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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.

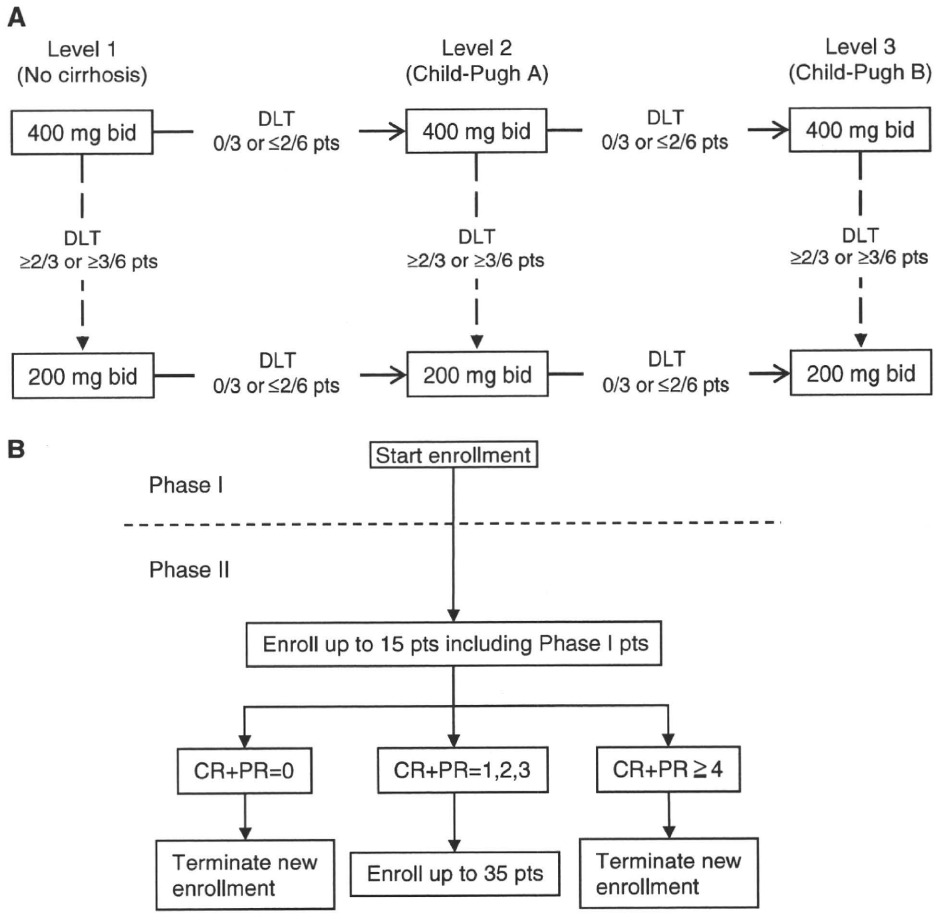

Fig. 1 TSU-68 phase I/II study schema. **a** In phase I, patients were stratified into three groups based on hepatic function, and the toxicity and pharmacokinetics were assessed from Level 1 (no cirrhosis) to Level 3 (Child–Pugh B) by enrolling three patients at each level. *Bid* twice daily, *DLT*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

