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ORIGINAL ARTICLE

A phase I/II trial of the oral antiangiogenic agent TSU-68 in patients with advanced hepatocellular carcinoma

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Abstract
Purpose We studied the safety and effectiveness of TSU-68, an oral tyrosine kinase inhibitor of vascular endothelial growth factor receptor-2, platelet-derived growth factor receptor and fibroblast growth factor receptor, in patients with advanced hepatocellular carcinoma (HCC).
Methods Patients with unresectable or metastatic HCC were eligible for enrollment. In phase I, the safety, tolerability and pharmacokinetics were assessed in patients stratified based on liver function, from no cirrhosis to Child–Pugh class B. The safety and effectiveness were assessed in phase II at the dose determined in phase I.
Results Twelve patients were enrolled in phase I. Dose-limiting toxicities were found with TSU-68 at the dose of 400 mg bid in Child–Pugh B patients, and 200 mg bid was established as the phase II dose. Phase II included 23 additional patients, and the safety and efficacy were evaluated in a total of 35 patients. One patient (2.9%) had a complete response. Two patients (5.7%) had a partial response, and 15 patients (42.8%) showed a stable disease. The median time to progression was 2.1 months, and the median overall survival was 13.1 months. Common adverse events were hypoalbuminemia, diarrhea, anorexia, abdominal pain, malaise, edema and AST/ALT elevation. The analysis of angiogenesis-related parameters suggests that serum-soluble vascular cell adhesion molecule-1 is a possible marker to show the response.
Conclusions TSU-68 at a dose of 200 mg bid determined by stratification into liver function, showed promising preliminary efficacy with a high safety profile in patients with HCC who had been heavily pre-treated.

Keywords Advanced HCC · Liver function · TSU-68 · Pharmacokinetics · Tolerability · Angiogenesis

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide, with ~626,000 new cases reported annually [1]. Potentially curative treatments such as surgical therapy (resection and liver transplantation) and locoregional procedures (radiofrequency ablation) are indicated in early stage HCC. However, disease that is

diagnosed at an advanced stage or with progression after locoregional therapy has a dismal prognosis owing to the underlying liver disease [2]. Although no systemic therapy was effective for advanced HCC, two randomized, placebo-controlled studies have proven the survival benefits of sorafenib in such patients [3, 4].

TSU-68 is an orally administered, small-molecule, multiple receptor tyrosine kinase inhibitor that targets vascular endothelial growth factor receptor-2 (VEGFR-2), platelet-derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR) [5–9]. As HCC is a highly vascular tumor, several antiangiogenic agents have been tested for the treatment of HCC [3, 4]. Since it is a potent antiangiogenic agent, TSU-68 is also expected to be effective against HCC. However, most patients with HCC have accompanying liver cirrhosis or hepatitis. Therefore, its safety must be reevaluated in the presence of liver function impairment [10, 11]. In particular, concerns have been expressed about impairment of the pharmacokinetics of TSU-68, which is eliminated predominantly through hepatic metabolism, oxidation and glucuronidation [12, 13].

From three phase I studies that have been conducted in Japan on patients with solid tumors, the administration of TSU-68 twice daily after meals was selected as the recommended dose regimen [14, 15]. In this regimen, although no dose-limiting toxicity (DLT) exists at dose levels of 200–500 mg/m^2/dose, the higher dose showed some unacceptable adverse events for an antitumor drug that is administered for long-term consecutive treatment. No obvious dose-dependent increases were detected in the maximum concentration (C_{max}) or the area under the curve ($AUC_{0\text{-}t}$) over the dose range, which was probably due to a saturation of absorption. Consequently, a dose of 400 mg/dose bid was determined to be the recommended dosage of TSU-68 [14, 15].

In the phase I step of our trial, the safety, tolerance and pharmacokinetics (PK) of TSU-68 at the recommended dose were assessed in successive cohorts of patients with various degrees of liver function: no cirrhosis, Child–Pugh class A and Child–Pugh class B cirrhosis, allowing for dose reduction when necessary. In phase II, we evaluated the effectiveness of TSU-68 against advanced HCC.

Patients and methods

Eligibility criteria

The eligibility criteria were histologically confirmed HCC; no indication for or no response to resection, ablation or transcatheter arterial chemoembolization (TACE); age 20–74 years old; World Health Organization performance status of ≤2; life expectancy of ≥90 days; and white blood cells ≥3,000/μl or neutrophils ≥1,500/μl; hemoglobin ≥8.0 g/dl; platelets ≥75,000/μl; liver function Child–Pugh A or B; total bilirubin ≤2.5 mg/dl; AST and ALT ≤ 200 U/l; albumin ≥3 g/dl; prothrombin time [%] ≥40 and serum creatinine ≤1.5 mg/dl. The criteria for patients in Level 1 of phase I were platelets ≥130,000/μl, AST and ALT ≤ 100 U/l; total bilirubin below or equal to the upper limit of normal and albumin equal to or over the lower limit of normal.

Patients were not eligible if they had received ablation, TACE, chemotherapy or radiotherapy within 4 weeks or surgery within 6 weeks. Patients were excluded if they had clinical evidence of central nervous system metastasis, severe cardiovascular disorders, hepatic encephalopathy, uncontrollable pleural effusion or ascites or a serious infection. Patients who needed prophylactic variceal ligation or sclerotherapy were excluded.

All patients were informed of the purpose and methods of the study and provided written informed consent in accordance with national and institutional guidelines. The study was approved by the institutional review board at each of the three participating hospitals and was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

Study design and treatment

This was an open-label phase I/II study. In phase I, eligible patients were stratified into three groups based on hepatic function: Level 1, no cirrhosis; Level 2, Child–Pugh class A; and Level 3, Child–Pugh class B. The safety, tolerability and PK were evaluated in each successive cohort. DLT was defined as grade 3 or 4 non-hematological toxicity or grade 4 hematological toxicity according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 2. As shown in Fig. 1a, the dosage of 400 mg bid was first assessed in three patients at Level 1, each treated for one cycle (28 days). If no DLT was observed, three patients at Level 2 were treated with the same dosage. However, if one patient developed DLT, another three patients at Level 1 were added, based on a 3 + 3 study design [16]. If DLT was observed in no more than two of the six patients, three patients at Level 2 were enrolled. By contrast, if more than one of the first three patients or more than two of the six patients developed DLT, the other three patients at Level 1 were treated with half the dosage. The level transition and dose reduction were planned similarly. Drug administration was continued until no evidence of disease progression was observed, unacceptable drug-related toxicity occurred or the patient withdrew consent.

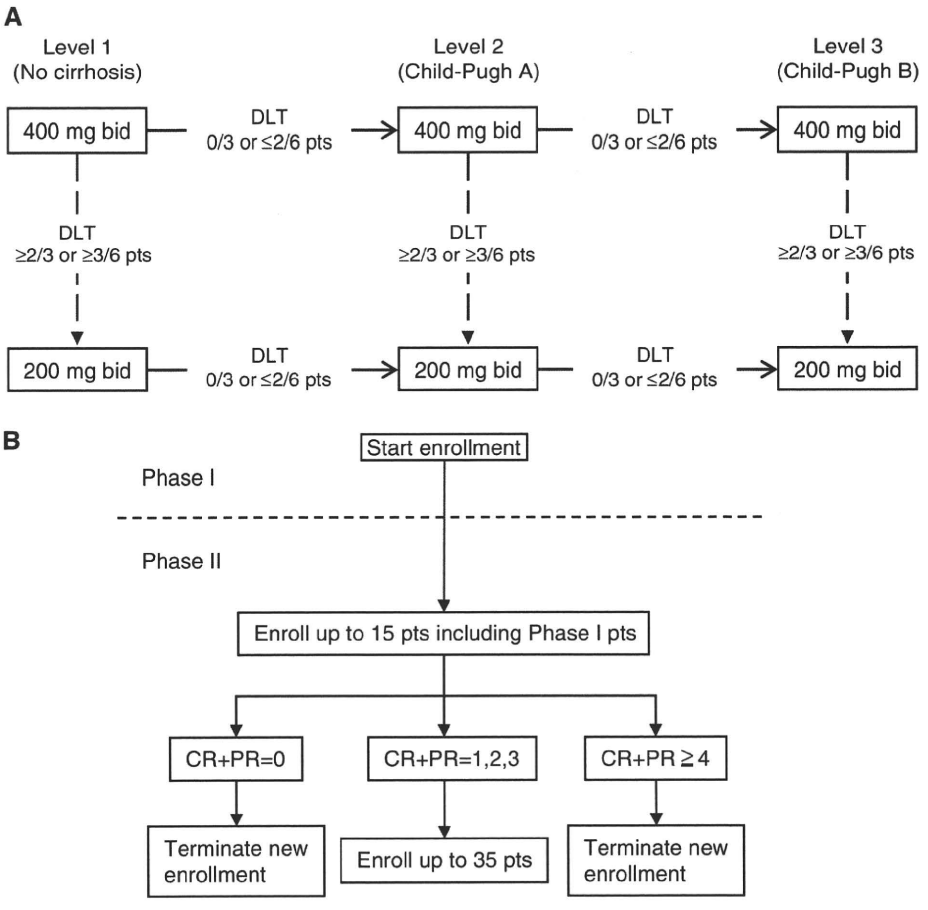

Fig. 1 TSU-68 phase I/II study schema. **a** In phase I, patients were stratified into three groups based on hepatic function, and the toxicity and pharmacokinetics were assessed from Level 1 (no cirrhosis) to Level 3 (Child–Pugh B) by enrolling three patients at each level. *Bid* twice daily, *DLT* dose-limiting toxicity, *pts* patients. **b** Patient enrollment procedure based on the two-step method of Fleming [17]

Patients were accrued using Fleming's optimal two-stage method [17], allowing for an interim evaluation that would be performed when 15 patients (including phase I) were enrolled (Fig. 1b). TSU-68 would be judged "effective" if efficacy (complete or partial response) was observed in four or more patients and "ineffective" if efficacy was observed in none. If efficacy were confirmed in one to three patients, phase II would be performed at the dosage determined in phase I using 20 additional patients (35 patients in total).

Drug administration

TSU-68 (Z)-3-[2,4-dimethyl-5-(2-oxo-1,2-dihydro-indol-3-ylidenemethyl)-1H-pyrrol-3-yl]-propionic acid was obtained from Taiho Pharmaceutical Inc. Co. (Tokyo, Japan). Twice-daily administration was given within 1 h after meals with about 12-h intervals between doses. TSU-68 was taken for 28 consecutive days and was continued in case of stable disease or disease remission after this period for as long as no disease progression and/or no unacceptable drug-related toxicity were seen. TSU-68 administration was immediately interrupted upon the occurrence of DLT.

