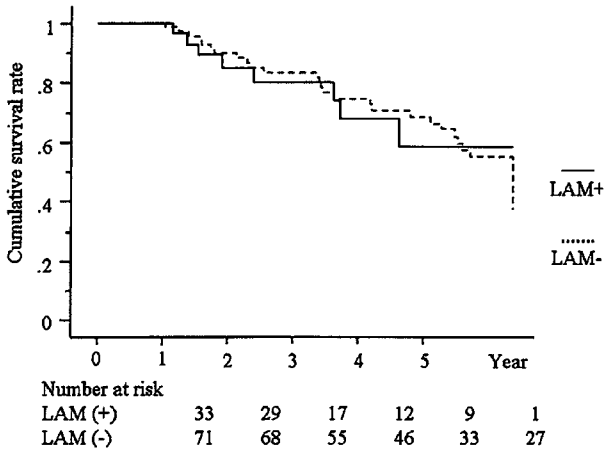

Fig. 4 Kaplan–Meier curves comparing the cumulative survival rates in LAM-treated and control group. Statistical analysis was done with log-rank test

We observed no significant differences in the overall and recurrence-free survival between LAM and nontreatment groups. The baseline liver function was poorer and AFP, a strong predictor of recurrence [21], was higher in the LAM group. Noninferior survival in the LAM group may suggest some beneficial effects of LAM administration. However, we did not find significant effects of LAM in multivariate analysis, possibly due to the small number of events (40 deaths). To conclude the efficacy of LAM treatment on recurrence-free or overall survival, either much larger cohort studies or well-designed randomized control trials will be required. The current study demonstrated the safety of LAM administration in HCC patients after HCC treatment, including the data on LAM resistance similar to a previous report [22].

In conclusion, LAM treatment after ablation therapy for HBV-related HCC improved liver function without any particular untoward effects. Its effects on survival remain to be studied in future studies.

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Annals of Surgical Oncology (© 2007)
DOI: 10.1245/s10434-007-9705-0

Systemic Therapy for Hepatocellular Carcinoma: Cytotoxic Chemotherapy, Targeted Therapy and Immunotherapy

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Conventional cytotoxic chemotherapy has not provided clinical benefit or prolonged survival for patients with advanced HCC. This review summarizes the results of prospective clinical trials of several categories of systemic therapy, with emphasis on the more promising results from recent trials of biologically targeted therapeutic agents in HCC.

Key Words: Hepatocellular—Hepatoma—Chemotherapy—Chemoresistance—Clinical trials—Biologic therapy.

Hepatocellular carcinoma (HCC) is currently the fifth most common solid tumor worldwide, and the fourth leading cause of cancer-related death.[1] It is a lethal disease, as the annual incidence roughly equals the annual mortality.[2,3] Eighty percent of new cases occur in developing countries, but the incidence is rising in economically developed regions including Japan, Western Europe, and the United States.[4] More than 80% of patients present with advanced or unresectable disease, and for those patients who do undergo resection, the recurrence rates can be as high as 50% at 2 years.[5–7] Thus, many patients will seek systemic therapy. A 1997 meta-analysis evaluating the results of 37 randomized clinical trials of systemic and regional chemotherapy in 2803 HCC patients concluded that nonsurgical therapies were ineffective or minimally effective at best.[8] Most HCC patients have underlying cirrhosis and hepatic dysfunction, which complicates safely administering systemic therapy and conducting trials of new agents in this patient population.

Received June 1, 2007; accepted October 23, 2007
Proceedings of the 5th International Meeting Hepatocellular Carcinoma: Eastern and Western Experiences held in Houston, TX, January 11–13, 2007.
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CYTOTOXIC CHEMOTHERAPY FOR HCC: REASONS FOR LACK OF EFFICACY

Most published studies of systemic chemotherapy report response rates of 0% to 25%, and there is no published evidence that systemic chemotherapy improves overall survival in any subset of HCC patients.[9–11] HCC comprises clinically chemotherapy-resistant tumors, and this observation is supported by low response rates across a wide variety of cytotoxic agents (Table 1). The most widely used agent has been doxorubicin, both as a single agent and in

TABLE 1. *Selected clinical trials in patients with advanced hepatocellular carcinoma*

Study	Regimen	Phase	Sample size	Response rate (%)	Median survival (mo)
Cytotoxic chemotherapy					
Yeo et al.[15]	PIAF vs. Adriamycin	3	94/94	20.9 vs. 10.5	8.6 vs. 6.83
Mok et al.[66]	Nolatrexed vs. doxorubicin	2	37/17	0	4.9 vs. 3.7
Posey et al.[67]	TI38067 vs. Adriamycin	2/3	169/170	NA	5.7 vs. 5.6
Gish et al.[16]	Nolatrexed vs. doxorubicin	3	444	1.4 vs. 4.0	5.5 vs. 8 (P = .0068)
Patt et al.[68]	Thalidomide	2	37	6%	6.8
Pastorelli et al.[69]	Pegylated doxorubicin + gemcitabine	2	35	23%	8.8
Immunotherapy/hormonal therapy					
Barbare et al.[70]	Tamoxifen vs. BSC	2	210/210	NA	4.8 vs. 4.0
O'Neil et al.[71]	Octreotide LAR	2	17		TTTF 3.5; OS 10
Lee et al.[61]	Dendritic cells	2	31	12.9%	1-y survival 40%
Targeted biologic therapy					
Llovet et al.[33]	Sorafenib vs. placebo	3	602	2.3%	10.7 vs. 7.9 (P = .00058)
Abou-Alfa et al.[32]	Sorafenib	2	137	2.2	9.3
Philip et al.[44]	Erlotonib	2	38	9%	13
Thomas et al.[43]	Erlotonib	2	40	0%	10.75
Thomas et al.[50]	Bevacizumab + erlotonib	2	34	20.6%	19 (PFS 9)
Zhu et al.[72]	Cetuximab	2	30	0%	PFS 6 wk; OS 22 wk
O'Dwyer et al.[73]	Gefitinib	2	31	3%	PFS 2.8; OS 6.5
Combination cytotoxic + biologic therapy					
Sun et al.[74]	Capecitabine, oxaliplatin, bevacizumab	2	30	11%	PFS 5.4
Zhu et al.[75]	Gemox + bevacizumab	2	33	20	9.6

