Table 2 Univariate analysis of prognostic factors in patients with hepatocellular carcinoma treated by transcatheter arterial infusion chemotherapy using cisplatin suspended in lipiodol

	n	Median survival (years)	2-year survival (%)	Hazard ratio	*p* value
Host-related variables					
Age (years)					
≥60	67	2.5	65		
<60	27	2.6	54	0.98 (0.60–1.59)	0.93
Gender					
Female	32	2.7	66		
Male	62	2.4	60	0.99 (0.61–1.56)	0.97
Blood transfusion					
Present	28	2.5	60		
Absent	66	2.7	63	0.77 (0.48–1.24)	0.28
Alcohol abuse[a]					
Present	11	2.0	55		
Absent	83	2.6	63	0.63 (0.33–1.20)	0.16
Smoking habit[b]					
Absent	63	2.5	59		
Present	31	3.4	69	0.79 (0.50–1.27)	0.33
HBs Ag					
Negative	80	2.5	64		
Positive	14	1.8	46	0.77 (0.40–1.49)	0.45
HCV Ab					
Negative	18	1.9	47		
Positive	76	2.5	65	0.93 (0.53–1.64)	0.81
Ascites					
Present	14	1.4	21		
Absent	80	2.8	69	0.29 (0.16–0.53)	**<0.01**
WBC ($\times 10^4/mm^3$)					
≤4.0	51	2.5	61		
>4.0	43	2.5	64	0.76 (0.49–1.19)	0.23
Hemoglobin (g/dL)					
<10	17	2.4	59		
≥10	77	2.6	63	0.69 (0.40–1.19)	0.18
Platelet ($\times 10^4/mm^3$)					
<7.5	36	2.5	67		
≥7.5	58	2.5	59	0.89 (0.57–1.37)	0.59
Total bilirubin (mg/dL)					
≥2.0	13	1.8	46		
<2.0	81	2.7	65	0.59 (0.32–1.09)	0.09
Albumin (g/dL)					
<3.0	33	1.6	35		
≥3.0	61	4.0	76	0.29 (0.18–0.47)	**<0.01**
AST (U/L)					
≥85	24	2.4	58		
<85	70	2.8	63	0.63 (0.38–1.04)	0.07
ALT (U/L)					
≥92	21	2.4	57		
<92	73	2.7	63	0.74 (0.44–1.24)	0.25
LDH (U/L)					
≥500	9	1.8	44		
<500	85	2.5	64	0.76 (0.36–1.58)	0.46
Prothrombin time (%)					
<70	41	2.4	58		
≥70	53	2.7	65	0.93 (0.60–1.45)	0.76
ICG R15 (%)					
≥30	46	2.2	52		
<30	43	3.4	71	0.68 (0.43–1.07)	0.09
Tumor-related variables					
Number of tumors					
Multiple	53	2.0	51		
Single	41	2.8	76	0.63 (0.41–0.98)	**<0.05**
Tumor distribution					
Bilateral	24	1.1	27		
Unilateral	70	2.8	73	0.39 (0.24–0.65)	**<0.01**
Maximum tumor size (cm)					
>3.0	40	1.6	42		
≤3.0	54	3.2	76	0.41 (0.26–0.66)	**<0.01**
Portal vein invasion					
Present	7	1.0	17		
Absent	87	2.6	65	0.36 (0.15–0.84)	**<0.05**
Alpha-fetoprotein (ng/mL)					
≥100	46	2.4	57		
<100	48	2.6	67	0.66 (0.42–1.02)	0.06
PIVKA II (mAU/mL)					
≥100	14	1.1	34		
<100	80	2.7	67	0.53 (0.29–0.97)	**<0.05**

p values lesser than 0.05 are given in bold

HBs Ag hepatitis B surface antigen, *HCV Ab* hepatitis C antibody, *WBC* white blood cell count, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactic dehydrogenase, *ICG* indocyanine green test, *PIVKA II* protein induced by vitamin K absence or antagonist-II

[a] Ethanol intake ≥80 g/day for ≥5 years

[b] >20 cigarettes/day for >10 years

patients. Neither severe toxicity including renal dysfunction or thrombocytopenia, nor complication or treatment related death were seen in the present study.

Univariate and multivariate analysis

The median survival times, two-year survival, hazard ratios and *p* values of the survival time for univariate analysis are shown in Table 2. Among the host-related factors, absence of ascites and a serum albumin level of >3.0 g/dL were

significantly associated with a longer survival time. Among the tumor-related factors, single nodule, unilateral distribution of tumors, maximum tumor size <3.0 cm, absence of portal vein invasion, and PIVKA II level <100 mAU/mL were significantly associated with a longer survival time. The results of multivariate analysis using the Cox proportional hazard model are shown in Table 3. In the multivariate analyses, only those variables identified as significant by the univariate analysis were entered. Serum albumin ≥3.0 g/dL, maximum tumor size <3.0 cm, absence of ascites, and unilateral distribution of the tumors were significantly associated with favorable survival.

Risk groups based on the regression model

For the clinical application of these findings, a prognostic index was calculated based on the regression coefficients derived from the four variables identified by multivariate analysis (Table 3), as follows: prognostic index = score for albumin (0 for ≥3.0, 1 for <3.0 g/dL) + score for ascites (0 for absence, 1 for presence) + score for maximum tumor size (0 for ≤3.0, 1 for >3.0 cm) + score for tumor distribution (0 for unilateral, 1 for bilateral). The index values ranged from 0 to 4. The patients were then classified into three groups according to the prognostic index, as follows: good prognosis group (Group A: prognostic index = 0, $n = 31$ patients) (equivalent to patients with none of the four prognostic factors); intermediate prognosis group (Group B: prognostic index = 1, $n = 28$ patients) (equivalent to patients with one of the four prognostic factors); poor prognosis group (Group C: prognostic index ≥ 2, $n = 35$ patients) (equivalent to patients with two or more of the four prognostic factors). The survival curves for the three groups are shown in Fig. 2. The median survival times in the good, intermediate, and poor prognosis groups were 4.3, 2.7, and 1.1 years, respectively. There were significant differences in the survival time among the three groups ($p < 0.01$).

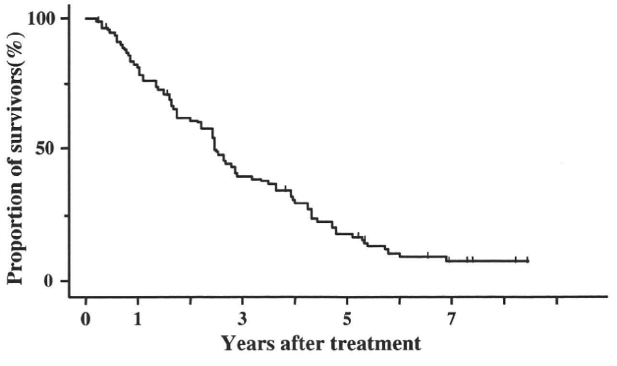

Fig. 1 Overall survival curve for all patients with hepatocellular carcinoma treated by transcatheter arterial infusion chemotherapy using cisplatin suspended in lipiodol. *Tick marks* indicate censored cases