Response assessment

The objective response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST). Naïve untreated lesions were selected as targets for evaluation. At the end of each cycle, a three-phase computed tomography protocol consisting of early arterial, late arterial and portal venous phases was performed, obtaining contiguous transverse sections with a thickness of 5–7 mm. Responses were assessed independently.

Pharmacokinetics

In phase I, blood samples were collected from a total of 12 patients at 0 (pre-dose), 1, 2, 3, 4, 6 and 9 h post-dose on days 1 and 2 of cycle 1 and at pre-dose on day 1 of cycle 2. The plasma TSU-68 concentration was determined using high-performance liquid chromatography (HPLC). Briefly, an aliquot of plasma was mixed with acetate buffer and methanol including an internal standard. After centrifugation, the supernatant was mixed with ammonium acetate and applied to a Zorbax Eclipse XDB C18 column (3.5 μm, 3 cm × 4.6 mm; Agilent Technologies, Mississauga, ON, Canada) of a Waters Alliance 2690 HPLC

system (Waters, Milford, MA, USA), and the effluent was monitored at 440 nm. The lower limit of quantification was 0.1 μg/ml. Non-compartmental PK parameters, including AUC, C_{max}, time to maximum concentration (T_{max}) and elimination half-life ($T_{1/2}$), were calculated using PhAST (version 2.3; MDS Pharma Services, Montreal, Quebec, Canada).

Angiogenesis-related markers

Blood samples were collected at baseline and at day 28 of cycle 1. The following were measured; platelet-derived growth factor (PDGF)-BB, basic fibroblast growth factor (bFGF), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble endothelial-leukocyte adhesion molecule-1 (sELAM-1) in serum and vascular endothelial growth factor-A (VEGF-A) in plasma were analyzed using enzyme-linked immunosorbent assays (ELISAs; R&D Systems, Minneapolis, MN, USA); plasma interleukin-8 (IL-8), with ELISA (BioSource Europe, Nivelles, Belgium); plasma tissue plasminogen activator (t-PA), with a soluble t-PA ELISA kit (Oncogene Science, Cambridge, MA, USA); plasma plasminogen activator inhibitor-1(PAI-1), with a latex photometric immunoassay (LPIA; LPIA t-PAI test, Mitsubishi Kagaku Iatron, Tokyo, Japan); and plasma factor VIII, with Pathromtin SL (Dade Behring, Marburg, Germany).

Statistical analysis

The primary endpoint of phase I was to evaluate the safety and PK, whereas the primary endpoint of phase II was to determine the best overall response rate based on RECIST. Secondary endpoints of both phases were to evaluate the tumor necrotic effect and the relationship between blood angiogenesis-related molecules and clinical effects. We adopted the 3 + 3 study design generally used in phase I dose-escalation studies [16]. Patients were accrued using Fleming's method [17]. The target number of patients was 35, with an interim evaluation planned for the first 15 patients. The statistical power was 86% with an expected response rate of 20%, and the lower margin of efficacy and one-sided α-level were both 5%. Time to progression (TTP) was defined as the interval between the first day of treatment and tumor progression or death due to any cause. Overall survival (OS) was calculated from the first day of treatment to death. TTP and OS were calculated using the Kaplan–Meier method.

The basal level of angiogenesis-related parameters to predict the response was evaluated by receiver operating characteristic (ROC) analysis. The optimal cut-off value for differentiation of responders and non-responders was defined by the point of the ROC curve (Youden index method). After ROC analysis, logistic regression analysis was performed. The t test was used to compare baseline levels of angiogenesis-related parameters in term of responders.

This study is registered at ClinicalTrials.gov, number NCT 00784290.

The data were analyzed using SAS version 8.1 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics

From September 2003 through February 2007, 35 patients were enrolled at the University of Tokyo Hospital, Mitsui Memorial Hospital and the National Cancer Centre, all located in Tokyo, Japan. Baseline demographics and disease characteristics are summarized in Table 1. Phase I consisted of 12 patients: three patients each at Level 1 (no cirrhosis) and Level 2 (Child–Pugh A), and six patients at Level 3 (Child–Pugh B). The other 23 patients were enrolled in phase II.

In the overall study population, 29 (82.9%) of 35 patients were HCV-positive, and four (11.4%) were HBV-positive. For liver function, three (8.6%) of 35 patients were non-cirrhotic; 24 (68.6%) had Child–Pugh A cirrhosis; and eight (22.9%) had Child–Pugh B cirrhosis. Extrahepatic metastasis was found in 19 (54.3%) patients. Table 1 shows the disease stages according to the TNM classification [18, 19]: 20 (57.1%) patients were stage C (advanced), and 15 (42.9%) patients were stage B (intermediate) according to the Barcelona Clinic Liver Cancer (BCLC) Staging System [2, 20]. The patients had been treated previously a mean of 8.2 (range, 1–20) times using various modalities, including surgery, RFA and TACE. No patients ever received Sorafenib.

Safety and pharmacokinetics

The toxicity of TSU-68 was assessed using NCI-CTC (version 2.0) in 12 patients enrolled in phase I (Table 2). Since no DLT was found with 400 mg bid at Level 1 (no cirrhosis) or Level 2 (Child–Pugh A), the same dosage was used in Level 3 (Child–Pugh B) patients (Fig. 1a). However, patients at Level 3 on 400 mg bid experienced DLT (grade 3 abdominal pain and ascites); the dose was reduced by half, to 200 mg bid, in an additional three patients at Level 3, among whom DLT was not observed. The most common drug-related adverse events observed in phase I were hypoalbuminemia, diarrhea, abdominal pain, fever and AST/ALT elevation.

Table 1 Patient characteristics

	Phase I		Phase II	All
	400 mg bid	200 mg bid	200 mg bid	
No. of patients	9	3	23	35
Gender				
Male	8	2	19	29
Female	1	1	4	6
Age, years				
Median	66	73	69	68
Mean	66.0	68.7	65.2	65.7
Range	53–74	60–73	49–74	49–74
ECOG performance status				
0	6	3	21	30
1	3	0	2	5
Viral markers				
HBs Ag^+, HCV Ab^-	2	0	2	4
HBs Ag^-, HCV Ab^+	6	3	20	29
HBs Ag^-, HCV Ab^-	1	0	1	2
Child–Pugh status				
Chronic hepatitis	3	0	0	3
A (5/6)[a]	3 (3/0)	0	21 (15/6)	24 (18/6)
B (7/8/9)[a]	3 (2/1/0)	3 (3/0/0)	2 (2/0/0)	8 (7/1/0)
Prior treatments[b]				
Median	8	4	9	8
Mean	8.9	6.0	8.2	8.2
Range	5–16	3–11	1–20	1–20
Disease stage[c]				
II	2	1	3	6
III	3	1	5	9
IVa	0	0	1	1
IVb	4	1	14	19
Extrahepatic metastasis				
Yes	4	1	14	19
No	5	2	9	16
Portal vein thrombosis				
Yes	0	0	1	1
No	9	3	22	34

[a] Child-Pugh score (points)

[b] Number of pre-treatments with surgery, radio-frequency ablation, transcatheter arterial chemoembolization, chemotherapy or radiotherapy

[c] Stage is based on the TNM classification [18, 19]

The PK levels were examined in nine patients (3 each at Levels 1–3) receiving 400 mg bid and in three patients (Level 3) receiving 200 mg bid, after the first dose (day 1) and the third dose (day 2; Table 3). The C_{max} and AUC_{0-9h} did not increase with poorer liver function. In all patients, the C_{max} and AUC_{0-9h} on day 2 were lower than those on day 1. In Level 3, in which both 200 and 400 mg TSU-68 were evaluated, no appreciable difference in the exposure was observed on day 2 between the two dose levels. TSU-68 had not accumulated at any level when measured immediately before administration on day 29 (data not shown).

Table 2 shows all of the drug-related adverse events reported in ≥10% of the patients. The most common adverse events, regardless of grade, were hypoalbuminemia (57%), diarrhea (37%), anorexia (34%), abdominal pain (31%), malaise (29%), edema (29%), AST/ALT elevation (29%) and fever (23%); most were grade 1 or 2. Four patients (11.4%) experienced grade 3 or higher toxicity, and the most common grade 3–4 adverse event was AST/ALT elevation (14%). Reducing the dose of TSU-68 from 400 to 200 mg bid decreased the incidence of diarrhea, abdominal pain, fever and hypoalbuminemia. TSU-68 administration was discontinued in one patient because of anemia. However, this patient was later diagnosed with bleeding from the peritoneal dissemination of HCC invading into the colon. Most adverse events were mild, and TSU-68 was well tolerated at the dose of 200 mg bid.

Efficacy and survival

The antitumor effect of TSU-68 was assessed independently in the 35 patients using RECIST (Table 4). One patient at 200 mg bid achieved a complete response (CR; Fig. 2, patient 1), two patients at 200 mg bid had a partial response (PR), 15 patients had stable disease (SD), and 16 patients had progressive disease (PD). The response rate (CR + PR) was 8.6%, and the disease control rate (CR + PR + SD) was 51.4%. Disease control was maintained for >6 months in six patients. One patient did not complete the first cycle and was not evaluated (NE).

Tumor necrosis (TN) was confirmed by independent radiologists in nine patients (25.7%). Figure 2 (patient 2) is an example in which the lack of contrast enhancement and marked central hypoattenuation within the metastatic masses were consistent with TN. The magnitude of necrosis in nine patients was quantified with bi-dimensional measurements of target lesions (RECIST). The baseline mean TN was 0%, and the follow-up mean TN was 35% (5–71%). In the overall study population of 35 patients, the median TTP was 2.1 months (95% confidence interval, 1.2–2.9 months; Fig. 3a), and the median OS was 13.1 months (95% confidence interval, 6.9–26.6 months; Fig. 3b).