PIAF, cisplatinum, interferon, doxorubicin, and 5-fluorouracil; OS, overall survival; PFS, progression-free survival; TTTF, time to treatment failure.

combination with other drugs.[12–14] An early randomized trial against best supportive therapy showed greatly increased survival, but this was only in the order of weeks. A pivotal phase 3 trial of doxorubicin combination chemotherapy (cisplatinum, interferon, doxorubicin, and 5-fluorouracil, PIAF) showed a statistically significant difference in response rate favoring PIAF, but no survival difference.[15] Another study in which doxorubicin was the control arm in a randomized phase 3 trial against nolatrexed showed a highly statistically significant survival benefit in favor of the control doxorubicin arm.[16] The variable results from trials summarized in Table 1 have contributed to the lack of consensus regarding "standard" chemotherapy for patients with advanced HCC; they have also resulted in ongoing debate regarding the best control arm for future randomized trials.

Furthermore, the definition of *drug activity* has changed over the years. It is now well recognized that the conventional markers of radiographic response (World Health Organization [WHO] or RECIST criteria) are poorly related to tumor cell kill in liver tumors and that end points other than radiographic tumor shrinkage, such as time to tumor progression, progression-free survival, and certainly overall survival, are more meaningful measures of therapeutic benefit.[17]

The third set of reasons is related to HCC tumor biology and intrinsic or acquired drug resistance. Large HCCs commonly develop areas of central necrosis, which may inhibit drug delivery to actively growing parts of the tumor. Topoisomerase IIa encodes an enzyme that is the target for anticancer chemotherapeutic agents such as doxorubicin, and mutations are associated with resistance.[18] There is upregulation of topoisomerase IIa in doxorubicin-resistant HCC cell lines, and its expression is associated with an aggressive tumor phenotype.[19] Cancer cells, including HCC cells, often have intrinsic drug resistance mediated by enhanced cellular drug efflux of several cytotoxic agents. This phenomenon is associated with increase in a drug transporter family, the adenosine triphosphate–binding cassette proteins that include MDR1, p-glycoprotein (p-gp), and the multidrug resistance protein (MRP).[20,21] Both these are overexpressed in HCC.[21,22] Overexpression of MDR1 accompanied by a decrease in doxorubicin accumulation levels has been observed in certain HCC cell lines.[23] The *H19* gene is believed to induce p-gp expression and MDR1-associated drug resistance in HCC cells through regulation of MDR1 promoter methylation.[23] Coexpression of p53 and p-gp may contribute to HCC drug resistance in HCC cell lines.[24] In addition, recent evidence suggests that hypoxia, MDR1 expression, and an angiogenic HCC phenotype may be linked.[25,26]

Clearly, to improve the outcome for patients with advanced HCC, alternatives to traditional cytotoxic chemotherapy agents must be explored.

HCC IN THE ERA OF TARGETED THERAPIES

In recent years, several molecular targets, including oncogenes, oncoproteins, and cellular receptors, have been identified in a variety of cancers as being key elements in carcinogenic pathways. Consequently, several agents have been developed that, by a variety of mechanisms, interfere with cell signaling and have demonstrated anticancer activity. In some cancers, the molecular target–targeted agent relationship is well understood—for example, the monoclonal antibody trastuzumab is only effective in tumors in which the her-2/neu oncoprotein is amplified. Conversely, there are several agents that target the transmembrane epidermal growth factor receptor (EGFR) and have demonstrated survival benefit in a broad range of tumor types, yet little is understood regarding the relationship between target expression and agent efficacy or lack thereof. Several targeted or novel biologic agents are now being tested in HCC patients. This discussion focuses on those aspects of hepatocarcinogenesis that are sufficiently well understood to provide a rational basis for developing clinical trials that use existing novel or targeted therapies in HCC.

TARGETING CARCINOGENIC PATHWAYS IN HCC

Hepatocarcinogenesis is known to be a complex multistep process that results in a large number of heterogeneous molecular abnormalities, and thus numerous potential targets for existing therapeutic agents. The pathways summarized below represent rational targets for existing therapeutic agents in HCC.

Mitogen-Activated Protein Kinase Pathway

The mitogen-activated protein kinase pathway (MAPK) pathway involves a cascade of phosphorylation of four major cellular kinases; ras, raf, MAP, and ERK (MAP, mitogen-activated protein kinase; ERK, extracellular-signal-regulated kinase), which is responsible for cellular proliferation and differentiation. These intermediates are found to be high in both HCC cell lines and human specimens.[27–31] Therapeutic agents that target this pathway include sorafenib (targets both raf and vascular endothelial growth factor receptor, VEGFR) and farnesyl transferase inhibitors (targeting ras). A phase 2 trial of sorafenib demonstrated antitumor activity in advanced HCC patients. This study did not meet its primary end point of response on the basis of WHO criteria, with limited response rate of 2.2%. However, many patients (33.6%) had stable disease for at least 4 months, with many showing central tumor necrosis.[32] On the basis of the encouraging overall survival of 9.2 months reported in the phase 2 trial, a placebo-controlled international trial was conducted in HCC patients with Childs-Pugh A cirrhosis. Preliminary data presented in abstract form from the phase 3 trial showed better survival in the sorafenib arm (10.7 months) compared with placebo (7.9 months).[33] These results indicate that this agent offers a survival advantage compared with placebo and with several cytotoxic agents (based on historical controls), but this may be comparable to survival observed with other biologic agents (Table 1).

