが問題点としてあげられた．わが国にこの結果をそのまま導入してよいかという点について考察が必要である．肝細胞癌の病因については，日本では70%程度でC型肝炎感染が関連しているが，B型肝炎は15%程度である[6]．今回の試験ではC型肝炎関連は30%弱であり，病因による治療効果や有害事象の差はないかどうか検証する必要がある．しかし，欧米ではC型肝炎が非常に増えており，日本の状況と類似してきていることも確かである．Child-Pugh Aのみで試験が実施されたことについては，国際試験であり，肝障害による影響を可能な限り除外し，sorafenibの効果をより確実に評価したいという考えがあったかと推測される．わが国で行われた肝細胞癌患者でのsorafenibの第I相試験では，同数のChild-Pugh AとBで治療を行ったが，両者で有効性や安全性に大きな差は認めなかった．実地診療ではChild-Pugh Bも十分忍容性があり，適応可能と考えられる．

肝細胞癌の全身化学療法は，一定の抗腫瘍効果が得られても肝障害や静脈瘤出血などの有害事象により生存期間の改善につながらないことが多くみられる．今回の試験では重篤な有害事象はsorafenib群54%，placebo群52%と高頻度に認められたが，治療関連と考えられる有害事象はそれぞれ13%と9%と低く，差も認められていない．肝細胞癌患者では全身化学療法実施時の有害事象は原病や肝硬変など背景障害肝から生じる合併症の関与がきわめて大きいものと考えられる．つまり，sorafenibは高い忍容性が確認されているが，肝細胞癌での適応においては，薬物有害反応以外のさまざまな症状・合併症に注意を払う必要がある．

まとめ

今後，欧米を初め多くの国々，地域でsorafenibの肝細胞癌に対する適応承認が認められることが予想される．わが国でも他に有効な全身治療薬がない状況であり，すみやかな実地医療での適応承認が期待される．さらにより有効な治療法の確立に向けて，sorafenibを参照治療とした比較試験やsorafenibへの上乗せ効果を期待する治療法の開発が考えられる．現在，日本では肝細胞癌の動脈塞栓化学療法後の無増悪期間の改善を目的とした補助療法としてのplacebo-control無作為化比較試験が行われている．今回のSHARP trialとはまったく異なった患者群とコンセプトであり，世界的にも大きく注目されていることから，早期に完遂されることが期待される．

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Regression of intestinal adenomas by vaccination with heat shock protein 105-pulsed bone marrow-derived dendritic cells in $Apc^{Min/+}$ mice

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Heat shock protein (HSP) 105 is overexpressed in various cancers, but is expressed at low levels in many normal tissues, except for the testis. A vaccination with HSP105-pulsed bone marrow-derived dendritic cells (BM-DC) induced antitumor immunity without causing an autoimmune reaction in a mouse model. Because $Apc^{Min/+}$ mice develop multiple adenomas throughout the intestinal tract by 4 months of age, the mice provide a clinically relevant model of human intestinal tumor. In the present study, we investigated the efficacy of the HSP105-pulsed BM-DC vaccine on tumor regression in the $Apc^{Min/+}$ mouse. Western blot and immunohistochemical analyses revealed that the tumors of the $Apc^{Min/+}$ mice endogenously overexpressed HSP105. Immunization of the $Apc^{Min/+}$ mice with a HSP105-pulsed BM-DC vaccine at 6, 8, and 10 weeks of age significantly reduced the number of small-intestinal polyps accompanied by infiltration of both $CD4^+$ and $CD8^+$ T cells in the tumors. Cell depletion experiments proved that both $CD4^+$ and $CD8^+$ T cells play a critical role in the activation of antitumor immunity induced by these vaccinations. These findings indicate that the HSP105-pulsed BM-DC vaccine can provide potent immunotherapy for tumors that appear spontaneously as a result of the inactivation of a tumor suppressor gene, such as in the $Apc^{Min/+}$ mouse model. (*Cancer Sci* 2007; 98: 1930–1935)