Discussion

TAE has been widely used for cases with unresectable HCC and is currently the mainstay of non-surgical treatment for HCC, because it has been shown to exert a marked antitumor effect against HCC and can be administered for any type of HCC, regardless of the size, location or number of tumors [1]. In addition, the survival benefit of this treatment modality has been verified by two meta-analyses [2, 3] of seven randomized controlled trials [4–10]. However, TAE has deleterious effects on liver functions, thereby impairing the baseline prognosis. On the other hand, TAI has milder hepatotoxicity, but also shows a lower antitumor efficacy against advanced HCC than TAE. However, in a randomized controlled trial of TAE versus TAI with zinostatin-stimalamer and lipiodol, TAI and TAE were reported to yield comparable survival [16]. Moreover, the result of our retrospective analysis of TAE versus TAI using cisplatin–lipiodol suspension indicated similar outcomes for the two modalities [17]. From the results of these two studies, we could not conclude that additional embolization is not necessary for the treatment of advanced HCC, but there may be a subset of patients of advanced HCC in which TAI alone may yield sufficient treatment efficacy and survival. Therefore, this analysis of prognostic factors was carried out to enable identification of appropriate candidates for TAI using cisplatin–lipiodol suspension among HCC patients with no prior treatment. This single-institution study was undertaken using a unified method for tumor staging and identical procedures for treatment, follow-up, and supportive care throughout the duration of the study, to enable us to obtain reliable results for confirming important

Table 3 Significant prognostic factors determined by multivariate analysis with the Cox proportional hazard model

Variable	Coefficient	Hazard ratio (95% confidence intervals)	*p* value
Albumin ≥3.0 g/dL	0.94	0.39 (0.23–0.66)	<0.001
Maximum tumor size ≤3.0 cm	1.01	0.37 (0.19–0.69)	0.001
Absence of ascites	0.81	0.45 (0.11–0.40)	0.002
Unilateral tumor distribution	0.77	0.46 (0.27–0.79)	0.004

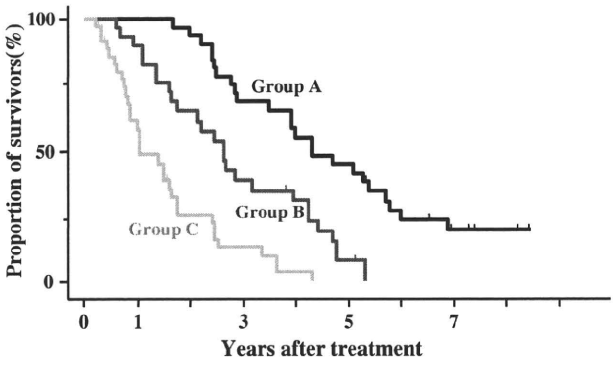

Fig. 2 Survival curves for the three groups determined by a prognostic index. *Group A* good prognosis (31 patients), *Group B* intermediate prognosis (28 patients), *Group C* poor prognosis (35 patients). *Tick marks* indicate censored cases

prognostic factors, predicting life expectancy and designing future clinical trials of TAI for HCC.

In this study, cisplatin was administered as the anticancer agent for TAI. Cisplatin has been reported to exert its actions by binding to the DNA in cancer cells, inhibiting DNA synthesis and subsequent cellular division. It is one of the key drugs for advanced HCC, that constituted a component of the combined chemotherapeutic regimen used in three of the seven randomized controlled trials of TAE reported until date [6, 7, 9]. In Japan, a favorable tumor response (33.8%) was reported in a clinical study of intra-arterial administration of cisplatin for advanced HCC [21], and the treatment has been approved for the treatment of HCC by the Ministry of Health, Labour and Welfare of Japan. Lipiodol has been used as a carrier for anticancer agents in targeting chemotherapy [13–15], and a suspension of cisplatin powder in lipiodol was used in this study. It has been reported that stronger antitumor effect is obtained by hepatic arterial administration of a combination of lipiodol and an anticancer agent than by that of an anticancer agent alone [26]. Recently, a lipophilic cisplatin derivative that can be suspended in lipiodol, SM-11355, was reported to show promising tumor efficacy (CR rate: 56%) in a phase II trial, and further trial is ongoing [27]. Therefore, combined therapy with cisplatin and lipiodol has been expected to become established as a valid option for the treatment of HCC. The response rate (51%: 95% confidence interval, 41–61%) at one month obtained in this study was more favorable than that in a clinical study of cisplatin alone, because TAI with an emulsion of an anticancer agent and lipiodol could be expected to exert more potent effects than an anticancer agent alone. However, follow-up at one month might be insufficient for evaluation of the rate/pattern of recurrence of HCC.

The median survival time and survival rates at two years in the current study were 2.5 years and 65.2%, respectively. These results were comparable or superior to those of TAE reported from the aforementioned seven randomized controlled trials [4–10]. Although the study was based on a retrospective cohort design, the treatment efficacy of TAI with cisplatin–lipiodol suspension was promising and comparable to that of TAE for HCC.

In regard to the host-related factors, absence of ascites and a serum albumin level >3.0 g/dL were found to be favorable prognostic factors by multivariate analysis. Ascites and albumin are the most important factors to consider when evaluating the hepatic reserve, being included in both the Okuda staging system [28] and Child-Pugh classification [29], and have been shown to be prognostic factor in previous studies of patients with advanced HCC [19, 20, 22–24]. In regard to the tumor-related factors, a maximum tumor size ≤3.0 cm and unilateral distribution of the tumors were identified as being significantly associated with a longer survival time by multivariate analysis. Increased tumor size and bilateral distribution of tumors are the well-known unfavorable prognostic factors in HCC patients, and have been shown to be correlated with increased tumor volume and poorer differentiation of HCC, which reflect a more advanced stage and higher malignant potential of the tumors [22]. However, these prognostic factors for TAI with lipiodol in this study were similar to those identified for TAI without lipiodol [19–21] or TAE in previous reports [22–24], and no specific prognostic factors for TAI could be identified in this study.

For clinical application of these findings, we propose a prognostic index based on the independent prognostic factors identified in this study. Patients could be classified into three groups: those with good, intermediate, and poor prognosis ($p < 0.0001$) (Fig. 2). This index consists of both hepatic reserve and tumor stage, like the modified JIS score [30], and it differs from the Child-Pugh stage or TNM stage which are, respectively, based on either only the hepatic reserve or tumor stage. An index based on both the hepatic reserve and tumor stage might enable a more accurate prediction of life expectancy and stratification of the group into more distinct prognoses. This index can be easily calculated, because it is based on variables obtained during routine examinations before TAI. It can, therefore, be used to stratify patients with HCC before TAI according to the predicted survival. Accordingly, patients with good prognosis may obtain sufficient treatment efficacy and survival with TAI alone. In contrast, patients with a poor prognosis may be treated with supportive care only because of the extremely short median survival (1.1 years) expected, or may be treated other more aggressive treatments, such as more intensive chemotherapy. Recently, systemic chemotherapy for advanced HCC has become an important treatment modality, because sorafenib has been proven to confer a survival benefit and to show promise as a standard

treatment for patients with advanced HCC [31]. To improve the treatment efficacy, further chemotherapy regimens, such as the combination therapy comprising TAI with cisplatin suspended in lipiodol and sorafenib or other molecularly targeted agents, remain as challenges to be met following further detailed investigations. These findings may be helpful in predicting the life expectancy in HCC patients treated with TAI and provide more information to stratify patients in future TAI trials. It is also important to validate this prognostic index by applying it to other populations of HCC patients.