PI3K/AKT/mTOR Pathway (Phosphoinositide-3 Kinase/Protein Kinase B/Mammalian Target of Rapamycin)

This kinase cascade is responsible for cellular proliferation and apoptosis, and is closely linked to cell cycle. PI3K is associated with cell surface growth factor receptors, and on ligand binding can trigger formation of PIP3, which in turn activates Akt and leads to a number of downstream events (mTOR being one of the targets). This pathway is known to be upregulated in a subset of HCC patients.[34–36] Molecular target therapy such as rapamycin, a naturally occurring mTOR inhibitor, showed promising results in HCC cell lines,[37,38] but no results from clinical trials of any agents that target mTOR in HCC patients are available.

Epigenetic Changes

Epigenetic modifications of the genome (mainly hypermethylation of CpG island and histone deacetylation) are accumulated during hepatocarcinogenesis in chronically injured liver. A large number of tumor suppressor genes have been shown to be inactivated by epigenetic mechanisms in HCC. Success in epigenetic therapy (such as 5-aza-2′-deoxycytidine and SAHA) had been achieved in both hematological malignancies and solid tumors. In HCC cell lines, chemosensitivity can be potentiated by epigenetic therapy.[39,40] A multicenter phase 1/2 trial on a novel histone deacetylase inhibitor, PXD-101, is currently underway in Hong Kong.

GROWTH FACTORS AS THERAPEUTIC TARGETS IN HCC

The epidermal growth factor receptor (EGFR) is frequently expressed in human hepatoma cells, and EGF may be one of the mitogens needed for the growth of hepatoma cells.[41,42] Several agents that inhibit EGF signaling are clinically available, including gefitinib, cetuximab, erlotinib, and panitumumab. Erlotinib is an orally active and selective inhibitor of the EGFR/HER1-related tyrosine kinase enzyme. EGFR/HER1 expression was detected in 88% of the patients in a phase 2 study of erlotinib.[43] In two phase 2 studies of this agent, the response rates were was < 10%, but the disease control rate was > 50%, and median survival times were 10.75 and 13 months, respectively.[43,44] Other studies of anti-EGFR agents in HCC are summarized in Table 1.

HCCs are generally hypervascular, and vascular endothelial growth factor (VEGF) promotes HCC development and metastasis.[45–49] Various agents targeting the VEGF circulating ligand or transmembrane receptor, including bevacizumab (Avastin), sorafenib (Nexavar), and TSU-68, have been studied in patients with HCC. Bevacizumab, a monoclonal antibody inhibitor of VEGF ligand, has been investigated in phase 2 studies alone or combination with other agents. These studies showed a high disease control rate of > 80% and a median progression-free survival of > 6 months.[50] Sorafenib, an oral multikinase inhibitor, blocks tumor cell proliferation mainly by targeting Raf/MEK/ERK signaling at the level of Raf kinase, and exerts an antiangiogenic effect by targeting VEGFR-2/-3.[51–54] TSU-68 is an oral antiangiogenesis compound that blocks VEGFR-2 (vascular endothelial growth factor receptor), PDGFR (platelet-derived growth factor receptor), and FGFR (fibroblast growth factor receptor); a phase 1/2 study has been conducted in Japan.[55]

IMMUNOTHERAPY OF HCC

Increasing evidence suggests that immune responses are important in the control of cancer and that the manipulation of the immune system to recognize and attack tumors may be a valuable form of therapy. HCC is attractive target for immunotherapy, because in addition to documented cases of spontaneous regression, lymphocytes can be seen infiltrating tumors and tumor-associated proteins such as alfa-fetoprotein (AFP) could act as targets for immune-mediated attack.[56,57] Given the limitations of current treatment modalities in the treatment of HCC, interest has been stimulated in immunotherapy of HCC, and a number of promising strategies have been developed in the laboratory, some of which have been applied in the clinical setting.

HCC are often infiltrated with lymphocytes, and patients with higher levels of tumor-infiltrating lymphocytes have a better prognosis after resection and transplantation.[56] A randomized, controlled clinical trial has shown that disease-free survival after HCC resection can be increased by infusion of lymphocytes activated by anti-CD3 and interleukin 2, suggesting a promising role for T cell adoptive immunotherapy.[58]

To generate a tumor-specific immune response, tumor-associated antigens must be presented to the immune system in an immunostimulatory context. Dendritic cells (DC) are the most efficient method of stimulating immune responses and are potent inducers of antitumor immunity when loaded with tumor-associated antigens. Animal models have shown encouraging results for DC-based vaccination strategies. DC transduced with adenovirus encoding AFP were able to prevent or delay growth of an AFP-producing tumor cell line in mice, and this was accompanied by the appearance of AFP-specific cytotoxic T lymphocytes.[59] By using fusions of DC and syngeneic hepatoma cells, Kawada and colleagues[60] were able to prevent the growth of implanted hepatoma cells and prevent local recurrence after surgical resection in rats.

The success of animal models in DC-based immunotherapy of HCC has lead to a number of clinical studies. These studies are all small and are mainly phase 1/2 studies designed primarily to assess feasibility and tolerability of this treatment modality. Currently reported DC vaccination studies have used DC loaded with autologous tumor or hepatoma cell line lysates.[61] DC have also been directly injected into tumors.[62] Clinical responses to these approaches have at best been modest, and the success of animal vaccination studies has not to date been replicated.

There are many reasons why this should be. First, the patients selected for clinical studies have been those with advanced disease and therefore may have tumor-induced immunosuppression. Additionally, questions still remain about the optimal route of administration of DC vaccines and the optimal method of loading tumor-associated antigens needs to be established. Importantly, the effect of concomitant viral infection, especially with hepatitis C virus, needs to be clarified.[63]

Currently the role for immunotherapy in HCC is limited, but from studies performed so far, we can be certain that future clinical studies should be ran-

domized and include patients with earlier disease and small tumor burden, to better identify potential benefit and truly identify the role of immunotherapy in HCC.