Angiogenesis-related markers

Multiple logistic regression analysis was performed. Independent variables were the data for VEGF, t-PA, sVCAM-

Table 2 Drug-related adverse events and laboratory abnormalities by grade occurring in at least 10% of patients ($n = 35$)

	Phase I ($n = 12$)								Phase II ($n = 23$)			All ($n = 35$)					
	Level 1 ($n = 3$) 400 mg bid		Level 2 ($n = 3$) 400 mg bid		Level 3 ($n = 3$) 400 mg bid		Level 3 ($n = 3$) 200 mg bid		200 mg bid								
Common toxicity criteria grade	All	3	All	3	All	3	All	3	All	3	4	All		3		4	
Adverse event	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	%	No.	%	No.	%
Treatment-related adverse events																	
Diarrhea	2		2		2		2		5			13	37				
Anorexia					2				10			12	34				
Abdominal pain	2				3	1	1		5			11	31	1	3		
Malaise	2								8			10	29				
Edema					1		1		8			10	29				
Fever	1		1		2				4			8	23				
Ascites					2	1	1		3			6	17	1	3		
Nausea					1				4			5	14				
Abdominal distension									4			4	11				
Laboratory abnormalities																	
Albumin decrease	2		3		3		1		11			20	57				
AST increase	1						2	1	7	4		10	29	5	14		
ALT increase	1						2	1	7	4		10	29	5	14		
Total bilirubin increase					1		1		6			8	23				
Alkaline phosphatase increase									7	1		7	20	1	3		
Erythropenia									7			7	20				
Hematocrit decrease	1				1				4	1		6	17	1	3		
Hemoglobin decrease	1				1				4	1	1	6	17	1	3	1	3
LDH decrease	1								5			6	17				
Thrombocytopenia	1								4	2		5	14	2	6		

Results are expressed as the worst adverse event possibly related to TSU-68 per patient based on the NCI-CTC version 2.0

Table 3 Pharmacokinetic parameters of TSU-68 corresponding to liver function levels (mean ± SD)

Hepatic function level ($n = 3$)	Dosing	T_{max} (h)	C_{max} (μg/mL)	AUC_{0-9h} (μg·h/mL)	$T_{1/2}$ (h)
Level 1 (400 mg bid)	Day 1 (1st)	3.7 ± 2.1	16.8 ± 7.1	70.1 ± 28.6	2.0[a]
	Day 2 (3rd)	3.0 ± 1.0	9.5 ± 1.8	44.4 ± 11.9	2.5 ± 0.8
Level 2 (400 mg bid)	Day 1 (1st)	4.7 ± 1.2	11.7 ± 2.5	60.6 ± 19.0	2.6[a]
	Day 2 (3rd)	4.0 ± 0.0	7.8 ± 1.4	36.7 ± 7.7	2.2 ± 0.9
Level 3 (400 mg bid)	Day 1 (1st)	4.0 ± 2.0	8.6 ± 4.1	46.4 ± 20.6	2.8[a]
	Day 2 (3rd)	3.7 ± 0.6	5.1 ± 1.6	26.0 ± 6.9	3.0 ± 1.4
Level 3 (200 mg bid)	Day 1 (1st)	4.0 ± 0.0	5.1 ± 1.6	28.9 ± 5.2	8.2[a]
	Day 2 (3rd)	3.7 ± 2.5	4.3 ± 1.4	20.7 ± 4.0	6.9[a]

AUC_{0-9h}, area under the concentration versus time curve for 0–9 h

[a] $n = 2$

1, PAI-1, sELAM-1, IL-8, PDGF, bFGF and plasma factor VIII levels, and dependent variables were the two groups based on each cut-off level (0, below the cut-off value or 1, above the cut-off value). By logistic regression analysis, we found that the sVCAM-1 level was an independent factor ($P = 0.014$; Table 5), and sVCAM-1 (odds ratio 16.0) had the strongest influence on responders (patients with CR + PR + SD). None of the rest of the

Table 4 Tumor response

Best response	Phase I (n = 12)		Phase II (n = 23)	Total (n = 35)	
	400 mg bid (n = 9) No.	200 mg bid (n = 3) No.	200 mg bid No.	No.	%
Complete response	0	0	1	1	2.9
Partial response	0	0	2	2	5.7
Stable disease	2	2	11	15	42.8
Progressive disease	6	1	9	16	45.7
Not evaluated[a]	1	0	0	1	2.9

[a] This patient did not complete cycle 1

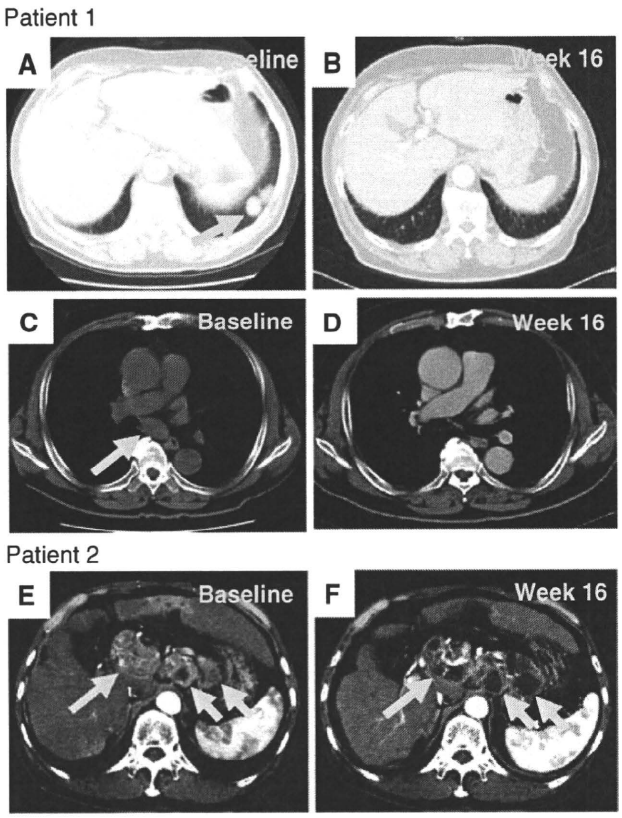

Fig. 2 Computed tomography images of responding lesions from patient 1, who achieved a complete response. Metastatic lesions in the lung (**a**) and lymph node (**c**) disappeared after four cycles (16 weeks) of TSU-68 treatment (**b**, **d**). Representative computed tomography images of a tumor showing necrosis in patient 2. Before treatment, several abdominal lymph node metastases were apparent (**e**). After four cycles of treatment (16 weeks), the lesions demonstrated a lack of enhancement and markedly lower attenuation, consistent with tumor necrosis (**f**)

angiogenesis-related parameters showed any variation with treatment (as the variation of the data for PAI-1 was so large, they were not analyzed; Table 5). The mean values of sVCAM-1 for responders (patients with CR + PR + SD; 1,944 pg/ml) were higher than that for non-responders (patients with PD + NE; 1,422 pg/ml), which was statistically significant (P = 0.026, t test).

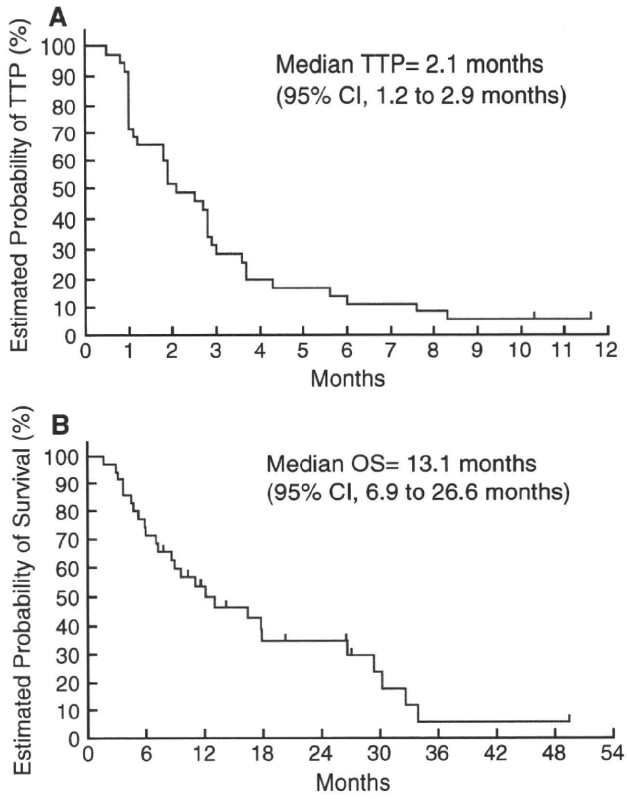

Fig. 3 **a** The independently assessed median time to progression in all 35 patients treated with TSU-68 was 2.1 months. **b** The investigator-assessed median overall survival in all 35 patients treated with TSU-68 was 13.1 months

Discussion

In this trial, special attention was paid to patients with HCC, who often have impaired liver function and might have the potential for reduced clearance of TSU-68, which is eliminated mainly by the liver [12, 13]. This study suggests that the adverse-event profile of TSU-68 in this trial was comparable to observations in other phase I trials examining patients with solid tumors [14, 15]. Although half of the patients experienced exacerbation of pre-existing hypoalbuminemia during the treatment, this was

Table 5 Logistic regression analysis of angiogenesis-related factors

Variable	Evaluation variable (cut-off point)	Odds ratio	95% CI	*P* value
VEGF	<47 × ≧47	0.480	0.095–2.426	0.375
t-PA	<2.3 × ≧2.3	2.250	0.574–8.824	0.245
VCAM-1	<2,370 × ≧2,370	16.000	1.735–147.541	0.014
ELAM-1	<70 × ≧70	0.716	0.187–2.744	0.626
IL-8	<10.0 × ≧10.0	3.250	0.761–13.889	0.112
PDGF	<1,450 × ≧1,450	3.666	0.907–14.813	0.068
Factor VIII	<181 × ≧181	0.545	0.140–2.120	0.382

The *t* test was used to compare baseline levels of angiogenesis-related parameters in terms of responders. A responder means a patient who showed CR, PR and SD; non-responders showed PD and NE

not associated with a worsening of liver function. The edema, associated with hypoalbuminemia, was managed with diuretics. The lack of hypertension as a toxic effect may have been due to the difference in the inhibitory profile between TSU-68, which strongly inhibits both PDGFR and VEGFR, and other antiangiogenic compounds, which predominantly inhibit VEGFR [21, 22].