In conclusion, TAI with cisplatin suspended in lipiodol exhibited favorable tumor efficacy and survival in patients with HCC. Although no specific prognostic factors for TAI could be identified in this study, the results of the prognostic factors and the prognostic index may be helpful for predicting life expectancy, determining the most appropriate treatment strategies, and designing future clinical trials.

References

1. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet. 2003;362:1907–17.
2. Cammà C, Schepis F, Orlando A, Albanese M, Shahied L, Trevisani F, et al. Transarterial chemoembolization for unresectable hepatocellular carcinoma: meta-analysis of randomized controlled trials. Radiology. 2002;224:47–54.
3. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: chemoembolization improves survival. Hepatology. 2003;37:429–42.
4. Lin DY, Liaw YF, Lee TY, Lai CM. Hepatic arterial embolization in patients with unresectable hepatocellular carcinoma—a randomized controlled trial. Gastroenterology. 1988;94:453–6.
5. Pelletier G, Roche A, Ink O, Anciaux ML, Derhy S, Rougier P, et al. A randomized trial of hepatic arterial chemoembolization in patients with unresectable hepatocellular carcinoma. J Hepatol. 1990;11:181–4.
6. Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire. A comparison of lipiodol chemoembolization and conservative treatment for unresectable hepatocellular carcinoma. N Engl J Med. 1995;332:1256–61.
7. Pelletier G, Ducreux M, Gay F, Luboinski M, Hagege H, Dao T, et al. Treatment of unresectable hepatocellular carcinoma with lipiodol chemoembolization: a multicenter randomized trial. J Hepatol. 1998;29:129–34.
8. Bruix J, Llovet JM, Castells A, Montana X, Bru C, Ayuso MC, et al. Transarterial embolization versus symptomatic treatment in patients with advanced hepatocellular carcinoma: results of a randomized controlled trial in a single institution. Hepatology. 1998;27:1578–83.
9. Lo CM, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, et al. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. Hepatology. 2002;35:1164–71.
10. Llovet JM, Real MI, Montana X, Planas R, Coll S, Aponte J, et al. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. Lancet. 2002;359:1734–9.
11. Schwartz JD, Beutler AS. Therapy for unresectable hepatocellular carcinoma: review of the randomized clinical trials-II: systemic and local non-embolization-based therapies in unresectable and advanced hepatocellular carcinoma. Anticancer Drugs. 2004;15:439–52.
12. Minagawa M, Makuuchi M. Treatment of hepatocellular carcinoma accompanied by portal vein tumor thrombus. World J Gastroenterol. 2006;12:7561–7.
13. Imaeda T, Yamawaki Y, Seki M, Goto H, Iinuma G, Kanematsu M, et al. Lipiodol retention and massive necrosis after lipiodol-chemoembolization of hepatocellular carcinoma: correlation between computed tomography and histopathology. Cardiovasc Intervent Radiol. 1993;16:209–13.
14. Takayasu K, Shima Y, Muramatsu Y, Moriyama N, Yamada T, Makuuchi M, et al. Hepatocellular carcinoma: treatment with intraarterial iodized oil with and without chemotherapeutic agents. Radiology. 1987;163:345–51.
15. Okusaka T, Okada S, Ueno H, Ikeda M, Yoshimori M, Shimada K, et al. Evaluation of the therapeutic effect of transcatheter arterial embolization for hepatocellular carcinoma. Oncology. 2000;58:293–9.
16. Okusaka T, Sato T, Hinotsu S, Shioyama Y, Kasugai H, Tanaka K, et al. Transarterial infusion chemotherapy alone versus transarterial chemoembolization for the treatment of hepatocellular carcinoma: results of a multicenter randomized phase III trial. J Clin Oncol 2007 ASCO Annual Meeting Proceedings. 2007;25:18S (abstr 4643).
17. Ikeda M, Maeda S, Shibata J, Muta R, Ashihara H, Tanaka M, et al. Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma. Oncology. 2004;66:24–31.
18. Okusaka T, Okada S, Ueno H, Ikeda M, Iwata R, Furukawa H, et al. Transcatheter arterial embolization with zinostatin stimalamer for hepatocellular carcinoma. Oncology. 2002;62:228–33.
19. Yamasaki T, Kimura T, Kurokawa F, Aoyama K, Ishikawa T, Tajima K, et al. Prognostic factors in patients with advanced hepatocellular carcinoma receiving hepatic arterial infusion chemotherapy. J Gastroenterol. 2005;40:70–8.
20. Uka K, Aikata H, Takaki S, Miki D, Kawaoka T, Jeong SC, et al. Pretreatment predictor of response, time to progression, and survival to intraarterial 5-fluorouracil/interferon combination therapy in patients with advanced hepatocellular carcinoma. J Gastroenterol. 2007;42:845–53.
21. Yoshikawa M, Ono N, Yodono H, Ichida T, Nakamura H. Phase II study of hepatic arterial infusion of a fine-powder formulation of cisplatin for advanced hepatocellular carcinoma. Hepatol Res. 2008;38:474–83.
22. Lladó L, Virgili J, Figueras J, Valls C, Dominguez J, Rafecas A, et al. A prognostic index of the survival of patients with unresectable hepatocellular carcinoma after transcatheter arterial chemoembolization. Cancer. 2000;88:50–7.
23. Ikeda M, Okada S, Yamamoto S, Sato T, Ueno H, Okusaka T, et al. Prognostic factors in patients with hepatocellular carcinoma treated by transcatheter arterial embolization. Jpn J Clin Oncol. 2002;32:455–60.
24. Changchien CS, Chen CL, Yen YH, Wang JH, Hu TH, Lee CM, et al. Analysis of 6381 hepatocellular carcinoma patients in southern Taiwan: prognostic features, treatment outcome, and survival. J Gastroenterol. 2008;43:159–70.
25. Shibata J, Fujiyama S, Sato T, Kishimoto S, Fukushima S, Nakano M. Hepatic arterial injection chemotherapy with cisplatin suspended in an oily lymphographic agent for hepatocellular carcinoma. Cancer. 1989;64:1586–94.
26. Yoshikawa M, Saisho H, Ebara M, Iijima T, Iwama S, Endo F, et al. A randomized trial of intrahepatic arterial infusion of 4′-epidoxorubicin with lipiodol versus 4′-epidoxorubicin alone in

the treatment of hepatocellular carcinoma. Cancer Chemother Pharmacol. 1994;33(Suppl):S149–52.
27. Okusaka T, Okada S, Nakanishi T, Fujiyama S, Kubo Y. Phase II trial of intra-arterial chemotherapy using a novel lipophilic platinum derivative (SM-11355) in patients with hepatocellular carcinoma. Invest New Drugs. 2004;22:169–76.
28. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer. 1985;56:918–28.
29. Pugh RNH, Murry-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg. 1973;60:646–9.
30. Luo KZ, Itamoto T, Amano H, Oshita A, Ushitora Y, Tanimoto Y, et al. Comparative study of the Japan Integrated Stage (JIS) and modified JIS score as a predictor of survival after hepatectomy for hepatocellular carcinoma. J Gastroenterol. 2008;43:369–77.
31. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. SHARP Investigators Study Group. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med. 2008;359:378–90.

