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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.

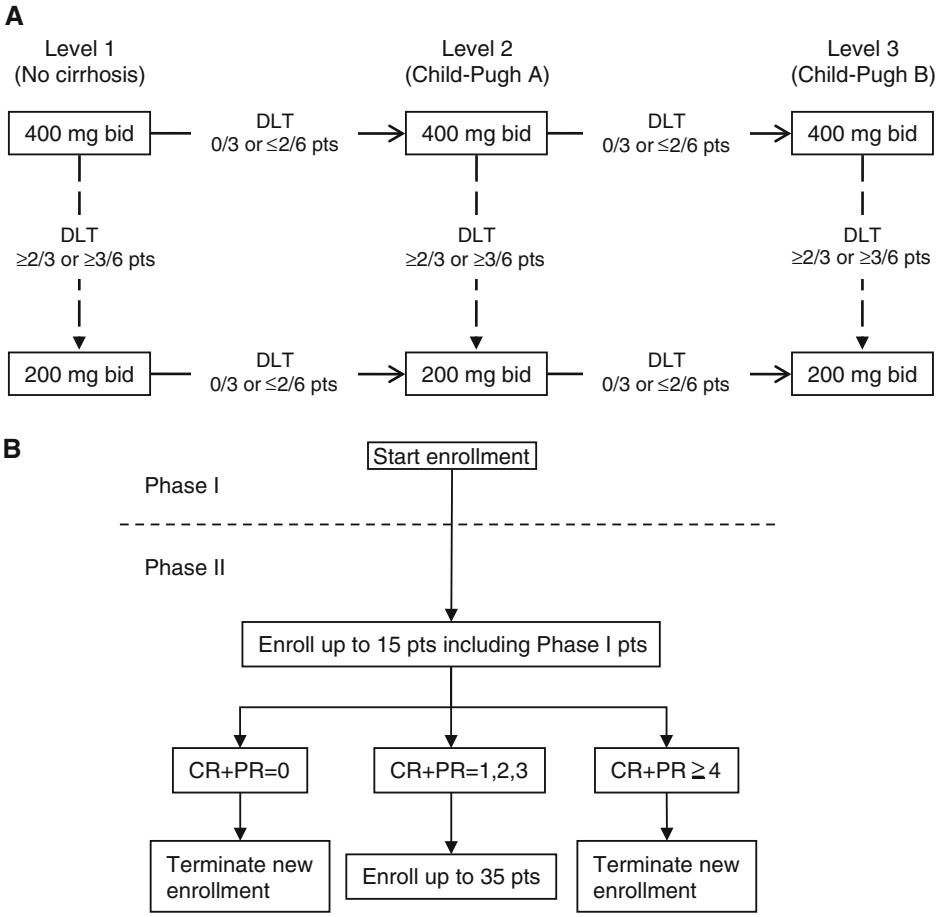

Fig. 1 TSU-68 phase I/II study schema. **a** In phase I, patients were stratified into three groups based on hepatic function, and the toxicity and pharmacokinetics were assessed from Level 1 (no cirrhosis) to Level 3 (Child–Pugh B) by enrolling three patients at each level. *Bid* twice daily, *DLT* 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

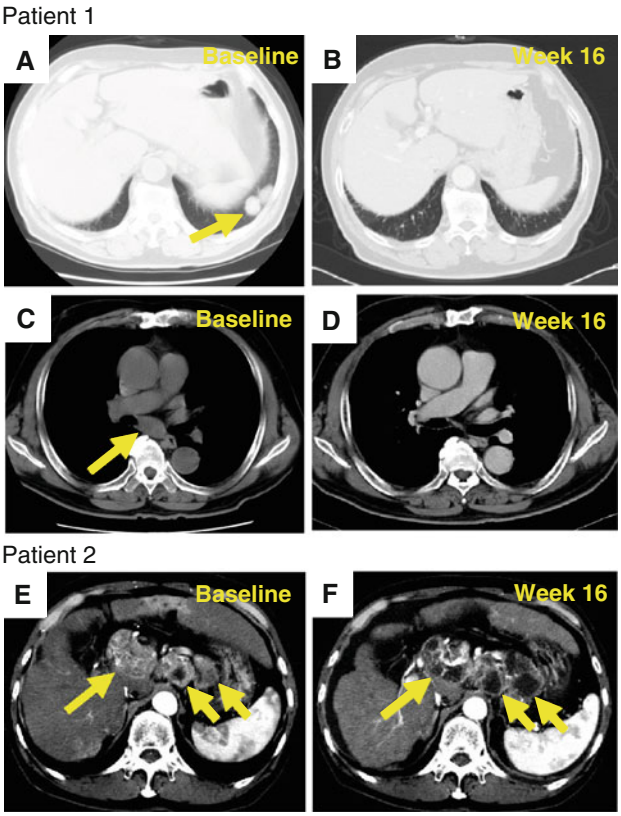

Fig. 2 Computed tomography images of responding lesions from patient 1, who achieved a complete response. Metastatic lesions in the lung (**a**) and lymph node (**c**) disappeared after four cycles (16 weeks) of TSU-68 treatment (**b**, **d**). Representative computed tomography images of a tumor showing 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**)

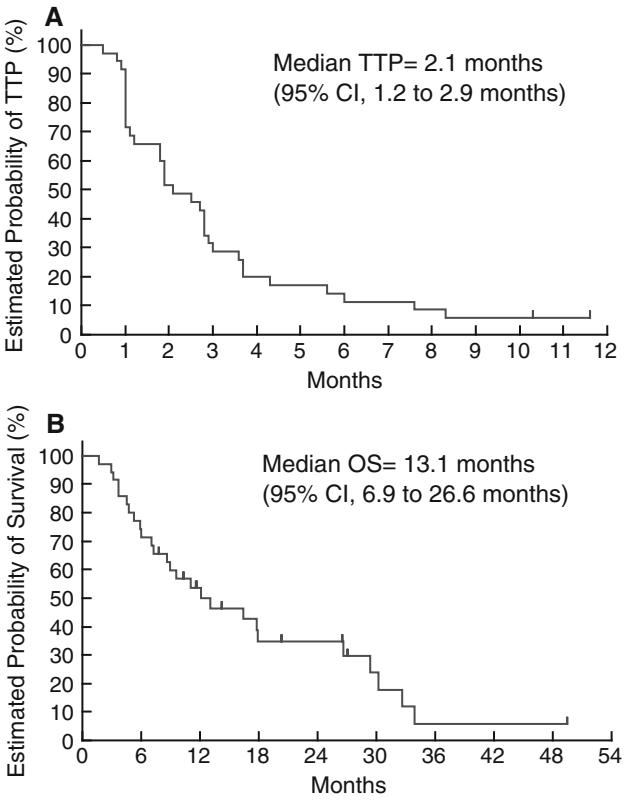

Fig. 3 **a** The independently assessed median time to progression in all 35 patients treated with TSU-68 was 2.1 months. **b** The investigator-assessed median overall survival in all 35 patients treated with TSU-68 was 13.1 months

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).

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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Conflict of interest statement The author(s) have nothing to disclose.

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