From the viewpoint of the pharmacokinetics of TSU-68, no trend was seen toward higher plasma exposure to TSU-68 with greater liver dysfunction (Levels 1–3). Furthermore, the exposure in the patients with HCC appeared to be similar to that in patients with advanced solid tumors that were not HCC in a phase I study [15]. These findings suggest that impaired liver function is unlikely to affect the pharmacokinetics of TSU-68. The present study indicated that the C_{max} and AUC were reduced by the repeated administration of TSU-68, which has also been observed in previous trials [14, 15]. This decrease was found to be due to TSU-68, which caused an induction of its own metabolism in the non-clinical studies [12, 13]. Although in this study, the pharmacokinetics of TSU-68 was not examined after long-term consecutive oral administration, the AUC on day 28 has been reported to be similar to that on day 2. This suggests that the decreased exposure, which reaches steady state on day 2, is maintained throughout the therapeutic cycle. In Level 3, no obvious decrease in the AUC on day 2 was observed by reducing the dose of TSU-68 from 200 to 400 mg, although these results are based on a small amount of data. In addition, the estimated daily AUC in the patients who received 200 mg TSU-68 bid was roughly similar to the AUC data showing a 50% inhibition of human xenograft tumor growth in mice (data not shown). However, these data should be interpreted cautiously because the majority of the patients who were included as Child-Pugh B had Child-Pugh scores of 7.

In this study, we selected the fixed-dose for both Child–Pugh A and B because hepatitis or Child–Pugh A patients experienced toxicities (abdominal pain and diarrhea), although no DLT was found when 400 mg bid TSU-68 was administered, and also because liver function may fluctuate between Child–Pugh A and B in the same patients. However, whether Child–Pugh A and B can be separated depends on the safety and PK profile of the drug. Patients with Child–Pugh A are initially recommended for clinical trials in HCC research [23], whereas the design of trials that include Child–Pugh B patients needs further investigation. In addition, whether Child-Pugh score is a good system for stratifying liver function with these types of drugs is open to argument.

Many agents targeting angiogenesis have been investigated in HCC [3, 4, 10, 11, 22, 24–27]. In an international phase III trial, sorafenib reduced the mortality hazard by 44% compared with placebo, with a median OS of 10.7 months (vs. 7.9 months with placebo) [3]. In an Asian phase III trial, patients who received sorafenib had a 35% disease control rate (vs. 16% with placebo), with a median TTP of 2.8 months (vs. 1.4 months) and a median OS of 6.5 months (vs. 4.2 months) [4]. The results mirrored those of the SHARP trial, although the Asia–Pacific patients had more advanced disease. In a phase I trial in Japan, sorafenib resulted in 4% PR and 83% SD, with a median TTP of 4.9 months and a median OS of 15.6 months [24]. Sunitinib, an inhibitor of VEGFR, PDGFR and c-Kit, was used against HCC in a phase II trial and produced a 3.9% PR and 38.5% SD, with a median progression-free survival of 3.9 months and a median OS of 9.8 months [22, 25]. Chemotherapy-naïve Child–Pugh A patients were enrolled in the sorafenib phase III trial [3, 4]. In our trial, eight Child–Pugh B patients were enrolled, and systemic chemotherapy had been already administered in 14 patients. The patients had been treated previously a mean of 8.2 times using various modalities. Although TTP in our trial is less than the reported data of SHARP [3] and similar to the Asian sorafenib trial in the placebo arms [4], these factors might affect the results.

The response rate (8.6%) and a median OS (13.1 months) of TSU-68 were comparable to those reported for these other agents. Some patients were

administered TSU-68 for more than 1 year after confirmed PD by independent review that was not determined by investigators, and the long-term treatment with TSU-68 might have contributed to the longer OS period. This warrants further study, but needs to be evaluated in a larger trial. Molecular-targeted agents, including TSU-68, generally show a relatively low response rate but a high disease control rate, indicating that a large proportion of patients reach SD. The treatment response assessed using RECIST may not accurately reflect the overall effect of these agents [23]. We had several cases in which necrosis was observed inside a tumor, despite the increase in tumor size. As an objective response is a weak surrogate of activity in phase II trials, a consensus conference endorsed by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver recommended the inclusion of TTP as the primary endpoint in phase II trials [23].

Molecular-targeted agents are being developed as systemic therapies for HCC in first- and second-line settings as monotherapy and in combination with locoregional therapies. The primary endpoint for phase III studies that assess primary HCC treatments is survival, and the control arm should be sorafenib. Comparison of single agents head to head with sorafenib might jeopardize study approval and the recruitment of patients for ethical reasons. For second-line treatments against advanced HCC, the new agents should be compared with placebo or best supportive care [23]. A phase II randomized study of TSU-68 in combination with TACE has been conducted (manuscript in preparation), and a phase III trial is being planned.

VEGF, PDGF and bFGF participate in the neovascularization of HCC [26, 27], and VEGF levels are thought to have a prognostic value [28]. IL-8 has proangiogenic activity in cancers, although its role in HCC is controversial [27]. Given that the primary target of TSU-68 is endothelial cells, we speculated that damaged vascular endothelial cells may release endothelial cell-specific markers such as sELAM-1 and sVCAM-1. As sVCAM-1 can be identified in the bloodstream, it is potentially useful as a non-invasive biomarker for the monitoring of disease progression in cancer [29]. A high level of VCAM-1 was significantly associated with an advanced disease stage and the presence of distant metastasis in gastric cancer [30] and also has been shown to be associated with angiogenesis and poor prognosis in breast cancer [31] and in HCC [32]. In this trial, we found higher baseline levels of sVCAM-1 in patients with good response (CR + PR + SD) after treatment with TSU-68. Although our data suggested that sVCAM-1 is a possible predictive marker for the response, the analysis is exploratory, and further study is necessary to confirm this possibility.

In conclusion, the step-wise study design based on hepatic function was useful in a safety assessment of TSU-68 in patients with HCC who had impaired liver function. The TSU-68 dosage of 200 mg bid has a favorable safety profile, even in patients with Child–Pugh B cirrhosis, and together with a high disease control rate, provides a rationale for its further evaluation in patients with HCC.

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A Conundrum for Randomized Controlled Trials: Experience from a Small Hepatocellular Carcinoma Trial

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Objective: The aim of this study was to explore why patients accepted or declined to participate in a randomized clinical trial, which was subsequently discontinued because of a low recruitment rate.

Methods: Forty-one patients were invited to participate in a randomized clinical trial that aimed to compare local ablation therapies and surgery to treat small asymptomatic hepatocellular carcinomas. These patients were then asked to answer a questionnaire that assessed patient perception and reasons for accepting or declining to enroll in the randomized clinical trial. When patients had a strong preference for a specific treatment, the questionnaire assessed why, how and when they had chosen it.

Results: The response rate was 6/6 (100%) and 30/35 (86%) for the participant and non-participant groups, respectively. Among the 30 non-participants, 23 had a strong preference for local ablation therapies, which was less invasive and offered shorter hospitalization. Patient preference for a specific treatment often stemmed from their consultations with a clinician who referred them to a specialist hospital. Patients without strong preference for a specific treatment participated in the randomized clinical trial because of altruistic motivations.

Conclusion: When new treatments that are innovative and less burdensome become widespread, they are difficult to compare with standard therapy utilizing a well-designed randomized clinical trial. Consequently, when an innovative treatment is developed, investigators should consider designing a randomized clinical trial as early as possible.

Key words: small asymptomatic hepatocellular carcinomas – local ablation therapies – liver resection – randomized clinical trial

INTRODUCTION

Randomized clinical trials (RCT) are the gold-standard to evaluate the safety and efficacy of proposed new treatments (1–3). When a new treatment shows benefits, it is introduced into general practice and is expected to improve the quality of care. However, an appropriate evaluation of an unproven new treatment through a RCT is difficult when it becomes integrated into general clinical practice because of its innovative and minimally burdensome nature (3). Consequently, the co-existence of a new treatment and a standard therapy often leads to diminished patient access to beneficial treatments.

Small asymptomatic hepatocellular carcinomas (HCC) are increasingly recognized as a problem in Japan since the initiation of periodic surveillance of high-risk populations (4). Surgical resection has been accepted as the first-line treatment for HCC. In addition, several local ablation therapies (LAT) have been developed to treat HCC, including percutaneous ethanol injection (PEI) (5) and radiofrequency ablation (RFA) (6). They are minimally invasive and have been recognized as an alternative to surgery in small HCC patients. Retrospective studies have reported that the prognosis of patients undergoing PEI (7–10) or RFA (6,11) for small HCC was equivalent to that of patients selecting surgery. However, the optimal therapeutic strategy for small HCC is under debate. Patient decisions regarding treatment are often guided by the expertise of their consulting clinician, which is frequently affected by sectionalism that is predominant in the Japanese medical community.

In 2002, a RCT (the parent study) was organized to settle the longstanding debate comparing the benefits of LAT relative to surgery in treating small HCC (i.e. three or fewer tumors, where each tumor is 3 cm in diameter or smaller). Table 1 shows the study outline. The trial was carried out in three cancer hospitals (Institutions A, B and C) and a university hospital (Institution D), where physicians and surgeons had the opportunity to build a framework for cooperation. We reached a consensus on what to include in the informed consent form and how to obtain it from patients. Specifically, we explained the clinical equipoise by noting: (i) the probability of 5-year disease-free survival associated with the two treatments was 25 and 10% for surgery and LAT, respectively; and (ii) the probability of 5-year survival associated with the two treatments was 62 and 59% for surgery and LAT, respectively (10). The purpose of the parent study and difference between two treatments were explained in informed consent form as follows; the purpose of this study is to compare the effectiveness, risk, burden and cost between surgery and LAT. Surgery has been usually performed for your type of cancer. LAT has been found to be effective and spread widely, but there is no solid evidence that LAT has a similar benefit to surgery. Currently, the proportion of recurrence in surgery is lower than LAT. However, there is little difference in long-term survival between surgery and LAT. LAT imposes less burden and invasiveness on patients than surgery. The comparative table of benefit, burden and cost in two treatments also was put on the form.

Table 1. Outline of the parent study

	Contents
Purpose	To compare local ablation therapies (RFA, PEI) with surgical resection
Eligibility	Hepatocellular carcinoma, three or fewer tumors each 3 cm in diameter or smaller, Child-Pugh class: A or B
	Age: ≥20, <80
Endpoints	
Primary endpoints	Overall survival and disease-free survival
Secondary endpoints	Medical costs, hospitalization period, Toxicity
Sample size	120 patients
Recruit period	2 year
Institutions	Cancer hospitals (Institution A, B, C), University hospital (Institution D)

Between October 2002 and April 2003, 41 patients were invited to participate in this study. Among these patients, six agreed and 35 refused to participate. Although a similar study was completed in China (12), the steering committee decided to discontinue the trial because of the low recruitment rate. Within this context, the aim of this study was to explore why patients accepted or declined to participate in the trial, and to use this information to provide insights for future research.