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ORIGINAL ARTICLE

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

Fumihiko Kanai · Haruhiko Yoshida · Ryosuke Tateishi · Shinpei Sato · Takao Kawabe · Shuntaro Obi · Yuji Kondo · Makoto Taniguchi · Kazumi Tagawa · Masafumi Ikeda · Chigusa Morizane · Takuji Okusaka · Hitoshi Arioka · Shuichiro Shiina · Masao Omata

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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

F. Kanai · H. Yoshida · R. Tateishi · S. Sato · T. Kawabe · S. Shiina · M. Omata
Department of Gastroenterology, University of Tokyo, Tokyo, Japan

F. Kanai
Department of Clinical Drug Evaluation, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

S. Obi
Department of Hepatology, Kyoundo Hospital, Tokyo, Japan

Y. Kondo · M. Taniguchi · K. Tagawa
Department of Gastroenterology, Mitsui Memorial Hospital, Tokyo, Japan

M. Ikeda · C. Morizane · T. Okusaka
Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, Japan

H. Arioka
Division of Medical Oncology, Yokohama Rosai Hospital, Yokohama, Japan

F. Kanai (✉)
Department of Gastroenterology, Chiba University Hospital, 1-8-1 Inohana, Chuo-ku, Chiba 260-8677, Japan
e-mail: kanaif@faculty.chiba-u.jp

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.

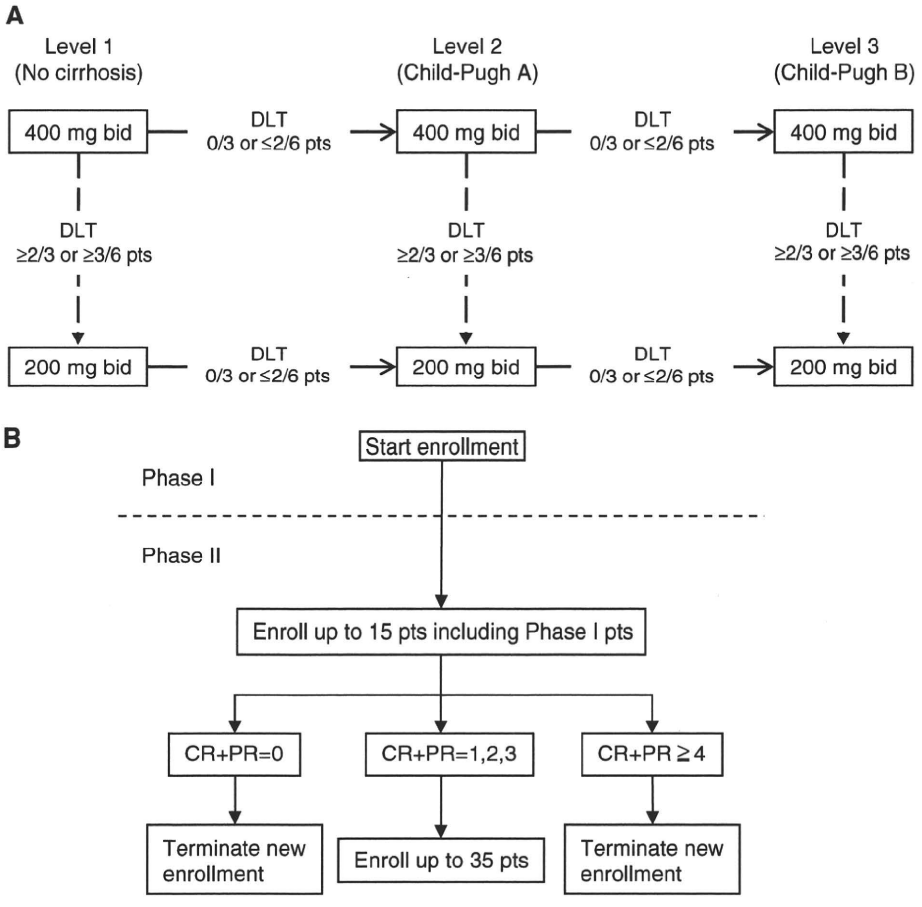

Fig. 1 TSU-68 phase I/II study schema. **a** In phase I, patients were stratified into three groups based on hepatic function, and the toxicity and pharmacokinetics were assessed from Level 1 (no cirrhosis) to Level 3 (Child–Pugh B) by enrolling three patients at each level. *Bid* twice daily, *DLT*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

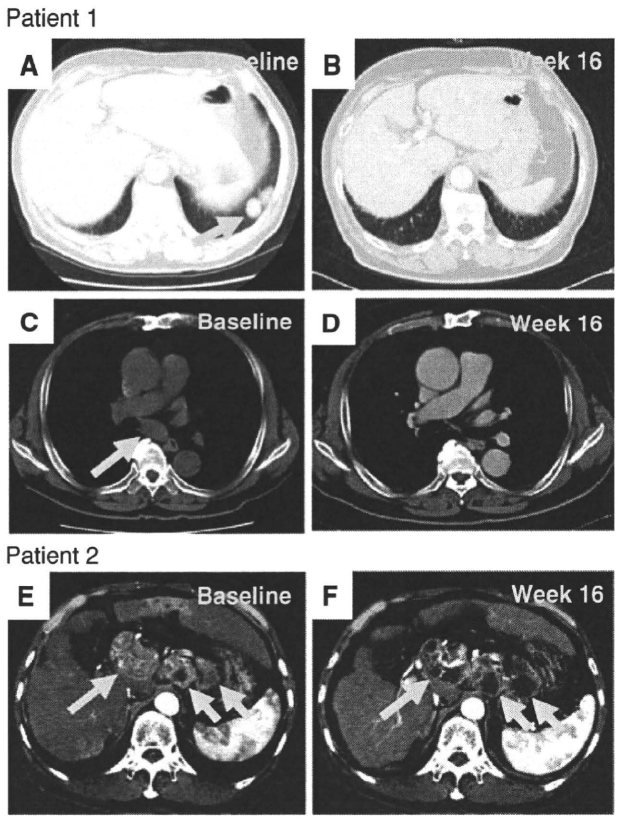

Fig. 2 Computed tomography images of responding lesions from patient 1, who achieved a complete response. Metastatic lesions in the lung (**a**) and lymph node (**c**) disappeared after four cycles (16 weeks) of TSU-68 treatment (**b**, **d**). Representative computed tomography images of a tumor showing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