Colorectal cancer is the third most common cancer and the fourth most frequent cause of cancer death worldwide. Every year, more than 945 000 people develop colorectal cancer worldwide, and approximately 492 000 patients die.[1] For patients with advanced stages of colorectal cancer, adjuvant systemic chemotherapy is a standard treatment. Major progress has been made by the introduction of regimens containing new cytotoxic drugs such as irinotecan and oxaliplatin; however, the new therapeutic regimens have led to only 8–9 months of progression-free survival.[2] Consequently, the development of new and effective therapeutic approaches, such as immunotherapy, is needed to expand treatment options.

The progression from normal epithelium to colorectal cancer is a multistep process involving the accumulation of multiple genetic alterations.[3] The *APC* gene, a tumor suppressor, is considered to be a gatekeeper in colon tumorigenesis,[4] and one of the earliest molecular events is the loss of function of the *APC* gene product.[5] APC forms a multimeric complex with the axis inhibition protein (AXIN)2 and glycogen synthase kinase 3β, which regulates the nuclear accumulation of β-catenin, a signal transducer of the wnt pathway.[6] When the APC–β-catenin complex is destabilized because of *APC* mutations, β-catenin binds and activates transcription factors that regulate the expression of potent oncogenes such as *c-Myc* and *c-Met*.[7] The importance of the *APC* gene product was confirmed by the demonstration that 80% of all sporadic colorectal cancers are characterized by one or more mutations in the *APC* gene, approximately 60% of which result in the expression of a truncated version of the APC protein.[8]

The $Apc^{Min/+}$ mouse has a nonsense mutation from T to A in the *Apc* gene at codon 850, homologous to the human germline and somatic *APC* mutation.[9] Although homozygous mice die before birth, all heterozygous mice develop multiple adenomas throughout their intestinal tract at an early age.[10] The $Apc^{Min/+}$ mouse model is unique in that tumors appear spontaneously in the intestinal tract, rather than as a result of induction by a carcinogen. This model is particularly advantageous for testing preventive agents targeted against early stage lesions because adenomas grow to a grossly detectable size within a few months on a defined genetic background.[10] Because $Apc^{Min/+}$ mice develop tumors due to the inactivation of the same tumor suppressor gene known to be involved in the pathogenesis of most colon cancers in humans, this model represents a clinically relevant model of human intestinal tumorigenesis.[10] Furthermore, germline mutations in the human *APC* gene cause FAP, whose symptoms resemble those of an $Apc^{Min/+}$ mouse. Therefore, this model provides useful information about not only colon cancer but also FAP.

Heat shock proteins are soluble intracellular proteins that are expressed ubiquitously, and their expression can be induced at much higher levels due to heat shock or other forms of stress. The essential functions of HSP are to bind and protect partially denatured proteins from further denaturation and aggregation.[11] A previous study reported that HSP105 (often called HSP110), identified with serological identification of antigens using the recombinant expression cloning (SEREX) method, is overexpressed in a variety of human cancers, including colorectal, pancreatic, thyroid, esophageal, and breast carcinoma, whereas HSP105 is expressed at lower levels in many normal tissues, except for the testis.[12,13] Immunotherapy targeted at HSP105 in the mouse prophylactic model, such as HSP105-pulsed BM-DC and *HSP105* DNA vaccines, induce antitumor immunity without causing an autoimmune reaction.[14,15] These findings indicate that HSP105 itself could be considered as a valuable tumor-associated antigen for immune-based treatment of various tumors.

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Abbreviations: APC, adenomatous polyposis coli; BM-DC, bone marrow-derived dendritic cell; COX, cyclooxygenase; DC, dendritic cell; ELISPOT, enzyme-linked immunospot; FAP, familial adenomatous polyposis; HSP, heat shock protein; mAb, monoclonal antibody; MBP, myelin basic protein; MHC, major histocompatibility complex; PBS, phosphate-buffered saline.