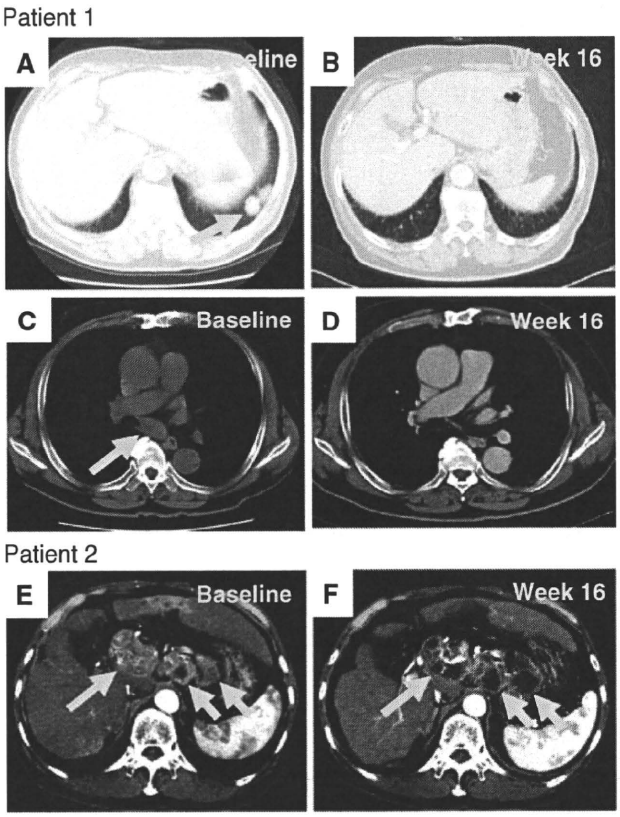

Fig. 2 Computed tomography images of responding lesions from patient 1, who achieved a complete response. Metastatic lesions in the lung (**a**) and lymph node (**c**) disappeared after four cycles (16 weeks) of TSU-68 treatment (**b**, **d**). Representative computed tomography images of a tumor showing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

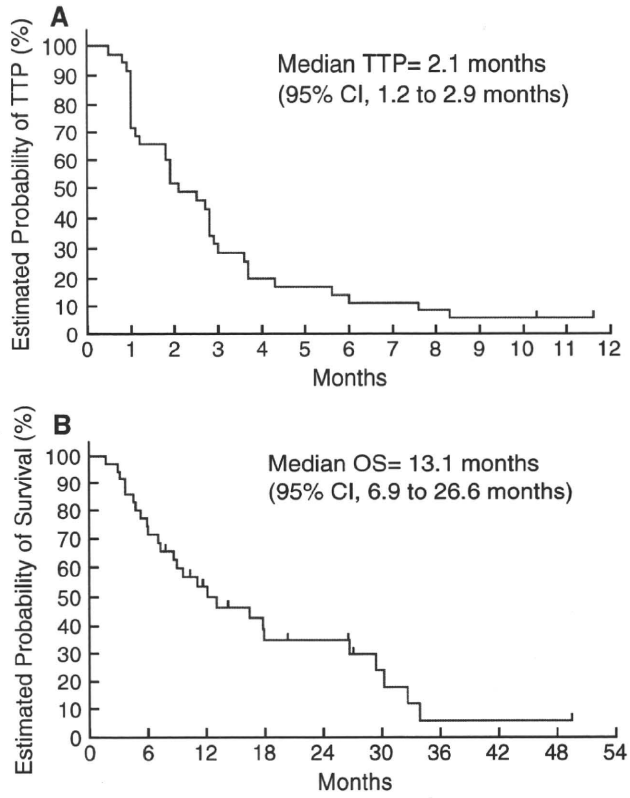

Fig. 3 **a** The independently assessed median time to progression in all 35 patients treated with TSU-68 was 2.1 months. **b** The investigator-assessed median overall survival in all 35 patients treated with TSU-68 was 13.1 months

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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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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REVIEW

Recent advances of radiofrequency ablation for early hepatocellular carcinoma

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Key words
Hepatocellular carcinoma, radiofrequency ablation, seeding, transarterial chemoembolization, CLIP, BCLC, JIS, des-gamma-crboxy prothrombin time (DCP), alpha-fetoprotein (AFP).

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Abstract

Hepatocellular carcinoma (HCC) is the third leading cause of death in the malignant neoplastic diseases in the world. Surgical operation is sometimes not indicated because of complicated liver cirrhosis and extrahepatic disorders. Radiofrequency ablation has been developed as a less invasive treatment for HCC since 1999, and long-term outcome has been shown. There are several complications which should be paid attention, and to improve the prognosis, combination treatment with transarterial chemoembolization should be discussed. Overall survival after between RFA and surgical resection should be compared prospectively. Establishment of staging system for treatment allocation of HCC and prevention of HCC recurrence is important issue to be examined.