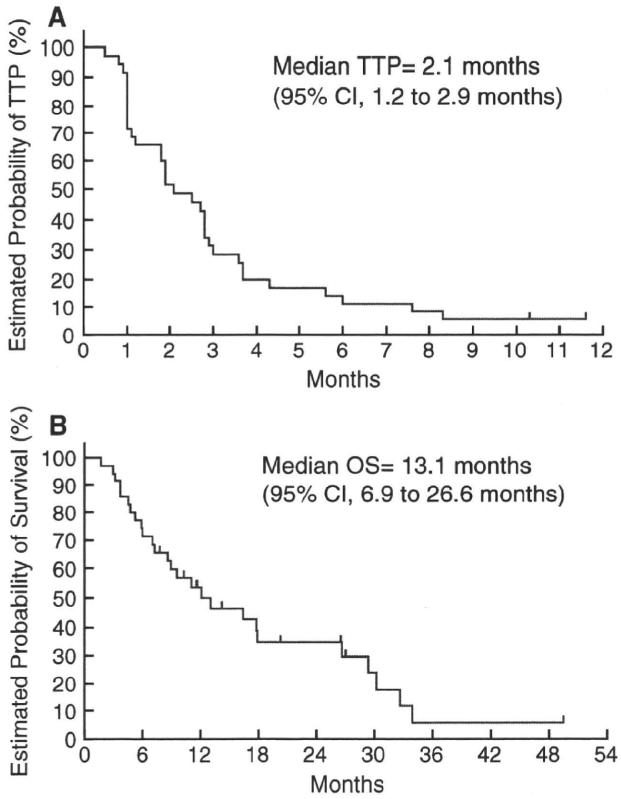

Fig. 3 **a** The independently assessed median time to progression in all 35 patients treated with TSU-68 was 2.1 months. **b** The investigator-assessed median overall survival in all 35 patients treated with TSU-68 was 13.1 months

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

References

1. Parkin DM, Bray F, Ferlay J et al (2005) Global cancer statistics, 2002. CA Cancer J Clin 55:74–108
2. Bruix J, Sherman M (2005) Management of hepatocellular carcinoma. Hepatology 42:1208–1236
3. Llovet JM, Ricci S, Mazzaferro V et al (2008) Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 359:378–390
4. Cheng AL, Kang YK, Chen Z et al (2009) Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 10:25–34
5. Laird AD, Vajkoczy P, Shawver LK et al (2000) SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. Cancer Res 60:4152–4160
6. Naumova E, Ubezio P, Garofalo A et al (2006) The vascular targeting property of paclitaxel is enhanced by SU6668, a receptor tyrosine kinase inhibitor, causing apoptosis of endothelial cells and inhibition of angiogenesis. Clin Cancer Res 12:1839–1849
7. Yorozuya K, Kubota T, Watanabe M et al (2005) TSU-68 (SU6668) inhibits local tumor growth and liver metastasis of human colon cancer xenografts via anti-angiogenesis. Oncol Rep 14:677–682
8. Solorzano CC, Jung YD, Bucana CD et al (2001) In vivo intracellular signaling as a marker of antiangiogenetic activity. Cancer Res 61:7048–7051
9. Kuenen BC, Giaccone G, Ruijter R et al (2005) Dose-finding study of the multitargeted tyrosine kinase inhibitor SU6668 in patients with advanced malignancies. Clin Cancer Res 11:6240–6246
10. Kanai F, Yoshida H, Teratani T et al (2006) New feasibility study design with hepatocellular carcinoma: a phase I/II study of TSU-68, an oral angiogenesis inhibitor [Abstract]. J Clin Oncol 24(Suppl):213S
11. Kanai F, Yoshida H, Tateishi R et al (2008) Final results of a phase I/II trial of the oral anti-angiogenesis inhibitor TSU-68 in patients with advanced hepatocellular carcinoma [Abstract]. J Clin Oncol 26(Suppl):235S
12. Kitamura R, Yamamoto Y, Nagayama S et al (2007) Decrease in plasma concentrations of antiangiogenic agent TSU-68 ((Z)-5-[(1, 2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2, 4-dimethyl-1H-pyrrole-3-propanoic acid) during oral administration twice a day to rats. Drug Metab Dispos 35:1611–1616

13. Kitamura R, Asanoma H, Nagayama S et al (2008) Identification of human liver cytochrome P450 isoforms involved in autoinduced metabolism of the antiangiogenic agent (Z)-5-[(1, 2-dihydro-2-oxo-3H-indol-3-ylidene)methyl]-2, 4-dimethyl-1H-pyrrole-3-propanoic acid (TSU-68). Drug Metab Dispos 36:1003–1009
14. Ueda Y, Shimoyama T, Murakami H et al (2010) Phase I and pharmacokinetic study of TSU-68, a novel multiple receptor tyrosine kinase inhibitor, by twice daily oral administration between meals with solid tumors (under submission)
15. Murakami H, Ueda Y, Shimoyama T et al. (2010) Phase I, pharmacokinetic, and biological studies of TSU-68, a novel multiple tyrosine kinase inhibitor, administered after meals with solid tumors (under submission)
16. Green H, Benedetti J, Crowley J (2002) Clinical trials in oncology, 2nd edn. Chapman & Hall, London
17. Fleming TR (1982) One-sample multiple testing procedures for phase II clinical trials. Biometrics 38:143–151
18. The Liver Cancer Study Group of Japan (2000) The general rules for the clinical and pathological study of primary liver cancer. Version 4. Kanehara & Co., Ltd., Tokyo
19. Makuuchi M, Belghiti J, Belli G et al (2003) IHPA concordant classification of primary liver cancer: working group report. J Hepatobiliary Pancreat Surg 10:26–30
20. Llovet JM, Bruix J (2008) Novel advancements in the management of hepatocellular carcinoma 2008. J Hepatol 48:S20–S37
21. Roodhart JM, Langenberg MH, Witteveen E et al (2008) The molecular basis of class side effects due to treatment with inhibitors of VEGF/VEGFR pathway. Curr Clin Pharmacol 3:132–143
22. Faivre S, Raymond E, Boucher E et al (2009) Safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma: an open-label, multicentre, phase II study. Lancet Oncol 10:794–800
23. Llovet JM, Di Bisceglie AM, Bruix J et al (2008) Panel of experts in HCC-design clinical trials. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 100:698–711
24. Furuse J, Ishii H, Nakachi K et al (2008) Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma. Cancer Sci 99:159–165
25. Zhu AX, Sahani DV, Duda DG et al (2009) Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. J Clin Oncol 27:3027–3035
26. Thomas MB, Abbruzzese JL (2005) Opportunities for targeted therapies in hepatocellular carcinoma. J Clin Oncol 23:8093–8108
27. Pang R, Poon RT (2006) Angiogenesis and antiangiogenic therapy in hepatocellular carcinoma. Cancer Lett 242:151–167
28. Poon RT, Lau C, Pang R et al (2007) High serum vascular endothelial growth factor levels predict poor prognosis after radiofrequency ablation of hepatocellular carcinoma: importance of tumor biomarker in ablative therapies. Ann Surg Oncol 14:1835–1845
29. Alexiou D, Karayiannakis AJ, Syrigos KN et al (2001) Serum levels of E-selectin, ICAM-1, and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumor surgery. Eur J Cancer 37:2392–2397
30. Alexiou D, Karayiannakis AJ, Syrigos KN et al (2003) Clinical significance of serum levels of E-selectin, intracellular adhesion molecule-1, and vascular cell adhesion molecule-1 in gastric cancer patients. Am J Gastroenterol 98:478–485
31. O'Hanlon DM, Fitzsimons H, Lynch J et al (2002) Soluble adhesion molecule (E-selectin, ICAM-1 and VCAM-1) in breast carcinoma. Eur J Cancer 38:2252–2257
32. Joanna WH, Ronnie TP, Cindy ST et al (2004) Clinical significance of serum vascular cell adhesion molecule-1 levels in patients with hepatocellular carcinoma. World J Gastroenterol 10:2014–2018

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Original Article

A Phase I/II Study of Combined Chemotherapy with Mitoxantrone and Uracil/Tegafur for Advanced Hepatocellular Carcinoma