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Another study reported that HSP105 is involved in tumorigenesis by protecting cancer cells from apoptosis.[16] The constitutive overexpression of HSP105 protein was found to be essential for various cancer cells to survive and, conversely, the apoptosis-inducing effect of HSP105 small interfering RNA (siRNA) is specific for cancer. In contrast, HSP can also stimulate an adaptive immune response against antigens bound to HSP,[17] provided that the vaccine forms a complex of recombinant HSP110 and target tumor-associated antigen.[18,19]

In the present study, $Apc^{Min/+}$ mice were used as a model of a cancer immunotherapy for human colorectal cancer. Because tumors in $Apc^{Min/+}$ mice strongly express HSP105, the efficacy of immunization with HSP105-pulsed BM-DC for preventing the development of tumors in $Apc^{Min/+}$ mice was investigated.

Materials and Methods

Mice and genotyping. Frozen embryos of $Apc^{Min/+}$ mice obtained from the Jackson Laboratory were transferred to C57BL/6J mice (purchased from Charles River Japan, Yokohama, Japan) at the Center for Animal Resources and Development, Kumamoto University. Mice at 4–5 weeks of age were characterized for the *Apc* genotype by polymerase chain reaction analysis of tail DNA with the use of allele-specific primers.[20] The concentrations of these primers were 1.0 μM (5′-TGAGAAAGACAGAAGTTA-3′), 1.0 μM (5′-TTCCACTTTGGCATAAGGC-3′), and 0.2 μM (5′-GCCATCCCTTCACGTTAG-3′). The amplification conditions were 5 min at 94°C before 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 5 min. The mice were maintained by breeding male $Apc^{Min/+}$ mice to female C57BL/6J mice. The mice were kept under specific pathogen-free conditions and these experiments were approved by the Animal Research Committee of Kumamoto University.

Production of recombinant proteins. Highly purified recombinant mouse HSP105 was produced from *Escherichia coli* strain BL21 cells transduced with the mouse *HSP105* gene expression vector, as described previously.[14,21] We also produced highly purified recombinant MBP as a negative control, which was prepared from bacterial lysate in the same way as the preparation of recombinant HSP105. Both recombinant HSP105 and MBP were estimated to be almost endotoxin free using a Limulus amebocyte lysate assay kit (BioWhittaker, Walkersville, MD, USA), and the endotoxin contents in the materials were <10 endotoxin U/mg.

Immunizations and scoring of tumors. HSP105-pulsed BM-DC were prepared as described previously.[14,22] The mice were inoculated intraperitoneally with HSP105-pulsed BM-DC (5×10^5) suspended in 200 μL PBS at 6, 8, and 10 weeks of age. The mice were treated with BM-DC alone, MBP-pulsed BM-DC, or PBS as controls. At 12 weeks of age the mice were killed and their small intestines were removed and fixed with formaldehyde. The intestines were then opened and stained with methylene blue and the number of tumors was counted.

Western blot and immunohistochemical analysis. Western blotting and the immunohistochemical detection of HSP105 were carried out as described previously.[12,16] Rabbit polyclonal antihuman HSP105 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as the primary antibody in this study. The immunohistochemical staining of $CD4^+$ and $CD8^+$ T cells was carried out as described previously.[14] mAb specific to CD4 (L3T4; BD PharMingen, San Diego, CA, USA) and CD8 (Ly-2; BD PharMingen) were used for staining.

Depletion of $CD4^+$ or $CD8^+$ T cells in mice. Rat mAb GK1.5 specific to mouse CD4 and 2.43 specific to mouse CD8 were used to deplete $CD4^+$ and $CD8^+$ T cells, respectively, *in vivo*. The 6-week-old $Apc^{Min/+}$ mice were injected with ascites (500 μg/mouse) from hybridoma-bearing nude mice six times intraperitoneally with an interval of 3–4 days between injection. Normal rat IgG (Chemicon, Temecula, CA, USA) was used as a control. The depletion of T cell subsets was monitored by a flow cytometric analysis, which showed a more than 90% specific depletion in the number of splenocytes.