Introduction

Radiofrequency ablation (RFA) has been utilized as a less invasive and curative treatment for the treatment of hepatocellular carcinoma (HCC), and the methods and procedure have been developed. In some countries, it has been chosen as first line treatment for early stage HCC. Long-term prognosis has been reported and the associated factors for the prognosis after RFA have been shown. Several complications were reported after RFA. The prognosis was compared between patients who were treated by between surgical resection and those treated by RFA. The recent developments and future perspective of RFA is discussed in this review.

Radiofrequency ablation method

Of all therapeutic apparatus compared and evaluated up to now, the RF 3000 generator system (Boston Scientific, Boston, USA) had the most positive therapeutic effects.[1] However, in many articles, an internally cooled single electrode was used.[2] When there was a risk of RFA incurred by the hepatocellular carcinoma (HCC) location, the therapeutic effects were reduced, in particular the complete response rate was low in the vicinity of the gall bladder and the stomach and intestine, as well as the diaphragm, and in the vicinity of large blood vessels.[3] However, it has been reported that, although the therapeutic effects are not reduced when tumors exist in the vicinity of large blood vessels or adjacent to the extrahepatic organs, attention should be paid to the prevention and control of complications.[4] RFA with the use of artificial ascites for HCC adjacent to the diaphragm and to the stomach and intestine produced sufficient therapeutic effects, thereby improving the sonic window.[5]

When performing RFA, the use of a guiding needle with an external insulated sheath was useful because it allowed for precise tumor targeting.[6] The use of laparoscopic RFA has allowed a sufficient therapeutic effectiveness to achieve complete tumor ablation in all cases when the HCC nodule is located with bulging or at subcapsular area, as well as an adequate safety margin, compared to percutaneous RFA.[7] As shown in Fig. 1, extra-hepatic protruding HCC nodule is the most appropriate indication for laparoscopic RFA, and complete necrosis could be achieved after one treatment session under laparoscopic ultrasound guiding. When RFA was performed under laparoscopy, complete necrosis is usually observed.[8]

Assessment of the therapeutic effect of RFA

Although the effect of RFA is, in general, evaluated by dynamic computed tomography (CT) scans taken 1 to 7 days after the procedure, it was possible to assess the therapeutic effect by multidetector row helical CT (MD-CT) immediately after RFA,