Eiichiro Suzuki[1,2,*], Junji Furuse[2], Masafumi Ikeda[1], Hiroshi Ishii[3], Takuji Okusaka[4], Kohei Nakachi[1], Shuichi Mitsunaga[1], Hideki Ueno[4] and Chigusa Morizane[4]

[1]Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital East, Chiba, [2]Department of Internal Medicine, Medical Oncology, Kyorin University School of Medicine, [3]Hepatobiliary and Pancreatic Section, Gastroenterological Division, Cancer Institute Hospital and [4]Hepatobiliary and Pancreatic Oncology Division, National Cancer Center Hospital, Tokyo, Japan

*For reprints and all correspondence: Eiichiro Suzuki, Department of Internal Medicine, Medical Oncology, Kyorin University School of Medicine, 6-20-2, Shinkawa, Mitaka, Tokyo 181-8611, Japan. E-mail: eisuzuki@ks.kyorin-u.ac.jp

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Objective: The aim was to determine the recommended dose of combined chemotherapy with mitoxantrone and uracil/tegafur (Phase I part) and to clarify its efficacy and safety in patients with advanced hepatocellular carcinoma at the recommended dose (Phase II part).
Methods: Patients eligible had histologically confirmed, chemo-naive advanced hepatocellular carcinoma and were amenable to established forms of treatment. The therapy consisted of mitoxantrone administered intravenously at one of three dosages (6, 8 and 10 mg/m^2/day) on day 1 and uracil/tegafur administered orally at 300 mg/m^2 from day 1 through day 21. The treatment was repeated every 4 weeks until evidence of tumor progression or unacceptable toxicity.
Results: A total of 25 patients were enrolled. In the Phase I part, dose-limiting toxicities occurred in all three patients, given mitoxantrone at the dosage of 10 mg/m^2/day, and the recommended mitoxantrone dosage was determined to be 8 mg/m^2/day. Among 19 patients administered the drug at the recommended dosage, 1 patient (5.3%) showed partial response, 8 patients (42.1%) showed stable disease and 10 patients (52.6%) showed progressive disease. The median survival and median progression-free survival were 8.4 and 2.5 months, respectively. The most common toxicities were Grade 3–4 leukopenia (63.2%) and neutropenia (68.4%).
Conclusions: Mitoxantrone at 8 mg/m^2 combined with uracil/tegafur at 300 mg/m^2/day was determined to be the recommended regimen. Although this regimen was generally well tolerated, it appeared to have little activity against advanced hepatocellular carcinoma. These findings do not support the use of this combination regimen in practice.

Key words: hepatocellular carcinoma – chemotherapy Phase I/II – mitoxantrone – uracil/tegafur

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most commonly occurring cancers worldwide (1,2). Surgical resection, liver transplantation and local ablation therapy, including radiofrequency ablation and ethanol injection, are considered as curative treatment for HCC (3). Transcatheter arterial chemoembolization (TACE) has been applied to patients with advanced incurable HCC (4,5). However, the majority of HCC patients develop recurrence or metastasis, regardless of the treatment modalities employed. Although patients with HCC at this advanced stage are generally treated by systemic therapy, the prognosis remains poor (6,7). Sorafenib

is an orally administered molecular-targeted drug that targets tumor cell proliferation and tumor angiogenesis by inhibiting the serine–threonine kinases Raf-1 and B-Raf and the receptor tyrosine kinase activity of vascular endothelial growth factor receptors 1, 2 and 3 and platelet-derived growth factor receptor β. This drug was reported to confer an overall survival advantage, with manageable toxicity, in comparison with placebo in a Phase III trial, and it has been accepted worldwide as the first-line chemotherapy for advanced HCC (8). But the advantage is modest. There is urgent need to develop more effective regimens.

5-Fluorouracil (5-FU) has been widely used for the treatment of various gastrointestinal malignancies, including advanced HCC (9,10). A high level of efficacy can be expected when the drug is given as a continuous intravenous infusion (11). However, this would necessitate a permanent intravenous access. Uracil/tegafur (UFT) is an orally administered drug which is a mixture of uracil and tegafur at a molar ratio of 4:1. Tegafur is a prodrug of 5-FU that is hydroxylated and converted to 5-FU by hepatic microsomal enzymes, and uracil prevents the degradation of 5-FU by inhibiting the enzyme dihydropyrimidine dehydrogenase, which results in an increased level of 5-FU in the plasma and tumor tissues (12,13). UFT has been reported to be as effective as intravenous 5-FU for the treatment of malignancies (14,15) and to be effective for the treatment of advanced HCC (16,17).

The therapeutic usefulness of doxorubicin in patients with advanced HCC has also been widely explored since the 1970s. A randomized trial in which doxorubicin was compared with supportive care alone for advanced HCC showed a significant survival benefit in the doxorubicin arm. However, treatment with this drug has not been accepted as a standard chemotherapy because of the high rate of fatal complications reported (18). Mitoxantrone, another anthracycline, has shown similar antitumor activity to that of doxorubicin in both human tumor cell lines and animal models of leukemia and has fewer myelotoxic and cardiotoxic effects than doxorubicin (19). Clinical trials of mitoxantrone have also demonstrated moderate activity against HCC, with a low incidence rate of adverse effects (20,21).

Combination chemotherapeutic regimens composed of a fluoropyrimidine and an anthracycline antibiotic have been reported to show moderate efficacy against HCC with tolerable toxicity (22–24), but combined chemotherapy with UFT and mitoxantrone has not yet been examined. We conducted Phase I/II studies to determine the recommended dosage of the combination of UFT with mitoxantrone (UFM regimen) and to clarify the efficacy and safety when administered at the recommended dose in patients with advanced HCC.

PATIENTS AND METHODS

Eligibility Criteria

The eligibility criteria for study enrolment were: (i) patients with histologically confirmed HCC, who were (ii) unsuitable for surgical resection, local ablation therapy or TACE, (iii) were ≥20 years old, (iv) had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2, (v) had adequate bone marrow function (white blood cell ≥3000 cells/mm^3, absolute neutrophil count ≥1500 cells/mm^3, platelet count ≥70 000 cells/mm^3 and hemoglobin ≥8.0 g/dl), renal function [serum creatinine concentration ≤upper limit of normal (ULN)] and hepatic function [serum albumin level ≥3.0 mg/dl, total bilirubin level ≤3.0 mg/dl, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels ≤5.0 × ULN], (vi) had a life expectancy of at least 12 weeks and (vii) provided written informed consent from each patient.

The exclusion criteria were: clinically evident congestive heart failure, serious cardiac arrhythmia, active or symptomatic coronary artery disease or ischemia, clinically serious infection, seizure disorder requiring medication, prior malignancy (any cancer treated curatively was permitted), clinically evident brain or meningeal metastasis, and pregnant/lactating women. This protocol was approved by the Institutional Review Board for clinical investigation of the National Cancer Center, in conformity with the provisions of the Declaration of Helsinki, Good Clinical Practice guidelines, and local laws and regulations.