CLINICAL TRIAL DESIGN FOR BIOLOGIC AGENTS IN HCC

As noted previously, the availability in the clinic of several novel biologic agents and the urgent need for effective therapies for advanced HCC has led to the evaluation of many of these agents in HCC, principally in phase 2 trials. The SHARP trial[33] was the first to demonstrate a statistically significant survival benefit for any chemotherapy agent in patients with HCC. This trial was, however, conducted in patients with excellent performance status and well-preserved liver function. The efficacy and safety of sorafenib in patients with more tumor-related symptoms and advanced hepatic dysfunction remains to be established.

A key objective going forward is to assess new agents and to integrate these and sorafenib into the treatment of all stages of HCC and patients with Child-Pugh A and B cirrhosis. The classic approach is to evaluate new agents in single-arm phase 2 studies and use classic radiological response criteria such as WHO or RECIST as a measure of activity and thereby identify promising agents to take forward into phase 3 clinical trial testing against an appropriate control group. This approach, however, is being questioned because traditional radiographic tumor responses may not occur with biologic agents, although they may cause other anticancer effects that may lead to meaningful patient benefit. This is especially true in HCC, where radiological assessment is notoriously difficult because of poor delineation of tumors in the liver[64] and tumor necrosis may occur without any change in overall tumor dimensions.

These observations have led some investigators to develop phase 2 studies with a major focus on correlative studies that may help delineate a mechanism of action for a particular drug (e.g., a kinase inhibitor along one of the different cell cycle pathways) such as downregulation of a downstream kinase which may predict response,[32] or by the use of novel radiological techniques that use changes in blood flow as criteria by which to assess biologic activity of antiangiogenic therapies.[32] Another option is to use the randomized phase 2 trial design that, by providing a contemporary control group, may permit a more confident assessment of the likelihood that a particular agent is worthy to progress to phase 3 trials.[65]

CONCLUSIONS

Conducting controlled clinical trials of systemic chemotherapy regimens in HCC patients is challenging. Obstacles include the multiple comorbidities of patients with cirrhosis, the intrinsic chemoresistance of HCC, the advanced nature of HCC at the time of presentation in most patients, the pharmacotherapeutic challenges of treating a cancer that arises in an already damaged liver, and the distribution of patients primarily in developing nations where multidisciplinary treatment of HCC may not be available. HCC is a heterogeneous disease in terms of its cause, underlying associations, and biologic and clinical behavior, which further complicates clinical trial design. The need for effective systemic therapies for HCC patients is clearly evident, and making progress in this area requires the talent and expertise of all of the medical disciplines involved in the care of HCC patients.

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Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma

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(Received July 31, 2007/Revised September 13, 2007/Accepted September 20, 2007/Online publication October 22, 2007)

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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doi: 10.1111/j.1349-7006.2007.00648.x

>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

doi: 10.1111/j.1349-7006.2007.00648.x

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

doi: 10.1111/j.1349-7006.2007.00648.x

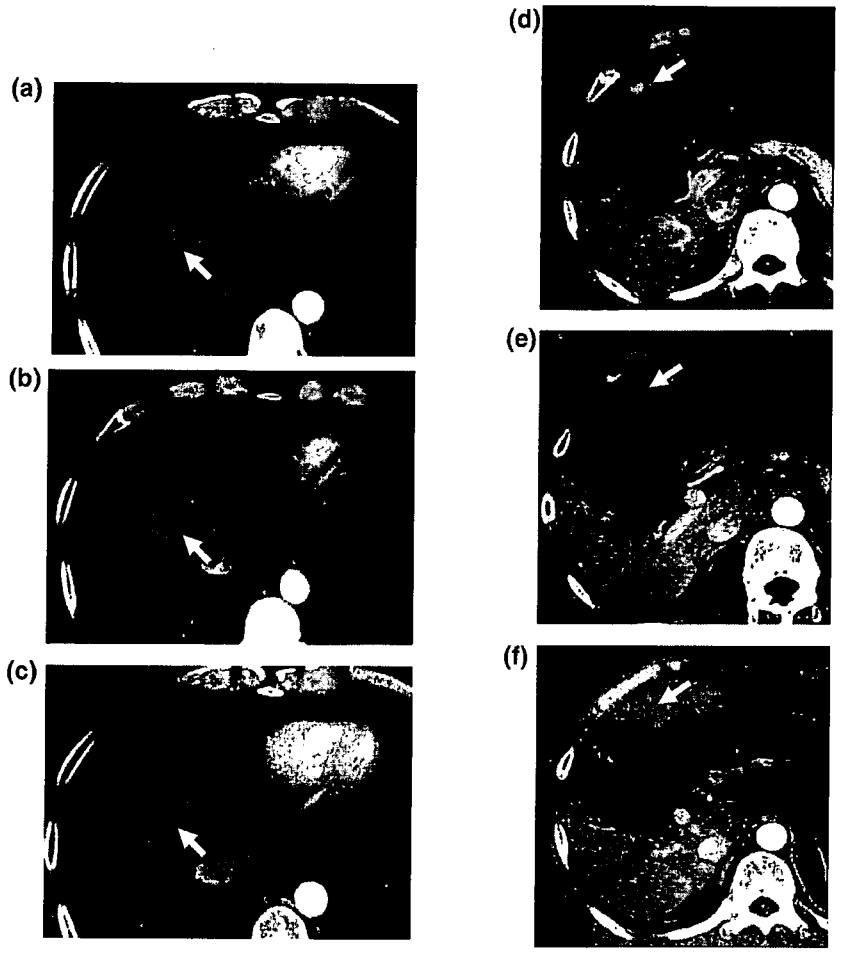

Fig. 1. A 48-year-old man with multiple tumors of hepatocellular carcinoma (HCC) after hepatectomy, percutaneous ethanol injection, and transcatheter arterial embolization. (a) Hypervascular HCC lesion, 1 cm in diameter, was revealed at the early phase of dynamic computed tomography (CT) before administration 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.