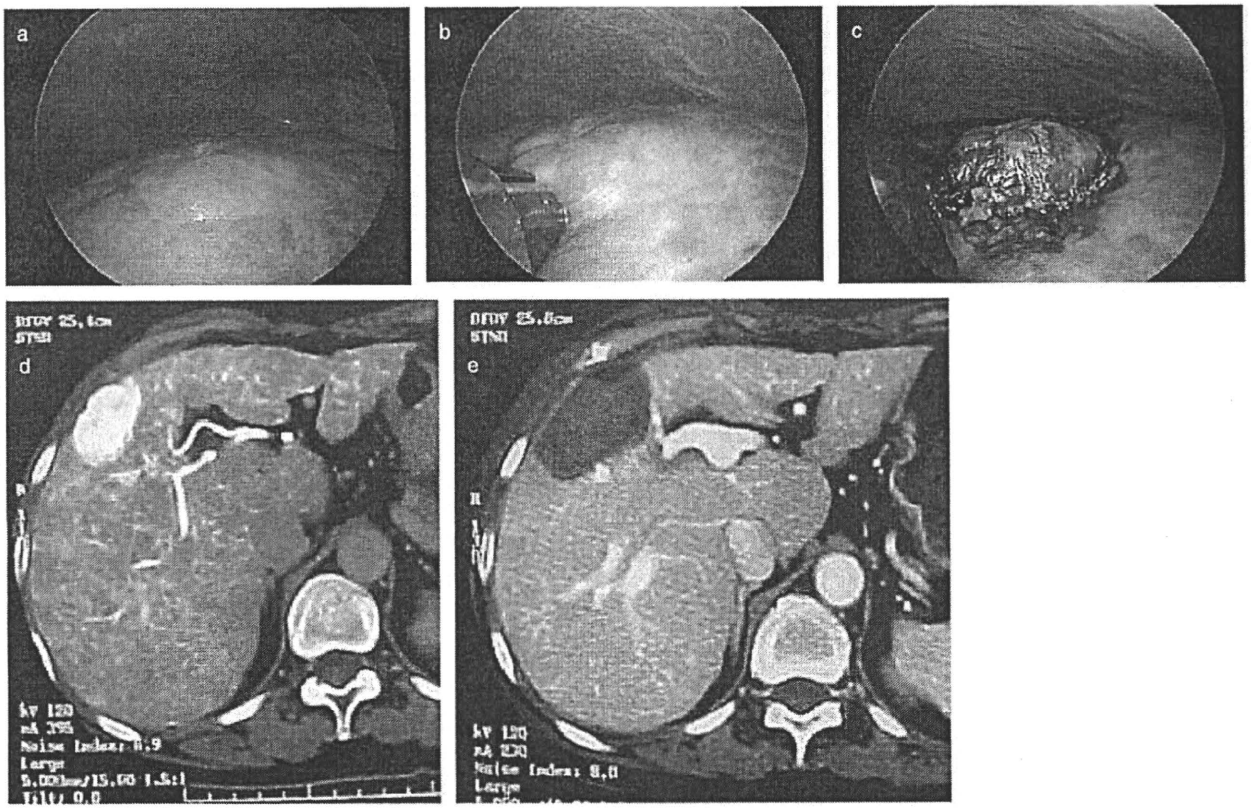

Figure 1 Hepatocellular carcinoma (HCC) nodule protruding from the liver surface is treated by laparoscopic radiofrequency ablation (RFA) under ultrasound guiding. (a) HCC nodule is directly observed under laparoscopy. (b) Under laparoscopic ultrasound guiding, RFA electrode is introduced to accurate position of the nodule, avoiding damage to diaphragm and intrahepatic vessels. (c) The entire HCC nodule was completely ablated by RFA. (d) Computed tomography (CT) scan before the treatment revealed hypervascular nodule with 2.6 cm in diameter at the surface of the liver. (e) After laparoscopic RFA, complete necrosis was confirmed by CT scan.

thereby achieving shorter hospital stays.[9] As well, one report indicates that it was possible to assess the therapeutic effect by contrast-enhanced ultrasonography immediately after RFA.[10] Contrast-enhanced sonography with abdominal virtual sonography was useful in monitoring the therapeutic effect and reducing the CT scan frequency.[11]

Prognosis after radiofrequency ablation

According to a report from a single institution in France, RFA was performed in 235 cases, with up to three HCC ≤ 5 cm in diameter, and achieved complete ablation in 222 cases. 67 cases were judged potentially resectable according to Barcelona Clinical Liver Cancer (BCLC) criteria; in these patients, RFA treatment produced 76% survival at 5 years. The factors contributing to survival were the prothrombin time and serum alpha-feto protein (AFP) levels. Conversely, the factors related to recurrence were multinodular tumors as well as the AFP level. In this report, RFA could be used as an effective first-line treatment in patients with a single nodule of 5 cm or less, a low serum AFP level, and well preserved liver function.[12]

According to a report from Italy, RFA was performed in 218 cases of single nodule HCC, measuring 2 cm or less in diameter, followed by an analysis of the prognosis. The 5-year survival rate was 68.5%, with a low 1.8% incidence of complications. Compared with resection, it was less invasive and could be conducted at a lower cost. It could therefore be considered the treatment of choice for resectable single HCC ≤ 2.0 cm.[13] In Japan, the prognosis of 1000 patients who had undergone RFA was analyzed; the 1, 3, and 5-year survival rates were 94.7%, 77.7% and 54.3%, respectively.[2] According to a report from China, the factors related to the prognosis after RFA were the tumor diameter, the number of tumors, the use of combination therapy with ethanol injection, the margin, and the Child-Pugh score.[14] According to the outcome of RFA treatment for a large single-institution series in Korea, the method had a local recurrence rate of 8.1% at 1 year and 11.8% at 3 years, and patient survival rates were 95.2% at 1 year, 69.5% at 3 years, and 58.0% at 5 years.[15] The five year survival after RFA was similar between Western and Eastern countries (Table 1).