Study Treatment

UFT was administered orally at the dose of 300 mg/m^2 per day in two divided doses for 21 consecutive days, followed by a rest period of 7 days (400 mg/body per day in patients with a body surface area of <1.50 m^2 and 500 mg/body/day in patients with a body surface area of ≥1.50 m^2). Mitoxantrone was given as a 60 min intravenous infusion on day 1. This cycle was repeated every 28 days. Patients continued to receive additional courses of this regimen until a cumulative dose of mitoxantrone of 100 mg/m^2, evidence of disease progression or the appearance of unacceptable toxicity.

Phase I Part

The objectives of the Phase I study were to investigate the frequency of dose-limiting toxicity (DLT) and to determine the recommended dose of mitoxantrone and UFT. The criteria of DLT included: Grade 4 leukopenia or neutropenia, Grade 3 neutropenia accompanied by fever (≥38°C) or infection (clinically or biologically confirmed), thrombocytopenia <25 000/mm^3 or necessity of transfusion, Grade 3 or 4 non-hematological toxicity (except nausea/vomiting, anorexia, fatigue and hyperglycemia), AST and ALT >10 times the ULN, suspension of UFT administration for over 3 successive weeks, or an over 6-week delay in the commencement of the next treatment cycle.

Three possible dosage levels of mitoxantrone (Level 1: 6 mg/m^2/day, Level 2: 8 mg/m^2/day and Level 3: 10 mg/m^2/day) were assigned for the Phase I part (Table 1). The first

Table 1. Dose-escalation schedules of mitoxantrone and uracil/tegafur

Dose level	Mitoxantrone (mg/m^2)	UFT (mg/m^2)	Number of patients enrolled
1	6	300	3
2	8	300	6
3	10	300	3

UFT, uracil/tegafur.

patient to enter the study was started at Level 1. At least three patients were treated at this level and observed for DLT. Dose escalation was continued until at least one-third of the patients in a given cohort showed DLT. If none of the first three treated patients developed DLT during the first cycle at a specific dose level, the dose escalation was continued. If one of the first three treated patients developed DLT at any dose level, three additional patients were entered at the same dose level; if only one or two of six patients at a given level experienced a DLT, the dose escalation was continued. The maximum tolerated dose (MTD) was defined as the dose level at which one-third or more of the patients experienced a DLT. The recommended dose for the Phase II study was defined as the dose level preceding the attainment of the MTD.

Phase II Part

The primary endpoint of the Phase II part was the objective response rate. The secondary endpoints were the overall survival, progression-free survival and the frequency and severity of adverse events. The Phase II part was begun after determination of the recommended dosage from the Phase I part.

Assessment of the Response and Toxicity

Physical examination including cardiac symptoms, complete blood cell counts, serum chemistries and urinalysis was performed at the baseline and at least once every 2 weeks after the start of the treatment. Dynamic computed tomography or magnetic resonance imaging was undertaken to evaluate the response at 4- to 6-week intervals after the start of treatment. Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors (25). Toxicity was graded according to the National Cancer Institute common toxicity criteria, version 2.0. Progression-free survival was calculated from the first day of treatment to the appearance of evidence of tumor progression, clinical progression or last date of follow-up. The overall survival was calculated from the first day of treatment until death due to any cause or date of last follow-up. Survival data were analyzed using the Kaplan–Meier method.

Statistical Analysis

In the Phase II part, the primary endpoint was the response rate, and data from at least 19 patients were accrued. The threshold response rate was set at 5% and the expected response rate at 15%. If no responses were observed in the 19 patients and the upper limit of the 90% confidence interval (CI) did not exceed the expected rate of 15%, the UFM regimen was judged to have no activity against HCC. If response was confirmed in one or more of the 19 patients, the decision of whether or not to proceed to a further study using the UFM regimen was taken on the basis of other factors, such as the safety and rate of response, overall survival and time to progression in this study.

RESULTS

Patients

From April 2004 to April 2007, 25 patients were registered for the present study: 12 patients completed the Phase I part (Level 1: 3 patients, Level 2: 6 patients and Level 3: 3 patients). Nineteen patients who received the recommended dose (6 patients received this dose during the Phase I part) were analyzed during the Phase II part. Table 2 shows the baseline characteristics of the patients in the Phase I and Phase II parts of the study of the UFM regimen. There were 19 males and 6 females with a median age of 67 years. All the patients had a good ECOG PS score of 0–1. There were 21 (84%) and 4 (16%) patients with the Child–Pugh Stages A and B, respectively. Thirteen (68%) patients had extrahepatic metastasis, and the major sites of metastasis were lymph node [$n = 7$ (28%)] and lung [$n = 6$ (24%)].

Treatments

In the Phase I part, there was no occurrence of DLT at the Level 1 and Level 2 doses, but all of the three patients who received the Level 3 dose experienced DLT; two of these patients developed Grade 4 neutropenia and one patient developed Grade 3 creatinine elevation. The additional three patients at the Level 2 dose did not experience any DLT. Therefore, Level 3 was considered as the MTD and Level 2 (UFT 300 mg/m^2 and mitoxantrone 8 mg/m^2) as the recommended dose for the Phase II part.

At the recommended dosage level, a total of 69 courses of the UFM regimen were administered with a median of three courses to each patient (range, 1–8 courses). The dose intensity was 98.9% of the planned dosage for mitoxantrone and 97.9% for UFT.

The reasons for treatment discontinuation in the Phase I and Phase II parts were disease progression in 19 patients, liver dysfunction in 1 patient, DLT according to this protocol in 3 patients during the Phase I part and an over 6-week delay in the start of the next course because of the development of leukopenia in 2 patients. After abandoning the UFM

Table 2. Profile of hepatocellular carcinoma patients population

	Phase I	Phase II
No. of patients	12	19
Gender		
Male	9	14
Female	3	5
Age (years)		
Median	63	67
Range	56–78	56–77
Performance status		
0	11	7
1	1	12
Viral marker		
Hepatitis C antibody+	7	7
Hepatitis B antigen+	2	5
Previous treatment		
Surgical resection	4	10
Percutaneous ablation therapy	3	3
Transcatheter arterial chemoembolization	5	8
Transcatheter arterial infusion	3	5
Radiation therapy	1	2
None	3	3
Child–Pugh classification		
A	8	17
B	4	2
UICC tumor stage[a]		
III	4	6
IVa	3	1
IVb	5	12
Portal vein tumor thrombosis		
(+)	5	4
Extrahepatic metastasis		
Lymph node	5	7
Lung	0	6
Bone	0	3
Adrenal gland	0	1
Peritoneum	0	1
None	7	6

[a]The International Union Against Cancer, 6th edition.

regimen, 10 patients received the second-line treatment. Five patients received systemic chemotherapy, one patient received UFT alone and four patients received a combined chemotherapy with UFT and doxorubicin. Two patients received transcatheter arterial infusion with cisplatin, one patient received salvage TACE because of HCC rupture during the follow-up period, one patient received salvage radiofrequency ablation because of rapid growth of HCC that needed control and one patient received immnunotherapy.