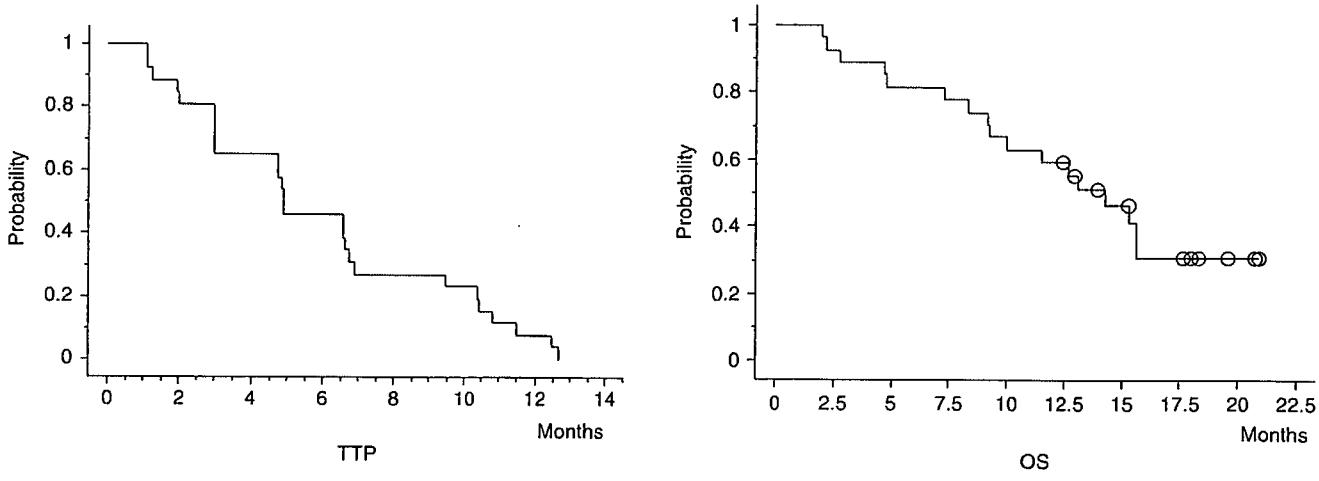

Fig. 2. Time to progression (TTP) in all 27 patients treated with sorafenib. The median TTP was 4.9 months, and the 6-month survival rate was 46.2%. Overall survival (OS) in the 27 patients treated with sorafenib. The median OS period was 15.6 months, and the 1-year survival rate was 59.3%.

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 1 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.

Acknowledgments

This study was supported by Bayer Yakuhin.

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Liver International ISSN 1478-3223

CLINICAL STUDIES

Obesity did not diminish the efficacy of percutaneous ablation for hepatocellular carcinoma

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Keywords
disease-free survival – hepatocellular carcinoma – obesity – percutaneous ablation – survival analysis

Abbreviations
AFP, α-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of AFP; DCP, des-γ-carboxy prothrombin; HCC, hepatocellular carcinoma; HCV-Ab, HCV antibodies; PEIT, percutaneous ethanol injection therapy; PMCT, percutaneous microwave coagulation therapy; RFA, radiofrequency ablation

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Received 27 October 2006
accepted 13 November 2006

DOI:10.1111/j.1478-3231.2006.01420.x

Abstract

Background: Overweight and hepatic steatosis can increase the risk of hepatocarcinogenesis. In addition, overweight may affect the treatment efficacy of ultrasound-guided percutaneous ablation. We evaluated the impact of overweight on the safety and efficacy of percutaneous ablation to hepatocellular carcinoma (HCC). *Methods:* We enrolled 743 patients with naïve HCC who were treated by percutaneous ablation including ethanol injection, microwave coagulation, and radiofrequency ablation (RFA) between 1995 and 2003. Patients were divided into two groups by body mass index (BMI): 219 overweight patients with BMI $> 25\,kg/m^2$ and 524 control patients with BMI $\leq 25\,kg/m^2$. The effects of BMI on complications of percutaneous ablation, HCC recurrence, and overall survival were analyzed, together with others including tumor and liver function-related factors. *Results:* The overweight group required a significantly larger number of sessions by RFA ($P=0.01$). Major complications were identified in 8.7% in the overweight group and 7.6% in the control group ($P=0.94$). There was no significant difference in cumulative recurrence rate and local tumor-progression rate between the two groups ($P=0.63$ and 0.44). Cumulative survival rates at 1, 3, and 5 years were 95.4%, 75.7%, and 57.8%, respectively, in the overweight group and 94.1%, 78.0%, and 58.8% in the control group ($P=0.99$). *Conclusions:* The results indicated that overweight did not increase complications nor affect HCC recurrence and overall survival. However, the number of sessions of RFA was significantly greater in overweight patients, suggesting that overweight was associated with minor technical difficulties.

Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer death worldwide, accounting for more than 500 000 annual deaths, with an increasing incidence throughout the world (1, 2). HCC usually develops in patients with advanced liver fibrosis as a result of chronic liver diseases such as chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, chronic alcohol abuse (2, 3), and hemochromatosis (4). In addition, several studies have shown a strong association between obesity and HCC development, reporting a relative risk of 1.7 in women and 4.6 in men (5–8). Obese patients often have hepatic steatosis and co-existent insulin resistance (9), the latter of which is associated with increased oxidative stress via lipid peroxidation and possibly with enhanced hepatocarcinogenesis (10).

In Japan and several other countries, chronic HCV infection is the predominant cause of HCC. It is known that recurrence after complete treatment of primary lesions is more frequent in HCV-related HCC than in HCC because of other etiologies (11), suggesting that multicentric carcinogenesis is a dominant form of recurrence. Overweight is reportedly associated with increased steatosis and accelerated fibrosis also in chronic hepatitis C (9, 12). Thus, overweight may be a risk factor of recurrence after complete ablation of HCV-related HCC.