PATIENTS AND METHODS

We invited 41 patients, who were originally asked to participate in the parent study, to take part in this study. These patients were then asked by an attending clinician to respond to a questionnaire accompanied by an envelope. Patients were directed to place the completed questionnaire into the envelope and deliver it to the hospital staff. This study was approved by the National Cancer Center Hospital research ethics committee.

The questionnaire contained both multiple-choice and open-ended questions that aimed to assess the reasons behind patient decisions to participate in the study. We also examined views of non-participants towards random allocation. When non-participants had a strong preference towards a specific treatment, we assessed their perception by inquiring why, how and when they developed this preference. The questionnaire, developed by the investigators, was pilot-tested with laypersons to ensure clarity and comprehensibility of the questions. The questionnaires are shown in the Supplementary data, Appendix, available at http://www.jjco.oxfordjournals.org.

RESULTS

The survey was performed between May and July of 2003. Among the six participants and 35 non-participants, 6 (100%) and 30 (86%) patients, respectively, responded to the questionnaire. Table 2 shows the number of patients who accepted or declined participation in the parent-trial. Table 2 also shows the number of non-participants who chose surgery or LAT. Only 15% of patients participated in the parent-trial. There were no differences among institutions. Among the 30 respondents who declined trial entry, four had surgery, 25 had LAT and the remaining one was unknown.

Table 2. Number of patients (Pt) who accepted or declined participation

	Pt invited to RCT	Participant (%)	Non-participant		
			Total	Surgery	Local ablation therapies
Institution A	10	3 (30)	7	1	6
Institution B	8	1 (12)	7	1	6
Institution C	12	1 (8)	11	0	11
Institution D	11	1 (9)	10	4	6
Total	41	6 (15)	35	6	29

Reasons for Participation or Non-participation

Table 3 summarizes participants' reasons for deciding to participate in the parent-trial. All participants answered that they thought participation in the trial would contribute to the development of medicine. When asked about their major reason for participation, three participants marked 'the contribution to medical development' and two participants noted 'clinicians asked me to participate'.

Table 4 shows non-participants' reasons for refusing to enroll in the parent-trial. Four patients (13%) answered that they preferred surgery to LAT whereas 23 (77%) noted that they preferred LAT. One of two patients who received LAT stated 'I disliked surgery'; although the other stated 'clinicians did not ask strongly to participate'. Twelve patients (40%) stated that they were not satisfied with the random allocation into a treatment group. Among these 12 patients, 7 (58%) answered that patients should decide their own treatment whereas 3 (25%) answered that clinicians should decide. Two patients (17%) answered that randomization was inhumane. One patient (8%) stated that random allocation was problematic when two treatments were very different. One patient (8%) stated that he/she could not understand randomization.

Table 3. The frequency of agreement to each statement according to participation among six patients

Statement[a]	Number of respondents (%)
I thought participation in the trial would contribute to the development of medicine	6 (100)
Clinician asked me to participate	2 (33)
I thought there were no differences between two treatments	1 (17)
Other	
I had no preference because my tumors were small	1 (17)
I could not decide which treatment to have	1 (17)

[a]More than one response was allowed.

Table 4. The reasons of 30 non-participants for refusal

Statement[a]	Number of respondents (%)
I was not satisfied to be assigned to the treatment by randomization	12 (40)
Patient should decide the treatment	7 (58)
Clinician should decide the treatment	3 (25)
Randomization was inhumane	2 (17)
Two treatments were very different	1 (8)
I could not understand randomization	1 (8)
I wanted to receive local ablation therapies	23 (77)
I wanted to receive surgery	4 (13)
Other	
Clinician did not ask me to participate	1 (3)
I disliked surgery	1 (3)

[a]More than one response was allowed.

Reasons for Refusing Trial Entry Among Non-participants

Table 5 shows non-participants' reasons for why they subsequently decided to undergo surgery or LAT. All four patients who received surgery and one patient who receive LAT answered that they had thought the probability of recurrences would be lower. Among the patients who had LAT, the majority (20/25, 75%) stated that LAT imposed a lower amount of burden and invasiveness to their body than surgery. In addition, about half of the non-participants (12/25, 48%) stated that the hospitalization period would be shorter with LAT than with surgery. One patient stated that the medical cost of LAT was fewer.

Table 6 summarizes the results of how non-participants made their treatment decisions. Among these four patients who had surgery, three answered that they followed their surgeons' recommendation and one answered he/she followed physicians' recommendation. Among these 25 patients who had LAT, 2 (8%) answered that they referred to their surgeons, 21 (84%) answered that they relied on their attending physicians' recommendation and 9 (36%) answered that they relied on general practitioners' recommendation. Thirteen out of 25 patients who had LAT answered they had already decided to obtain this treatment before they were invited to the trial.

DISCUSSION

In this study, we found that patients who declined trial entry had a strong preference for LAT, which was less invasive and offered a shorter hospitalization course. We also found that this patient preference had stemmed from patient consultations with either a clinician or general practitioner who

Table 5. The reasons of 30 non-participants for preferring surgery or local ablation therapies

Statements[a]	Number of respondents (%)	
	Pt with surgery ($n = 4$)	Pt with local ablation therapies ($n = 25$)
I thought the probability of recurrences would be lower	4 (100)	1 (4)
I thought the survival period would be longer	0	0
I thought the treatment was less burdensome	0	20 (80)
I thought the hospitalization period was shorter	0	12 (48)
I thought the medical cost was fewer	0	1 (4)
Other	0	
I heard that the prognosis were the same		1 (4)
I did not want to increase wound any more		1 (4)

[a]More than one response was allowed.

Table 6. What non-participants referred to when they made a decision

	Number of respondents (%)	
	Pt with surgery ($n = 4$)	Pt with local ablation therapies ($n = 25$)
What non-participants referred to[a]		
Informed consent form	0	13 (52)
Consultation with surgeon in charge	3 (75)	2 (8)
Consultation with physician in charge	1 (25)	21 (84)
Consultation with general practitioner	0	9 (36)
Opinion of other patients	0	2 (8)
Opinion of my family	1 (25)	3 (12)
Other		
My close friend who was clinician suggested	1 (25)	
My friend suggested		1 (4)
The explanation about the prognosis		1 (4)
The information from internet		1 (4)
The information from newspaper		2 (8)
When they made a decision		
Before invitation to the study	1 (25)	13 (52)
After invitation to the study	1 (25)	8 (32)
Do not know or no answer	2 (50)	4 (16)

[a]More than one response was allowed.

referred them to a specialist hospital. Non-participants who received surgery believed in the survival benefits from surgery and relied on surgeon recommendations. On the other hand, patients without strong preference participated in the trial largely because of altruistic motivations. In summary, we found that patients tended to choose less invasive treatment methods even if there is a lack of superiority evidence or an inferiority possibility compared with the standard treatment. Many studies have reported a number of complex barriers in appropriately conducting RCTs (13–18), and we found a couple of these factors that contributed to the incompletion of this trial.

One barrier is that LAT, which had been performed in patients with unrespectable hepatic malignancies, has become popular in treating patients with small HCC due to its superiority in local tumor control and minimal invasiveness. It has become so popular that even without appropriate evidence that LAT has equivalent survival benefits compared with surgery, many general practitioners have recommended it to their patients as an alternative therapy.

Another barrier was patient fear towards a possible allocation into a treatment group that they did not prefer. Although some studies reported that a barrier to trial entry was patient difficulty in understanding the randomization concept and associated patient uneasiness (19–21), our study did not find this as an issue. Only one in 12 respondents that disliked randomization could not understand the randomization concept. Consequently, unbiased and objective explanations by clinicians are crucial in the consent process. However, in our study, we found that the more we stressed the clinical equipoise, the more the patients preferred LAT.

Although the lack of participation was based on these simple reasons, the solution is not simple. In order to increase the number of participants, there are a few possible study designs. One is a randomized consent design, where patients are randomly allocated into a specific treatment group before they provide consent (22,23). If patients decline the allocated treatment, they are then possibly allocated to the other treatment. Even if we apply this design, apart from its ethical problems, the effort will likely fail because most patients allocated to the surgery group will decline. Another possible solution is a randomized trial with a non-randomized part. Specifically, consenting patients are randomized into the two treatment groups, and those that refuse their allocated treatment are enrolled into a non-randomized study. At the conclusion of such a study, the endpoints of the randomized group and the non-randomized group are compared. In such a design, the results may include biases. Moreover, if there is an imbalance in the number of patients between the treatment groups in the non-randomized study, it is difficult to obtain appropriate results.

Furthermore, when there is a discrepancy in results between the randomized and non-randomized study groups, there is difficulty in the interpretation of the results.

In conclusion, when innovative and less burdensome treatments become widespread, they are difficult to compare with standard therapy utilizing a RCT. In light of the increasing number of organ preserving therapies, investigators should evaluate the efficacy and safety of innovative treatments with RCTs as early as possible (24).

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Conflict of interest statement

None declared.

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Phase I/II study of the pharmacokinetics, safety and efficacy of S-1 in patients with advanced hepatocellular carcinoma

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S-1, an oral fluoropyrimidine derivative, has been shown to be clinically effective against various solid tumors, and preclinical studies have demonstrated activity against hepatocellular carcinoma. We conducted a phase I/II study in patients with advanced hepatocellular carcinoma to examine the pharmacokinetics, recommended dose, safety and efficacy of S-1. In phase I, the administered dose of S-1 was approximately 64 mg/m^2 per day in three patients (level 1) and approximately 80 mg/m^2 per day in six patients (level 2). There was no dose-limiting toxicity at level 1, but two patients had dose-limiting toxicity at level 2 (grade 3 anorexia and grade 2 rash requiring eight or more consecutive days of rest). The recommended dose was finally estimated to be 80 mg/m^2 per day. There were no significant differences in the pharmacokinetics of S-1 between patients with Child-Pugh A and those with B. In phase II, five of 23 patients (21.7%) had partial responses. The median progression-free survival and overall survival were 3.7 and 16.6 months, respectively. The most common toxicities of grade 3 or 4 were elevated serum aspartate aminotransferase levels, hypochromia and thrombocytopenia. In conclusion, S-1 showed an acceptable toxicity profile and promising antitumor activity for hepatocellular carcinoma, warranting further evaluation in randomized clinical trials. (*Cancer Sci*, doi: 10.1111/j.1349-7006.2010.01730.x, 2010)

Hepatocellular carcinoma (HCC) is one of the most common cancers in the world. Outcomes remain poor because the disease is usually advanced and associated with hepatic impairment at diagnosis, and because of the high rate of recurrence resulting from either intrahepatic metastases from the primary tumor or multicentric lesions. As for therapy, surgical resection and percutaneous ethanol injection (PEI) or radiofrequency ablation (RFA) are considered the mainstays of treatment in patients with potentially curable disease. Transcatheter arterial chemoembolization (TACE) is the treatment of choice for noncurative HCC. Despite numerous clinical trials of a wide variety of cytotoxic agents, survival remains dismal in HCC.[1] Recently, sorafenib, an oral multi-kinase inhibitor that targets mainly Raf kinases and receptor tyrosine kinases associated with angiogenesis (vascular endothelial growth factor receptor [VEGFR]-2/-3 and platelet-derived growth factor receptor [PDGFR]-β), provided a significant survival benefit in patients with advanced HCC enrolled in placebo-controlled, randomized, phase III trials, including Asian as well as European subjects.[2,3] An initial phase I study in Japanese patients with HCC associated mainly with hepatitis C virus (HCV) infection showed promising antitumor activity and a favorable tolerability profile.[4] However, further improvement in the treatment of advanced HCC is essential.