Table 3. Toxicity

Toxicity grade	Phase I part									Phase II part		
	Level 1 ($n = 3$)			Level 2 ($n = 6$)			Level 3 ($n = 3$)			Level 2 ($n = 19$)		
	1–2	3	4	1–2	3	4	1–2	3	4	1–2	3	4
Hematological toxicity												
Leukopenia	2	1	0	0	2	0	0	1	1	4	9	3
Neutropenia	0	1	0	0	2	0	0	0	2	4	11	2
Thrombocytopenia	1	1	0	0	0	0	1	0	0	4	1	0
Anemia	0	0	0	1	0	0	0	0	0	1	0	0
Non-hematological toxicity												
Nausea	3	0	0	0	0	0	2	0	0	3	0	0
Anorexia	0	0	0	2	0	0	1	0	0	3	0	0
Elevated bilirubin	2	0	0	0	1	0	1	0	0	6	0	0
Hypoalbuminemia	1	0	0	0	0	0	0	0	0	1	0	0
Fatigue	0	0	0	0	0	0	1	0	0	1	0	0
Hyperpigmentation	0	0	0	0	0	0	0	0	0	1	0	0
Constipation	0	0	0	0	0	0	0	0	0	1	0	0
Elevated creatinine	0	0	0	0	0	0	0	1	0	0	0	0
Elevated AST	0	0	0	1	0	0	0	0	0	2	1	1[a]
Elevated ALT	0	0	0	1	0	0	0	0	0	1	2	1[a]
Liver dysfunction	0	0	0	0	0	0	0	0	0	0	0	1[a]

AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[a]Death related to adverse event.

Toxicity

Table 3 summarizes the toxicities observed in the patients. At the recommended dose (level 2), the major Grade 3–4 hematological toxicities were leukopenia (63.2%) and neutropenia (68.4%). The most common non-hematological toxicities were elevated serum total bilirubin level (31.6%), elevated AST level (26.3%), elevated ALP level (26.3%) and anorexia (21.1%); however, no Grade 3–4 non-hematological toxicities were observed. One patient died of hepatic failure due to hepatitis B virus (HBV) reactivation.

Efficacy

Of the 19 patients who were administered the recommended dosage, 18 died during the follow-up period. All of the 19 patients administered the recommended dosage were evaluable for tumor response; of these, 1 patient achieved partial response (PR), with an overall response rate of 5.3% (95% CI, 0.0–26.0%). Eight patients (42.1%) had stable disease

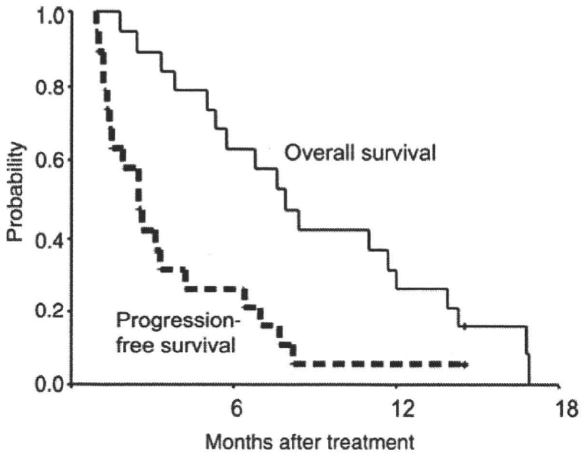

Figure 1. Overall survival and progression-free survival in 19 patients at the recommended dose. Tick marks indicate censored cases.

and 10 patients (52.6%) had progressive disease. The 1-year survival rate, median overall survival, median progression-free survival and time to progression were 26.3%, 8.4 months (95% CI, 5.4–11.4) and 2.5 months (95% CI, 1.5–3.5), respectively (Fig. 1).

DISCUSSION

Systemic chemotherapy for unresectable HCC is recognized as an important treatment modality, because some patients who have recurrent or very advanced disease are not suitable candidates for effective local treatments such as surgical resection, liver transplantation, local ablation therapy and TACE. Many patients with HCC have underlying chronic liver disease and impaired hepatic function, increasing the toxicity of standard doses of many chemotherapeutic agents and causing difficulty in delivering combination chemotherapies. The results, in terms of the therapeutic efficacy, of investigation of cytotoxic agents for advanced HCC have been disappointing, with few agents have yielded response rates of over 20%, and no cytotoxic agents have produced convincing survival benefits in the Phase III setting (26–28).

In Japan, only five anticancer agents, UFT, adriamycin, cytarabine, mitomycin and 5-FU, had been approved for the systemic chemotherapy of HCC by the Ministry of Health, Labor and Welfare of Japan before sorafenib has been approved. Among these drugs, the results of multiagent regimens containing both a fluoropyrimidine and an anthracycline antibiotic have shown favorable results for advanced HCC (22–24). Thus, it was expected that the combination of mitoxantrone and UFT (UFM regimen) would have effective anticancer activity, and we conducted a Phase I/II study to evaluate this regimen.

In the Phase I part, we determined the recommended dose of mitoxantrone as 8 mg/m^2 on day 1 and of UFT as 300 mg/m^2 from days 1 to 21 of a 28-day cycle. The DLTs observed at Level 3 were Grade 4 neutropenia (two patients) and Grade 3 creatinine elevation (one patient).

Patients with HCC tend to experience more severe myelosuppression and hepatic toxicity than those with other malignant diseases, because most have underlying cirrhosis, which is usually associated with compromised hepatic function, leukopenia and thrombocytopenia (24). In 19 patients treated at the recommended dose level, the most frequently encountered toxicities were leukopenia and neutropenia, which are well-known toxicities of the two drugs. When compared with that in trial of mitoxantrone or UFT for other malignancies, Grade 3 or 4 hematological toxicities occurred more frequently (29–31). However, these toxicities were reversible and generally well tolerated in patients with advanced HCC, except for one case of treatment-related death; this patient developed hepatic failure due to HBV reactivation, because no antiviral drug for HBV infection, such as lamivudine or entecavir, was given. This is a well-recognized complication in patients with HBV infection who received immunosuppressive therapy or chemotherapeutic agents (32,33). Thus, patients with HBV infection should receive prophylactic antiviral treatment before chemotherapy.

In the current study, 1 of the 19 patients showed a PR (response rate, 5.3%). However, the rate of progressive disease was 52.6%. In addition, the result of median time to progression was only 2.5 months. Those results were unfavorable when compared with those reported from other clinical trials (8,21–23). Therefore, this regimen is considered to be ineffective and cannot be recommended for use in clinical practice. There were several reasons for this negative result. One of the reasons was the number of anticancer drugs in the regimen. A regimen containing two drugs may have little activity, and three or more drugs may be needed to obtain activity against HCC, because many of the regimens that have been shown to exert anticancer effect against HCC contain three or more drugs. The other reason was the recommended doses of the drugs in this regimen. We set the criteria of DLT which had included Grade 4 neutropenia or leukopenia. Two patients experienced DLT based on these criteria. However, both recovered soon, with only observation. Therefore, the criteria may be too strict, although the two drugs have been used at these recommended doses for other malignancies. It may be possible to set higher dose levels to obtain higher antitumor effect.

Recently, increasing knowledge of the molecular pathogenesis of HCC as well as the introduction of molecular-targeted therapies has created an encouraging trend in the management of HCC. Combination regimens consisting of molecular-targeted agents such as sorafenib and cytotoxic agents have been reported as promising regimens for patients with advanced HCC and other malignancies (34–37). The UFM regimen itself has little antitumor activity, but the result may be useful in the setting of future clinical trials of cytotoxic agents used in combination with molecular-targeted agents.

In conclusion, the recommended dose was mitoxantrone at 8 mg/m^2 and UFT at 300 mg/m^2/day. A combined chemotherapy with mitoxantrone and UFT appeared to show