Current therapeutic options for HCC include surgical resection, orthotropic liver transplantation, and percutaneous tumor ablation. Surgical resection, considered to be the first choice treatment (13, 14), is frequently contra-indicated by underlying chronic liver diseases (3, 15). Liver transplantation, although ideal for treating both HCC and background liver, is limited in application by the scarcity of donor organs. Percutaneous tumor ablation such as ethanol injection

(PEIT), percutaneous microwave coagulation therapy (PMCT), and radiofrequency ablation (RFA) can achieve a complete local cure even in patients with impaired liver function (16–19). Percutaneous ablation is usually performed under ultrasound guidance. In obese patients, ultrasound beams may be attenuated by subcutaneous and intrahepatic fat and the tumor targeting may become difficult. This may result in increased complications and/or compromised treatment efficacy. In particular, the latter, together with increased risk of multicentric carcinogenesis caused by obesity, may negatively affect recurrence-free and overall survival. The aim of this study is to investigate the feasibility, efficacy, and long-term outcome of percutaneous ablation to obese HCC patients.

Patients and methods

Patients

A total of 758 patients received percutaneous ablation (PEIT, PMCT, or RFA) as the initial treatment for HCC at the authors' institution between January 1995 and December 2003. We enrolled 743 of them in this study, excluding 15 patients because of intractable ascites. Patients were divided into two groups by body mass index (BMI): overweight as BMI $> 25\,kg/m^2$ and controls as BMI $\leq 25\,kg/m^2$.

Diagnosis of HCC

HCC diagnosis was based on typical findings on imaging studies such as arterial hyperattenuation and portal hypo-attenuation on contrast-enhanced dynamic-computed tomography (CT) or angiography under CT (20, 21). Most nodules were also confirmed histopathologically with ultrasound-guided biopsy. Pathological grade was based on the Edmondson–Steiner criteria (22).

Treatment and follow-up

The inclusion criteria for percutaneous ablation were as follows: total bilirubin concentration $< 3.0\,mg/dl$; platelet count $\geq 50 \times 10^3/mm^3$; and prothrombin activity $\geq 50\%$. Patients with portal vein tumor thrombosis, massive refractory ascites, or extrahepatic metastasis were excluded. In general, we performed percutaneous ablation on patients with three or fewer lesions, all of which were $\leq 3.0\,cm$ in diameter. However, we also performed ablation on patients with more than three lesions or lesions larger than 3.0 cm if the procedure could be assumed to be clinically beneficial. The procedure was meticulously described elsewhere (23). We sometimes found difficulty in accurately targeting HCC nodules in those patients. In such cases, we first located a thin PEIT needle (22-gauge, 20 cm long) as close as possible to the tumor referring to CT images and observable vascular and biliary structures. Then, we checked the position of the needle tip by longitudinal and horizontal ultrasound probing, adjusting the position when required. PMCT/RFA needles were then inserted targeting the tip of the PEIT needle. A total of three (5.2%) patients in the PMCT group, and 28 (6.5%) patients in the RFA group needed this 'targeting' technique.

After several sessions of percutaneous ablation, dynamic CT was performed with a section thickness of 0.5 cm to evaluate treatment efficacy. Complete ablation was defined as hypo-attenuation of the whole lesion, together with the surrounding liver parenchyma as the safety margin. Patients received additional sessions of ablation until complete ablation was confirmed for each HCC nodule.

We defined a 'session' as a single intervention episode that consists of one or more ablations performed on one or more tumors, and a 'treatment' as the completed effort to ablate one or more tumors that consists of one or more sessions according to the working party report on the image-guided tumor ablation (24). Complications were assessed on the basis of number of treatments and sessions. Major complications were defined as those that, if left untreated, might threaten the patient's life, lead to substantial morbidity and disability, or result in hospital admission, or substantially lengthen hospital stay according to the previously described guideline (25–27). All other complications were considered minor. We compared the incidence of major complications between the two groups.

The follow-up consisted of monthly blood tests and monitoring of tumor markers at the outpatient clinic, with ultrasonography and dynamic CT scan performed every 4 months. Tumor recurrence was diagnosed according to the same criteria applied to the initial HCC. Intrahepatic HCC recurrence was classified as either recurrence at a site distant from the primary tumor or adjacent to the treated site (local tumor progression).

Analysis of recurrence

Tumor recurrence was analyzed among 685 of the 743 naïve HCC patients. In the excluded 58 patients, RFA was intended only to reduce tumor burden as it had been judged before the start of percutaneous ablation that curative treatment was not feasible, mainly because of HCC multinodularity. Recurrence time was defined as the interval between the first ablation and

the detection of HCC recurrence, death without recurrence, or the last examination until December 31, 2005, whichever came first. As tumor recurrence and death without recurrence are 'competing risks,' we used cumulative incidence estimation with competing risks described by Gray et al. (28). The risk for recurrence was assessed with a multivariate proportional hazard regression model described by Fine and Gray controlling for factors shown to be significant in univariate analysis (29).

Analysis of survival

Survival analysis was performed on patient basis, including all 743 patients in the analysis. Survival time was defined as the interval between the first treatment and the death or the last visit to the outpatient clinic until March 31, 2005. A cumulative survival curve was plotted according to the Kaplan–Meier method, and the difference between the groups was assessed by a log-rank test. The effect of overweight on survival was assessed by multivariate Cox proportional hazard regression adjusted for factors shown to be significant in univariate analysis.

Statistical analysis

We used the following variables obtained at the initial ablation therapy in the analysis of recurrence and survival: age, gender, tumor factors including size and number of nodules, aspartate aminotransferase (AST) levels, platelet counts, two HCC biomarkers, namely, α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), positivity for viral markers (hepatitis B virus surface antigen and anti-hepatitis C virus antibody), and liver function as classified by Child–Pugh scores (5–6 points, class A; 7–8 points, class B; and 9–12, points class C) based on albumin, bilirubin, prothrombin activity, and the presence of ascites or hepatic encephalopathy. Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) was excluded because this examination was introduced in April 1997 in our hospital. Nominal categorical data were represented by corresponding binary dummy variables. Continuous variables were transformed into ordinal categories. Categorical variables were compared by the χ^2 test, except the Child–Pugh score, which was compared by the Cochran–Armitage test. Continuous variables were compared with unpaired Student's *t*-test (for parametric variables) and Wilcoxon's test (for non-parametric variables). Data processing and analysis were performed using the S-PLUS 2000 (MathSoft Inc., Seattle, WA).