S-1 is a novel, orally administered drug that combines tegafur (FT), 5-chloro-2,4-dihydroxypyridine (CDHP) and oteracil potassium (Oxo) in a molar concentration ratio of 1:0.4:1.[5] CDHP is a competitive inhibitor of dihydropyrimidine dehydrogenase (DPD), a metabolizing enzyme of 5-fluorouracil (5-FU) that is expressed in the liver. Inhibition of DPD by CDHP results in prolonged effective concentrations of 5-FU in plasma and tumor tissue.[6] Oxo, a competitive inhibitor of orotate phosphoribosyltransferase, inhibits the phosphorylation of 5-FU in the gastrointestinal tract, thereby reducing serious 5-FU-related gastrointestinal toxicity.[7] Clinically, S-1 has been shown to be effective against a variety of solid tumors, with response rates ranging 21–49% in late phase II studies conducted in Japan.[8] S-1 has yet to be evaluated in patients with HCC. However, in nude rats with human HCC xenografts, S-1 has been confirmed to have antitumor activity.[9]

Patients with HCC usually have various degrees of liver dysfunction because of associated liver disease and replacement of liver tissue by tumor, leading to pathophysiological changes that influence drug disposition. Decreased hepatic blood flow, extrahepatic and intrahepatic blood shunting and hepatocyte loss also alter drug metabolism, and decreased protein synthesis reduces drug binding to plasma proteins. In fact, the maximal tolerated dose (MTD) of 5-FU given as a 5-day continuous infusion in patients with HCC is approximately 50% of that in patients with normal organ function, and patients with cirrhosis have significantly lower clearance of 5-FU than those without cirrhosis.[10] We therefore conducted a multicenter phase I/II study to evaluate the pharmacokinetics, safety and efficacy of S-1 monotherapy in patients with advanced HCC.

Materials and Methods

Eligibility. Eligible patients had histologically or cytologically proved HCC that was not amenable to treatment by resection, liver transplantation, RFA, PEI or percutaneous microwave coagulation therapy (PMCT) and was not expected to respond to TACE. A hypervascular mass on computed tomography (CT) or magnetic resonance imaging (MRI) associated with a serum alpha-fetoprotein level or a serum protein induced by vitamin K absence or antagonist (PIVKA-II) level of more than the upper limit of normal (ULN) was considered a sufficient non-invasive diagnostic criterion for HCC. At least one measurable lesion on CT or MRI (not including necrotic lesions caused by prior treatment) was required. Other eligibility criteria included: age of at least 20 years; Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2; estimated life expectancy of at least 60 days; adequate

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hematological function (white blood cells [WBC] ≥3000/mm^3, hemoglobin ≥ 9.0 g/dL, platelets ≥ 7.0 × 10^4/mm^3); adequate hepatic function (aspartate aminotransferase [AST] and alanine aminotransferase [ALT] ≤5 times the ULN, total bilirubin ≤2.0 mg/dL, serum albumin ≥2.8 g/dL, prothrombin activity ≥40%); adequate renal function (serum creatinine ≤ULN); and a Child-Pugh class of A or B. Prior treatment for HCC, such as resection, liver transplantation, RFA, PEI, PMCT and TACE was permitted if the treatment had been performed 30 or more days before registration in the study. Patients were excluded if they had: tumor involving more than 50% of the liver; brain or bone metastasis or vascular invasion of the main trunk and first-order branch(es) of the portal vein, hepatic veins, hepatic arteries or bile duct; severe complications; other malignancies; or inability to comply with the protocol requirements. Written informed consent was obtained from each patient. The study was approved by the local institutional review boards at all participating centers.

Study design. S-1 was supplied by Taiho Pharmaceutical Co., Ltd (Tokyo, Japan) in capsules containing 20 or 25 mg of FT. Individual doses were calculated according to body surface area. The calculated dose was rounded to derive the daily dose and the number of capsules to be dispensed per patient. At each dose level, S-1 was administered orally twice daily (after breakfast and dinner) for 28 consecutive days, followed by a 14-day recovery period. Each treatment cycle was 42 days. If grade 3 or higher hematological toxicity, grade 2 or higher non-hematological toxicity, grade 3 or higher elevations of AST or ALT, or grade 2 or higher increases in the serum creatinine concentration occurred, treatment with S-1 was temporarily suspended, the dose of S-1 was reduced, or both (minimum dose, 50 mg/day). Treatment continued until there was evidence of disease progression, or if the recovery period exceeded 28 days, the patient requested treatment to be discontinued or unacceptable toxicity developed and treatment was terminated at the discretion of the investigator. Drug compliance and accountability were carefully monitored; patients were requested to record their intake of S-1 and other medications in a diary.

During phase I, the starting dose of S-1 (level 1) was approximately 64 mg/m^2 per day twice daily (80% of the standard dose), level 2 was approximately 80 mg/m^2 per day and level 0 was approximately 50 mg/m^2 per day (80% of level 1). Patients were enrolled in cohorts of three for each dose level. The dose was escalated according to the cohort and was not increased in the same patient. If none of the first three patients had dose-limiting toxicity (DLT) during the first cycle, the dose was increased to level 2. If one or two of the first three patients had DLT, three additional patients were entered at the same dose level; if only one or two of the first six patients at level 1 had DLT, the dose was increased to level 2; if all of the first three patients or three or more of the first six patients had DLT, the dose was decreased to level 0; if none of the first three patients had DLT at level 0 or level 2, three additional patients were assigned to receive the same dose level. The DLT was defined as any of the following: (i) hematological toxicity ≥grade 4; (ii) non-hematological toxicity ≥grade 3; (iii) AST, ALT ≥15 times the ULN; or (iv) a rest period of 8 or more consecutive days was required. The recommended dose (RD) determined in the phase I part of this study was used in phase II.

Pharmacokinetics. Blood samples (5 mL) were obtained from each patient assigned to receive level 2 in the phase I part of the study. The samples were taken before and 1, 2, 4, 6, 8, 10 and 12 h after administration of S-1 on days 1 and 8 of the first treatment cycle. Plasma was separated from the whole-blood samples by centrifugation and stored at −20°C until analysis. Plasma FT concentrations were measured by high-performance liquid chromatography with ultraviolet detection. Plasma concentrations of 5-FU, CDHP and Oxo were measured by gas chromatography-negative ion chemical ionization mass spectrometry, as described previously.[11]

Pharmacokinetic data, including the maximum plasma concentration (C_{max}, ng/mL), time to reach C_{max} (T_{max}, h), area under the plasma-concentration–time curve for 0–12 h (AUC_{0-12}, ng h/mL) and the elimination half-life ($T_{1/2}$, h) were calculated by noncompartment model analysis using WinNonlin software, version 4.1 (Pharsight, Cary, NC, USA).

Assessment of efficacy and toxicity. All patients who received at least one dose of the study drug were included in the evaluations of response and toxicity. During each course of treatment, tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) by computed tomography (CT) or magnetic resonance imaging (MRI), with a slice thickness of no more than 5 mM.[12] The primary efficacy end-point in the phase II part of this study was the overall response rate, assessed on the basis of changes in tumor dimensions. The other end-points were overall survival (OS) and progression-free survival (PFS). The PFS was defined as the interval between the date of initiating treatment and the date on which disease progression was first confirmed or the date of death from any cause. Overall survival was defined at the interval from the date of initiating treatment to the date of death from any cause. Median OS and median PFS were

Table 1. Patient characteristics

	Level 1 (*n* = 3)	Level 2 (*n* = 23)
	n (%)	*n* (%)
Median age (range) (years)	67.0 (63–68)	68.0 (45–78)
Gender		
Male	2 (66.7)	21 (91.3)
Female	1 (33.3)	2 (8.7)
Virus marker		
HBs (+)	1 (33.3)	3 (13.0)
HCV (+)	1 (33.3)	14 (60.9)
HBs(−), HCV(−)	1 (33.3)	6 (26.1)
Child-Pugh classification		
A	3 (100)	16 (69.6)
B	0 (0)	7 (30.4)
Stage		
Stage II	1 (33.3)	3 (13.0)
Stage III	1 (33.3)	10 (43.5)
Stage IVB	1 (33.3)	10 (43.5)
Vascular invasion	0 (0)	2 (8.7)
ECOG PS		
0	3 (100)	21 (91.3)
1	0 (0)	2 (8.7)
Pretreatment		
TA(C)E	2 (66.7)	17 (73.9)
Surgery	1 (33.3)	8 (34.8)
RFA	0 (0)	7 (30.4)
HAI	2 (66.7)	6 (26.1)
PEI	0 (0)	4 (17.4)
Radiation	0 (0)	4 (17.4)
PMCT	0 (0)	3 (13.0)
Systemic chemotherapy	0 (0)	3 (13.0)
BCLC staging		
Early	0 (0)	1 (4.3)
Intermediate	2 (66.7)	11 (47.8)
Advanced	1 (33.3)	11 (47.8)

BCLC, Barcelona Clinic Liver Cancer Group; ECOG, Eastern Cooperative Oncology Group; HAI, hepatic arterial infusion; HBs, hepatitis B surface antigen; HCV, hepatitis C virus antibody; PEI, percutaneous ethanol injection; PMCT, percutaneous microwave coagulation therapy; PS, performance status; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization.