ELISPOT assay. The $Apc^{Min/+}$ mice were immunized with HSP105-pulsed BM-DC or BM-DC alone at 6 and 8 weeks of age. At 10 weeks of age, spleen cells were harvested and depleted of $CD4^+$ or $CD8^+$ T cells using a magnetic cell-sorting system with antimouse CD4 mAb and antimouse CD8a (Mittenyi Biotec GmbH, Bergisch Gladbach, Germany) mAb, respectively. The purity of these T-cell subsets exceeded 95% based on a flow cytometric analysis. $CD4^-$ T cells were used as a source of $CD8^+$ T cells and antigen-presenting cells, and $CD8^-$ T cells were used as a source of $CD4^+$ T cells and antigen-presenting cells. Five hundred thousand $CD4^-$ or $CD8^-$ T cells were added to each well in triplicate cultures of RPMI-1640 medium containing 10% fetal calf serum (FCS) together with 2 μg/mL HSP105, MBP, and one with medium only at 37°C for 24 h. Then ELISPOT assays were carried out as described previously.[12]

Statistical analysis. The statistical significance of differences between the experimental groups was determined using Student's *t*-test. The overall survival rate was calculated using the Kaplan–Meier method, and statistical significance was evaluated using Wilcoxon's test. A value of $P < 0.05$ was considered to be statistically significant.

Results

Overexpression of HSP105 in intestinal adenomas of the $Apc^{Min/+}$ mice. A previous study reported that mouse HSP105 is overexpressed in liver metastasis of a murine colorectal adenocarcinoma cell line (Colon26), and in lung metastasis of a murine melanoma cell line (B16-F10).[15] The expression of HSP105 in tumors of $Apc^{Min/+}$ mice were thereby analyzed. The small intestines of $Apc^{Min/+}$ mice were excised, and the expression level of HSP105 was evaluated by both western blot and immunohistochemical analyses. The $Apc^{Min/+}$ mice developed adenomatous polyps spontaneously, predominantly in and throughout the small intestine at 4 months of age (Fig. 1a). Both western blot and immunohistochemical analyses confirmed the strong expression of HSP105 in the tumors of $Apc^{Min/+}$ mice (Fig. 1b,c). Based on these observations, the $Apc^{Min/+}$ mouse was chosen as a murine model of cancer immunotherapy targeted at HSP105.

Immunization with HSP105-pulsed BM-DC vaccine reduced the number of small intestinal polyps in $Apc^{Min/+}$ mice. The preventive effects of HSP105-pulsed BM-DC vaccination on the development of adenomatous polyps in the $Apc^{Min/+}$ mice were investigated. The mice were divided into four groups consisting of 10 mice each, inoculated intraperitoneally with PBS (group 1), BM-DC (group 2), MBP-pulsed BM-DC (group 3), or HSP105-pulsed BM-DC (group 4) at 6, 8, and 10 weeks of age. Two weeks after the last immunization, the number of tumors in the small intestine was counted.

Tumors had already developed in the small intestine of $Apc^{Min/+}$ mice at the time of the first vaccination (6 weeks of age). Each mouse had a mean of 6.3 ± 3.4 tumors at that time. The mean number of tumors at 12 weeks of age was 20.9 ± 9.6 in group 4, which was significantly less ($P = 0.006$) than the numbers in group 1 (37.8 ± 11.0), group 2 (40.8 ± 11.0), and group 3 (34.8 ± 9.5) (Fig. 2a). It was therefore concluded that the HSP105-pulsed BM-DC vaccine has the potential to prevent the growth of tumors expressing HSP105. The survival time in group 4 (175.3 ± 32.6 days) tended to be longer than that in group 1 (146.7 ± 13.0 days) and in group 2 (152.7 ± 25.5 days); however, the difference between group 4 and group 2 was not statistically significant ($P = 0.081$; Fig. 2b). No apparent abnormalities, such as weight loss, hair abnormality, or paralysis, were observed in