Results

Patient profile

The baseline characteristics of patients, 496 males and 247 females, are shown in Table 1. The median age (25th–75th percentiles) was 66.6 (60.6–70.3) for the overweight patients and 67.8 (62.0–72.8) for the controls. The proportion of female patients was significantly larger in the overweight group ($P = 0.02$). As for background liver disease, the proportion of non-viral liver diseases was significantly larger in the overweight group (19.0% vs. 7.6%, $P < 0.001$). These results were compatible with a previous report that cryptogenic cirrhosis was found preferentially among obese or female patients (30).

Percutaneous ablation

The treatment modality was PEIT/PMCT/RFA in 77/14/128 patients in the overweight group and in 181/43/300 patients in controls ($P = 0.70$). The overweight group required a significantly larger number of sessions for achieving complete ablation by RFA but the difference was not significant for PEIT or PMCT (Table 2). Major complications were identified in 19/219 (8.7%) in the overweight group and 40/524 (7.6%) in the control group ($P = 0.94$) (Table 3). By right, obesity is defined as BMI $> 30\,kg/m^2$ according to World Health Organization (WHO) criteria (31). However, there were only 23 patients (3.1%, 95% CI: 2.1–5.3%) whose BMI exceeded $30\,kg/m^2$ among the patients in the current study. Although statistical analysis was not feasible because of the small number, the safety and efficacy of percutaneous ablation among these patients was comparable with those among the others: complete ablation was achieved in all patients with two (8.7%) major complications, neoplastic seeding, and hemoperitoneum. In overall procedures, there was no treatment-related death.

Analysis of recurrence

By the end of the follow-up, tumor recurrence was identified in 144/219 (65.8%) patients in the overweight group and 336/524 (64.1%) in controls. There was no significant difference in cumulative recurrence rate between the two groups ($P = 0.64$; Fig. 1). No significant difference was identified in local tumor progression rate between the two groups ($P = 0.44$). Recurrence was not evaluated in the 30 patients who were excluded from curative ablation and retained viable tumor. The distribution of BMI did not differ between these 58 patients ($> 25\,kg/m^2$ in 16 and $\leq 25\,kg/m^2$ in 42) and the others ($P = 0.74$). The

Table 1. Baseline characteristics of patients

	BMI > 25 kg/m^2 (N=219), overweight	BMI ≤ 25 kg/m^2 (N=524), control	P value
Age*	65.6 ± 8.2	67.3 ± 8.1	0.36†
Male	133 (60.7%)	363 (69.2%)	0.02‡
Viral infection	184 (84.0%)	484 (92.3%)	< 0.001‡
HBs-Ag positive	24 (11.0%)	42 (8.0%)	
Anti-HCV positive	156 (84.7%)	434 (82.8%)	
Both negative	35 (19.0%)	40 (7.6%)	
Drinking > 80 g/day	41 (18.7%)	90 (17.2%)	0.61‡
Child–Pugh classification			0.07§
Class A	139 (63.4%)	372 (71.0%)	
Class B	75 (34.2%)	147 (28.0%)	
Class C	5 (2.2%)	5 (1.0%)	
Tumor size (mm)*	27.4 ± 12.3	27.9 ± 13.1	0.65†
Number of nodules			0.38‡
Uninodular	119 (54.3%)	303 (57.8%)	
Multinodular	100 (46.7%)	221 (42.2%)	
AFP > 100 ng/ml	59 (26.9%)	127 (24.2%)	0.43‡
DCP > 100 mAU/ml	46 (21.0%)	86 (16.4%)	0.13‡
AFP-L3 > 15%	23 (10.5%)	64 (12.2%)	0.42‡
Treatment modalities			0.70‡
PEIT	77 (35.2%)	181 (34.5%)	
PMCT	14 (6.4%)	43 (8.2%)	
RFA	128 (58.4%)	300 (57.3%)	

AFP, α-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of AFP; DCP, des-γ-carboxyprothrombin; BMI, body mass index; HBs-Ag, HBV surface antigen; HCV, hepatitis C virus; PEIT, percutaneous ethanol injection therapy; PMCT, percutaneous microwave coagulation therapy; RFA, radiofrequency ablation.

*Expressed as standard deviation (SD).

†Unpaired Student's t test.

‡χ^2 tests.

§Cochran–Armitage test.

Table 2. Number of sessions

Variable, n (%)	BMI > 25 kg/m^2 (N=219)	BMI ≤ 25 kg/m^2 (N=524)	P value
PEIT	6.8 ± 3.6	6.7 ± 3.7	0.79†
PMCT	2.2 ± 2.1	2.8 ± 2.5	0.26†
RFA	1.9 ± 1.1	1.7 ± 1.1	0.01†

BMI, body mass index; PEIT, percutaneous ethanol injection therapy; PMCT, percutaneous microwave coagulation therapy; RFA, radiofrequency ablation.

*Expressed as mean ± standard deviation.

†Wilcoxon's test.

median recurrence-free times of 23 patients whose BMI exceeded 30 kg/m^2 were 2.29 ± 1.63 years. We also compared patients with BMI > 27 kg/m^2 and those with BMI ≤ 27 kg/m^2 and found no significant difference in recurrence (P = 0.54, respectively).

Univariate analysis identified HCV positivity (P < 0.001), platelet count < 150 × 10^3/mm^3 (P = 0.05), tumor size > 3.0 cm (P = 0.001), multinodular HCC (P = 0.001), AFP > 400 ng/ml (P < 0.001), and DCP > 100 mAU/ml (P = 0.001) as significant predictors for recurrence after curative ablation. Multivariate analysis also showed HCV positivity (P = 0.002), platelet count < 150 × 10^3/mm^3 (P = 0.05), tumor size > 3.0 cm (P = 0.004), multinodular HCC (P = 0.007), and AFP > 400 ng/ml (P = 0.02) as significant predictors. However, overweight (BMI > 25 kg/m^2) was not significant for a cumulative recurrence event after adjusting for the significant predictors (P = 0.88; Table 4).