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Table 2. Toxic effects

Toxicity	Level 1 (n = 3)		Level 2 (n = 23)		Child Pugh A (n = 16)		Child Pugh B (n = 7)	
	All grades	≥G3	All grades	≥G3	All grades	≥G3	All grades	≥G3
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
All adverse events	3 (100.0)	0 (0.0)	23 (100.0)	10 (43.5)	16 (100.0)	8 (50.0)	7 (100.0)	2 (28.6)
Hematological								
Erythropenia	1 (33.3)	0 (0.0)	21 (91.3)	1 (4.3)	14 (87.5)	1 (6.3)	7 (100.0)	0 (0.0)
Hypochromia	1 (33.3)	0 (0.0)	19 (82.6)	4 (17.4)	12 (75.0)	4 (25.0)	7 (100.0)	0 (0.0)
Leukopenia	2 (66.7)	0 (0.0)	18 (78.3)	1 (4.3)	12 (75.0)	1 (6.3)	6 (85.7)	0 (0.0)
Lymphopenia	2 (66.7)	0 (0.0)	12 (52.2)	3 (13.0)	7 (43.8)	3 (18.8)	5 (71.4)	0 (0.0)
Neutropenia	1 (33.3)	0 (0.0)	17 (73.9)	1 (4.3)	12 (75.0)	1 (6.3)	5 (71.4)	0 (0.0)
Reduced hematocrit	1 (33.3)	0 (0.0)	19 (82.6)	1 (4.3)	12 (75.0)	1 (6.3)	7 (100.0)	0 (0.0)
Reduced prothrombin content	1 (33.3)	0 (0.0)	19 (82.6)	0 (0.0)	14 (87.5)	0 (0.0)	5 (71.4)	0 (0.0)
Thrombocytopenia	1 (33.3)	0 (0.0)	18 (78.3)	4 (17.4)	12 (75.0)	4 (25.0)	6 (85.7)	0 (0.0)
Non-hematological								
Elevated alkaline phosphatase	0 (0.0)	0 (0.0)	8 (34.8)	1 (4.3)	7 (43.8)	1 (6.3)	1 (14.3)	0 (0.0)
Elevated lactate dehydrogenase	0 (0.0)	0 (0.0)	15 (65.2)	0 (0.0)	9 (56.3)	0 (0.0)	6 (85.7)	0 (0.0)
Elevated serum AST	1 (33.3)	0 (0.0)	8 (34.8)	4 (17.4)	6 (37.5)	3 (18.8)	2 (28.6)	1 (14.3)
Elevated serum bilirubin	0 (0.0)	0 (0.0)	18 (78.3)	3 (13.0)	13 (81.3)	2 (12.5)	5 (71.4)	1 (14.3)
Hyponatremic	0 (0.0)	0 (0.0)	8 (34.8)	0 (0.0)	5 (31.3)	0 (0.0)	3 (42.9)	0 (0.0)
Reduced cholinesterase	2 (66.7)	0 (0.0)	18 (78.3)	0 (0.0)	13 (81.3)	0 (0.0)	5 (71.4)	0 (0.0)
Reduced serum albumin	0 (0.0)	0 (0.0)	18 (78.3)	2 (8.7)	12 (75.0)	1 (6.3)	6 (85.7)	1 (14.3)
Reduced total protein	0 (0.0)	0 (0.0)	11 (47.8)	0 (0.0)	8 (50.0)	0 (0.0)	3 (42.9)	0 (0.0)
Anorexia	1 (33.3)	0 (0.0)	18 (78.3)	2 (8.7)	13 (81.3)	1 (6.3)	5 (71.4)	1 (14.3)
Ascites	0 (0.0)	0 (0.0)	7 (30.4)	0 (0.0)	3 (18.8)	0 (0.0)	4 (57.1)	0 (0.0)
Diarrhea	0 (0.0)	0 (0.0)	10 (43.5)	0 (0.0)	8 (50.0)	0 (0.0)	2 (28.6)	0 (0.0)
Fatigue	0 (0.0)	0 (0.0)	19 (82.6)	2 (8.7)	13 (81.3)	2 (12.5)	6 (85.7)	0 (0.0)
Pigmentation	0 (0.0)	0 (0.0)	20 (87.0)	0 (0.0)	14 (87.5)	0 (0.0)	6 (85.7)	0 (0.0)
Rash	0 (0.0)	0 (0.0)	8 (34.8)	0 (0.0)	5 (31.3)	0 (0.0)	3 (42.9)	0 (0.0)
Stomatitis	0 (0.0)	0 (0.0)	7 (30.4)	0 (0.0)	5 (31.3)	0 (0.0)	2 (28.6)	0 (0.0)

Dosage level, level 1, 2 (n = 3, 23); AST, aspartate aminotransferase.

Table 3. Efficacy in patients who received dose level 2

	Child-Pugh A (n = 16)	Child-Pugh B (n = 7)	Total (n = 23)
Partial response†	4	1	5
Stable disease‡	5	2	7
Progressive disease	7	3	10
Not evaluable	0	1	1
Response rate (90%CI)§ (%)	–	–	23.1 (9.0–40.4)
Response rate (95%CI) (%)	25.0 (7.3–52.4)	14.3 (0.4–57.9)	23.1 (7.5–43.7)
Median PFS (95% CI) (months)	3.3 (2.3–5.1)	3.7 (2.5–7.4)	3.7 (2.5–5.1)
Median OS (95% CI) (months)	17.8 (14.0–NA)	14.5 (9.6–18.7)	16.6 (14.0–24.5)
1-year survival (95% CI) (%)	–	–	69.6 (50.8–88.4)
1.5-years survival (95% CI) (%)	–	–	43.0 (22.6–63.5)
Disease control rate¶			
6W (95% CI) (%)	–	–	47.8 (26.8–69.4)
12W (95% CI) (%)	–	–	26.1 (10.2–48.4)
24W (95% CI) (%)	–	–	21.7 (7.5–43.7)

†Partial response was re-evaluated after at least 4 weeks in patients with a partial response. ‡Stable disease was reassessed after at least 6 weeks. §Response rate (90% CI) is a primary end-point. ¶Disease control rates were respectively estimated by dividing the number of patients with no disease progression by the total number of patients. Disease control was defined as a response of complete response, partial response or stable disease. CI, confidence interval; NA, not available; OS, overall survival; PFS, progression-free survival.

estimated using the Kaplan–Meier method. Physical findings and the results of serum chemical and urine analyses were assessed at 2-week intervals; vital signs were assessed as necessary. Patients were observed until death or at least 1 year after registration to determine survival status. The severity of all adverse events was evaluated according to the Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE, Ver. 3.0). The duration of all adverse events and their relation to S-1 were initially assessed by the attending physicians. Subsequently, an independent review committee reviewed data on objective response and adverse events.

Statistical considerations. With the response rate as the primary end-point, a total sample size of at least 23 patients was estimated to be required in the phase II portion to allow the

study to have a one-sided 5% significance level of 0.05 and a power of 70%, assuming a threshold response rate of 5% and an expected response rate of 20%.

Results

Patient characteristics and treatment. Between May 2006 and April 2007, a total of 26 patients (nine in phase I and 17 in phase II) were enrolled at four centers in Japan. All patients were eligible for the evaluation of toxicity and efficacy. The first six patients who received dose level 2 (80 mg/m^2 per day) during the phase I part of this study were included in the phase II assessment, along with the 17 other patients (a total of 23 patients in the phase II assessment). The characteristics of patients are summarized in Table 1. At the study entry, 11 of 26 (42.3%) had metastatic disease. Six patients (23.1%) had single extrahepatic metastases (lung metastases, three patients; lymph node metastasis, three patients). Four patients had two sites of metastases, including the lung, lymph nodes and adrenal glands. Of the 26 patients, 23 had received some prior treatment, including three who had received systemic chemotherapy.

Dose-limiting toxicity and RD. None of the three patients who received dose level 1 (64 mg/m^2 per day) in the phase I part of the study had DLT. At dose level 2 (80 mg/m^2 per day), one patient with Child-Pugh class B had grade 3 anorexia during the first course of treatment, but the other two patients in the same cohort had no DLT. Three additional patients were enrolled to confirm safety, and one patient with Child-Pugh class B had a grade 2 rash; recovery required eight or more consecutive days of rest. Because two of the six patients who received level 2 had DLT, level 2 was defined as the RD for the phase II part of the study.

Treatment delivered. Twenty-three patients received a total of 85 cycles of treatment at dose level 2 (median, three cycles per patient; range, 1–15). The dose of S-1 was reduced in seven patients (30.4%) or a total of nine cycles (10.6%). The most common reasons for dose reductions were rash in four patients, and elevated serum bilirubin concentrations and anorexia in two patients each (some overlap among patients). Treatment was delayed because of toxicity in 12 patients (20 cycles), most often in cycles 1 or 2. The most common reasons for toxicity-related treatment delays were fatigue (five patients), rash (four patients) and elevated serum bilirubin concentrations (three patients). The reasons for terminating treatment were progressive disease in 19 patients (82.6%), adverse reactions in two patients (8.7%) and other reasons in two patients (8.7%; one required 28 or more consecutive days of rest, and one withdrew consent).

Toxicity. Drug-related adverse events occurring in all 26 patients in the phase I/II portion of the study are shown in Table 2. Treatment with S-1 was generally well tolerated throughout the study. Grade 3 or 4 toxicity occurred in 10 of the 23 patients (43.5%) who received level 2. Most toxic effects were laboratory abnormalities. There was no grade 3 or 4 toxicity at level 1. The most common grade 3 or 4 hematological toxic effects were hypochromia (17.4%), thrombocytopenia (17.4%) and lymphopenia (13.0%); the most common grade 3 or 4 nonhematological toxic effects were elevated serum AST levels (17.4%) and elevated serum bilirubin concentrations (13.0%).