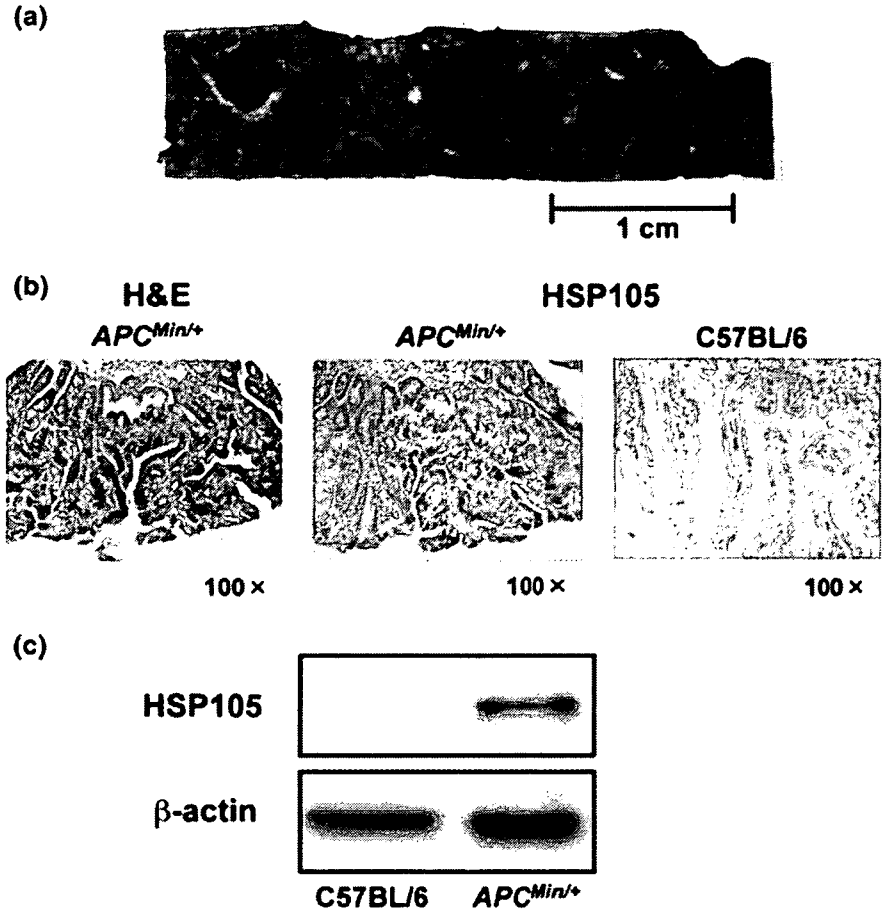

Fig. 1. Overexpression of heat shock protein (HSP) 105 in adenomatous polyps of $Apc^{Min/+}$ mice. (a) Macroscopic polyps in the small intestine of 4-month-old $Apc^{Min/+}$ mice. (b) A microscopic analysis of polyps in the small intestine of 12-week-old $Apc^{Min/+}$ mice stained with hematoxylin–eosin (left) and anti-HSP105 monoclonal antibody (middle). A normal small intestine was stained with anti-HSP105 monoclonal antibody as a negative control (right). Objective magnification was ×100. (c) Western blot analysis of HSP105 in the small intestine of 4-month-old $Apc^{Min/+}$ mice. The samples were small intestines of $Apc^{Min/+}$ and C57BL/6J mice homogenized in lysis buffer. The small intestines of three mice per group were pooled.

the mice immunized with HSP105-pulsed BM-DC, suggesting that serious autoimmunity was not observed in the mice. A histological analysis of the major organs (brain, lung, heart, liver, small intestine, kidney, and testis) of the immunized mice revealed no pathological inflammation (data not shown).