We also performed subanalysis among tumors larger than 3.0 cm. The results showed no significant difference between overweight and control groups in tumor recurrence rate and local tumor progression rate between the two groups (P = 0.83 and 0.47, respectively).

Analysis of survival

There were 223 deaths during the observation period: 72 deaths in the overweight group and 151 deaths in the control group. The causes of death were HCC progression in 128 patients, hepatic failure in 56, upper gastro-intestinal bleeding in nine, and liver-unrelated causes in 30 (Table 5). There was no significant difference in the proportion of causes of deaths between the two groups (P = 0.84).

Table 3. Major complications of percutaneous ablation

Variable, *n* (%)	BMI > 25 kg/m^2 (*N* = 219)	BMI ≤ 25 kg/m^2 (*N* = 524)
Liver infarction	3	2
Liver abscess	2	7
Neoplastic seeding	5	9
Hemoperitoneum	5	5
Hemobilia	1	6
Pneumothorax	0	1
Perforation of the gastrointestinal tract	1	2
Pleural effusion	0	7
Sepsis	1	0
Myocardial infarction	0	1
Brain infarction	1	0
Total	19 (8.7%)	40 (7.6%)

BMI, body mass index.

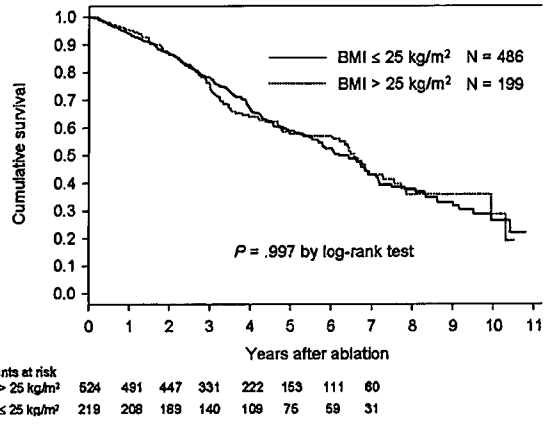

Fig. 1. Cumulative probability of recurrence and local tumor progression in the body mass index (BMI) ≤ 25 kg/m^2 group and in the 25 kg/m^2 < BMI group.

Cumulative survival rates at 1, 2, 3, 4, and 5 years were 95.4%, 87.2%, 75.7%, 64.0%, and 57.8%, respectively, in the overweight group and 94.1%, 86.6%, 78.0%, 66.5%, and 58.8% in the control group (Fig. 2). There was no significant difference in cumulative survival, as assessed by the log-rank test, between the two groups ($P = 0.82$). The median overall survival times of 23 patients whose BMI exceed 30 kg/m^2 were 4.16 ± 2.76 years. We also compared patients with BMI > 27 kg/m^2 and those with BMI ≤ 27 kg/m^2 and found no significant difference in survival ($P = 0.13$, respectively).

Univariate analysis identified age over 68 years old ($P = 0.002$), platelet counts > 100×10^3/mm^3 ($P = 0.03$), Child–Pugh B or C ($P < 0.001$), AFP > 400 ng/ml ($P < 0.001$), DCP > 100 mAU/ml ($P < 0.001$), AFP-L3 > 15% ($P < 0.001$), tumor size > 3.0 cm ($P < 0.001$), and multinodular HCC ($P < 0.001$) as significant factors for survival. Multivariate analysis also showed age over 68 years old ($P < 0.001$), platelet counts > 100×10^3/mm^3 ($P = 0.04$), Child–Pugh B or C ($P < 0.001$), tumor size > 3.0 cm ($P = 0.007$), multinodular HCC ($P = 0.01$), AFP > 400 ng/ml ($P < 0.001$), and DCP > 100 mAU/ml ($P < 0.001$) as significant factors for survival. However, overweight was not a significant predicting factor for survival even after adjusting for these significant factors ($P = 0.45$; Table 6).

Table 4. Multivariate analysis of recurrence after curative ablation

Variable	Hazard ratio (95%CI)	*P* value
BMI > 25 kg/m^2	0.98 (0.82–1.19)	0.88
HCV positive	1.54 (1.17–2.01)	0.002
Platelet count > 150×10^3/mm^3	0.79 (0.63–1.00)	0.05
Tumor size > 3.0 cm	1.39 (1.11–1.74)	0.004
Multinodular	1.30 (1.07–1.58)	0.007
AFP > 400 ng/ml	1.75 (1.09–2.81)	0.02
DCP > 100 mAU/ml	1.29 (0.98–1.70)	0.07

AFP, α-fetoprotein; BMI, body mass index; HCV, hepatitis C virus; DCP, des-γ-carboxyprothrombin.

Table 5. Liver-unrelated death

Variable, *n* (%)	BMI > 25 kg/m^2 9 (12.5%)	BMI ≤ 25 kg/m^2 21 (13.9%)
Cerebrovascular disease	2	6
Renal failure	1	1
Cardiovascular disease	0	1
Lung cancer	1	0
Pancreatic cancer	0	3
Respiratory failure	1	4
Ileus	1	1
Suicide	0	1
Accident	0	1
Unknown	3	3

BMI, body mass index.

Discussion

We found no significant influences of obesity on ablation-related complications, HCC recurrence, or overall survival among patients treated by percutaneous ablation. The only difference was found for the number of treatment sessions in case of RFA, suggesting a surmountable difficulty in tumor ablation in overweight patients. Obesity is defined as BMI > 30 kg/m^2 according to World Health Organization (WHO) criteria.(31) However, there were only 23 patients (3.1%, 95% CI: 2.1–5.3%) whose BMI exceeded 30 kg/m^2 among the patients in this study. The Ministry of Health, Labour and Welfare of Japan