Efficacy. A response could be evaluated in 26 patients in the phase I/II portion of the study. In the phase I part of the study (dose level 1), one patient had a partial response, one had progressive disease and the other was not evaluable. Of the 23 patients in the phase II part of the study, five (21.7%; 90% confidence interval [CI], 9.0–40.4%) responded to treatment Among the 23 patients in whom a response could be evaluated, five had a partial response, seven had stable disease, and 10 had progressive disease (Table 3). The remaining patient underwent imaging studies, but treatment was completed after one course, and continuation of stable disease for at least 6 weeks could not be

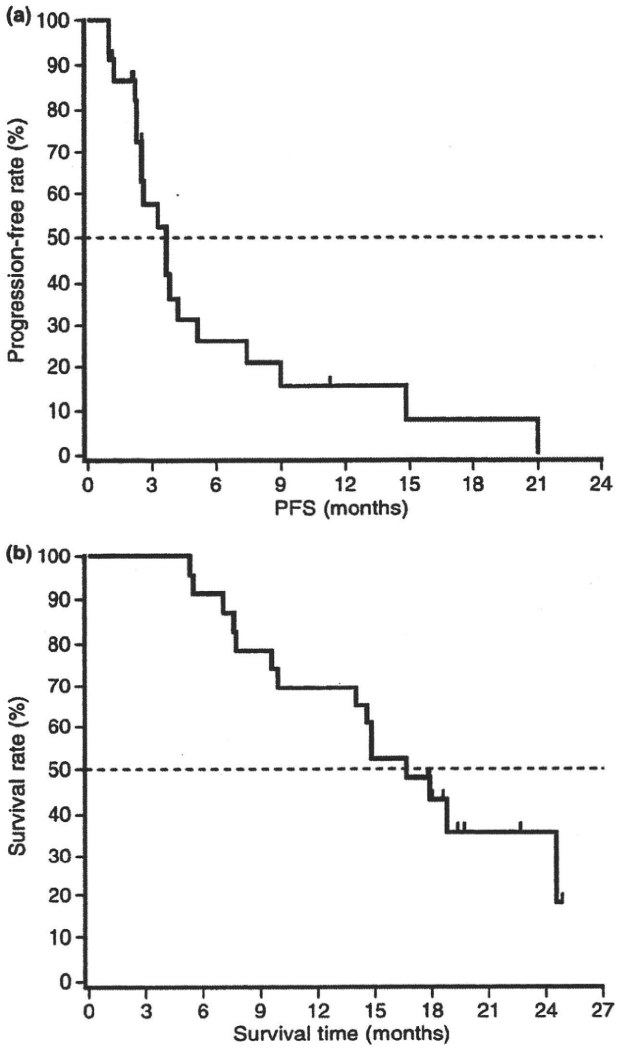

Fig. 1. Progression-free survival (PFS) (a) and overall survival (b) in patients who received dose level 2 of S-1 (n = 23). The median progression-free survival and overall survival were 3.7 and 16.6 months, respectively.

Table 4. Pharmacokinetics of FT, 5-FU, CDHP and Oxo on day 1 and day 8 in patients with HCC who received dose level 2

		C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}12}$ (ng h/mL)	$T_{1/2}$ (h)
FT	Day 1	2032 ± 437	3.3 ± 1.0	17070 ± 5139	10.1 ± 2.8
	Day 8	4365 ± 1712	3.7 ± 0.8	42399 ± 18137	12.7 ± 5.0
5-FU	Day 1	114.5 ± 35.5	4.3 ± 0.8	695.3 ± 223.6	2.3 ± 1.0
	Day 8	145.8 ± 31.4	4.3 ± 0.8	936.6 ± 292.3	2.4 ± 1.0
CDHP	Day 1	267.2 ± 76.8	3.3 ± 1.0	1424.8 ± 414.2	3.3 ± 0.9
	Day 8	281.0 ± 113.8	3.3 ± 1.0	1694.4 ± 603.5	3.4 ± 0.9
Oxo	Day 1	38.5 ± 1.8	3.7 ± 0.8	231.6 ± 69.8	4.0 ± 2.1
	Day 8	33.4 ± 9.5	4.0 ± 0.0	241.5 ± 115.6	4.0 ± 2.0

Parameters are represented as mean ± SD. CDHP, 5-chloro-2,4-dihydroxypyridine; 5-FU, 5-fluorouracil; FT, tegafur; Oxo, oteracil potassium.

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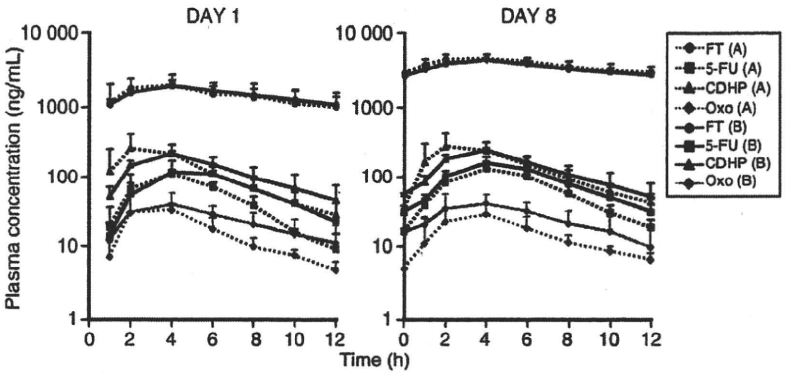

Fig. 2. Plasma-concentration–time profiles of tegafur (FT), 5-fluorouracil (5-FU), 5-chloro-2,4-dihydroxypyridine (CDHP) and oteracil potassium (Oxo) on day 1 and day 8 were similar in patients with Child-Pugh class A ($n = 3$) and those with Child-Pugh class B ($n = 3$).

confirmed. The duration of the five responses was 42, 147, 188, 238 and 371 days, respectively.

The median PFS was 3.7 months (95% CI, 2.5–5.1 months). The disease control rates at 6, 12 and 24 weeks were 47.8% (95% CI, 26.8–69.4%), 26.1% (95% CI, 10.2–48.4%) and 21.7% (95% CI, 7.5–43.7%), respectively. The PFS and OS are shown in Figure 1. The median OS was 16.6 months (95% CI, 14.0–24.5 months). Survival rates were 69.6% (95% CI, 50.8–88.4%) at 1 year and 43.0% (95% CI, 22.6–63.5%) at 1.5 years.

Pharmacokinetic analysis. Table 4 shows the pharmacokinetic data for the components of S-1 and 5-FU at level 2 on days 1 and 8. Compared with day 1, the C_{max} and $AUC_{0\text{-}12}$ of FT increased markedly on day 8; however, these increases were within the expected range given the slow elimination of FT, and repeated administration of S-1 had no effect on the T_{max} or $T_{1/2}$ of FT. There was no evidence of accumulation of 5-FU, CDHP or Oxo on day 8.

Figure 2 compares the plasma-concentration–time profiles of S-1 components and 5-FU between patients with Child-Pugh class A and those with Child-Pugh class B on days 1 and 8. The plasma-concentration–time profiles of FT, 5-FU, CDHP and Oxo were similar in patients with Child-Pugh class A and those with Child-Pugh class B on both days.

Discussion

There has been no established standard therapy for patients with advanced HCC refractory to surgery, transplantation, local ablation and TACE.[13,14] Some cytotoxic regimens have produced encouraging response rates, but survival benefits have been minimal compared with control groups, at the cost of clinically unacceptable adverse effects.[1,15]

S-1 is an anticancer drug consisting of FT, CDHP and Oxo. The conversion of FT to 5-FU is mediated mainly by hepatic cytochrome CYP2A6.[16] 5-FU is rapidly metabolized by DPD in the liver after the intravenous administration of 5-FU alone, but S-1, which includes a DPD inhibitor (i.e. CDHP), produces prolonged, effective concentrations of 5-FU in the blood. Thus, the liver plays an important role in the metabolism of FT.

The RD of S-1 in patients with HCC was estimated to be 80 mg/m^2 per day in phase I, which is similar to the dose recommended for the treatment of other solid tumors. However, in patients with HCC, Ueno *et al.*[10] reported that the DLT of 5-FU administered as a 5-day continuous infusion was stomatitis. Moreover, the MTD was equivalent to approximately 50% of that of 5-FU in patients with normal organ function,[10] suggesting that 5-FU-related gastrointestinal toxicity was reduced by Oxo in the formulation of S-1. We did not determine the MTD in this study because S-1 was approved for the treatment of other cancers. The pharmacokinetic properties of S-1 components and 5-FU in patients with HCC were similar to those in patients with pancreatic cancer or biliary tract cancer.[17,18]

Hematological toxic effects and symptomatic events such as pigmentation (87.0%), fatigue (82.6%), anorexia (78.3%) and ascites (30.4%) were more common than previously reported for S-1 in patients with other cancers. Nonetheless, severe toxic effects were comparable among patients with HCC and those with other cancers. Nonhematological toxic effects related to hepatic function were also more frequent than reported previously for S-1 in patients with other types of cancer, but such effects may have been caused by differences in underlying liver disease.

The pharmacokinetics of S-1 did not obviously differ between patients with Child-Pugh class A and those with Child-Pugh class B, suggesting that hepatic dysfunction associated with Child-Pugh class B did not affect the pharmacokinetics of S-1 components or 5-FU. The sample size of the pharmacokinetic evaluations was small because the primary end-point was to determine the RD as the evaluation of DLT in phase I. At dose level 2, DLT occurred in two patients with Child-Pugh class B (Grade 3 anorexia in one, and a Grade 2 rash requiring 8 or more consecutive days of rest in the other). There was no DLT at level 1 (given only to patients with Child-Pugh class A). However, the patient who had DLT of grade 3 anorexia had renal dysfunction at baseline, and the plasma 5-FU concentrations in this patient on day 8 were higher than those in other patients, perhaps contributing to the development of DLT (data not shown). In addition, there were no obvious differences in the incidence or grade of drug-related adverse events between patients with Child-Pugh class A and those with Child-Pugh class B, consistent with the results of pharmacokinetic analysis. These results suggested that there were no clinically meaningful differences in pharmacokinetics or safety according to Child-Pugh class or between patients with HCC and those with other cancers, and that S-1 was well tolerated in patients with HCC, similar to patients with other cancers. However, our study had several limitations: only a very small number of patients with Child-Pugh class B were included; among the patients with Child-Pugh class B, the score was heterogeneous, ranging from 7 to 9; and only patients with better scores were studied. Therefore, extra care should be taken when S-1 is given to patients with Child-Pugh class B.

As for efficacy, five of 23 patients had partial responses at dose level 2. Compared with previously reported response rates obtained with single-agent chemotherapy in patients with HCC, our results are good. In particular, the median OS appeared to be longer than that obtained with other agents in non-Japanese studies. The reason for the better OS in Japanese patients might be similar to that previously reported for sorafenib.[4] The median OS in our study was similar to that in a Japanese phase I study of sorafenib.[4] In studies of sorafenib in non-Japanese and