Both CD4⁺ and CD8⁺ T cells are required for antitumor immunity. To determine the role of $CD4^+$ and $CD8^+$ T cells in the reduction of tumor development in $Apc^{Min/+}$ mice immunized with HSP105-pulsed BM-DC, mice were depleted of $CD4^+$ or $CD8^+$ T cells by treatment with anti-CD4 or anti-CD8 mAb, respectively, *in vivo*. During the depletion procedure, the mice were immunized with PBS or HSP105-pulsed BM-DC vaccine (Fig. 3a). In the group of mice immunized with HSP105-pulsed BM-DC, together with inoculation of anti-CD4 mAb (35.5 ± 10.8) or anti-CD8 mAb (30.2 ± 9.6), the tumor numbers were significantly larger than those in the mice given rat IgG (18.8 ± 5.9) or left untreated (19.9 ± 7.7). The differences in the tumor numbers between the anti-CD4 mAb-treated group and the rat IgG-treated group ($P = 0.002$), and between the anti-CD8 mAb-treated group and the rat IgG-treated group ($P = 0.013$) were statistically significant. In the group of mice inoculated with PBS, the numbers of tumors in the mice given either anti-CD4 mAb (38.1 ± 5.7) or anti-CD8 mAb (38.1 ± 5.6) did not differ significantly from those in the mice given rat IgG (37.8 ± 4.8) or in the untreated mice (40.8 ± 6.1) (Fig. 3b). These results suggest that both $CD4^+$ and $CD8^+$ T cells play a crucial role in the protective antitumor immunity induced by the HSP105-pulsed BM-DC vaccine, because the HSP105-pulsed BM-DC vaccine was not effective in the mice showing a depletion of either $CD4^+$ or $CD8^+$ T cells.

Detection of HSP105-specific T cells in mice immunized with the HSP105-pulsed BM-DC vaccine. The $Apc^{Min/+}$ mice were immunized with HSP105-pulsed BM-DC or BM-DC at 6 and 8 weeks of age. At 10 weeks of age, spleen cells were harvested and depleted of $CD4^+$ or $CD8^+$ T cells using magnetic cell-sorting system, and the ELISPOT assay was carried out. The ELISPOT assay showed that the $CD8^-$ cells ($CD4^+$ T cells and antigen-presenting cells) derived from the mice immunized with HSP105-pulsed BM-DC produced a significantly larger amount of interferon-γ in response to HSP105 than did $CD8^-$ cells derived from mice immunized with BM-DC. Similar results were observed for the $CD4^-$ cells ($CD8^+$ T cells and antigen-presenting cells) (Fig. 4a). These observations clearly indicate that both HSP105-specific $CD4^+$ and $CD8^+$ T cells were induced in the mice immunized with HSP105-pulsed BM-DC vaccine.

To investigate the antitumor effect of the HSP105-pulsed BM-DC vaccination, the tumor was evaluated histopathologically. The small intestines derived from the mice used for the ELISPOT assay were stained with anti-CD4 or anti-CD8 mAb. Both $CD4^+$ and $CD8^+$ T cells infiltrated into the tumors of mice immunized with HSP105-pulsed BM-DC; however, this was not the case in tumors derived from the mice immunized with BM-DC (Fig. 4b). These results suggest that HSP105-pulsed BM-DC have the potential to sensitize many HSP105-specific $CD4^+$ and $CD8^+$ T cells to kill tumor cells.

Discussion

In the present study, the HSP105-pulsed BM-DC vaccine could sensitize HSP105-specific T cells *in vivo* and inhibited the spontaneous development of intestinal tumors overexpressing HSP105 in $Apc^{Min/+}$ mice. For diseases of germline mutations that cause malignancy throughout the body, such as FAP, novel strategies for the prevention of cancer are needed urgently because there is no satisfactory treatment for FAP. Therefore,

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