Prognosis after RFA and the staging system

The Cancer of the Liver Italian Program (CLIP) score and BCLC scoring system more accurately predicted the prognosis than the Okuda score in patients with early-intermediate HCC, undergoing

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Table 1 5-year overall survival after radiofrequency ablation in the patients with operable HCC nodule

			Overall survival	
Investigator	Diameter of the nodule	Patient number	3 y	5y
N'Kontchou G[16]	≤ 5 cm	235		76%
Livraghi T[17]	≤ 2 cm	218		68.5%
Tateichi R[18]	≤ 3 cm	1000	77.7%	54.3%
Peng ZW[19]	≤ 5 cm	281	57.1%	37.1%
	≤ 3 cm		65.7%	58.6%
Choi D[20]		570	69.5%	58.0%

non-surgical therapy, such as RFA.[21] The results of an analysis in Japan demonstrated that, regardless of the CLIP score, the combination of transarterial chemoembolization (TACE)—RFA had the highest 5-year survival.[22] In Japan, where early-stage HCC is prevalent, the majority of cases are classified into CLIP stage 1 of CLIP scores and, as such, the Japanese integrated staging (JIS) score was proposed as a new early HCC staging system.[23] The results of the validation done in many cases demonstrated that the JIS score yielded a better prediction of the prognosis than the CLIP score.[24] It has also been reported from in Korea that the JIS score is the most appropriate score for predicting the prognosis.[25]

Tumor markers

The tumor marker relevant to the prognosis after RFA is des-gamma-carboxy prothrombin time (DCP) levels; wherein, high DCP levels predicted a poor prognosis after RFA.[16,17] However, the same institution also reported that a comparison of AFP, DCP and AFP-leptin 3 (AFP-L3) demonstrated that AFP-L3 was the most useful indicator of the overall survival and disease-free survival.[18] It was pointed out that the AFP mRNA levels in the blood after RFA are also an objective index of recurrence.[19] On the other hand, blood vascular endothelial growth factor (VEGF) levels have also been reported to be related to the prognosis.[20]

Recurrence

Local tumor recurrence after RFA is 9.0% at 1 year and 17.7% at 3 years; therefore, local recurrence is relevant to the prognosis for survival.[26] Evaluation of the therapeutic effects of RFA by contrast enhanced CT scans or by enhanced magnetic resonance imaging (MRI) here demonstrated that the procedure provides good local control and the recurrence rate is low in cases in which the post-ablation margin was 0.4 cm or more and the tumor size was smaller than 2.5 cm.[27] The overall local recurrence rate after RFA was 12.8% and the tumor diameter of >2.5 cm was a significant independent factor.[28] However, another report indicates that even when local recurrence occurred, it did not adversely affect the survival prognosis.[29] Utilizing the RF 3000 generator system has been reported more positive effects than cool-tip electrode.[30]

On the other hand, the cumulative rate of intrahepatic distant recurrence was reported as 10.4% and 77.0% at 1 and 5 years, respectively. In a multivariate analysis, AFP and DCP values, as well as the safety margin, were significant independent factors.[31] The intra-hepatic distant recurrence was associated with multi-nodular lesions and hepatitis C virus (HCV), even after curative ablation was achieved.[32] Recurrence at a distant site is an important, poor prognostic factor.[33] Although it is possible to ensure long-term survival by carrying out repeat RFA after recurrence,[34] the more frequently recurrences occur, the higher the risk for subsequent recurrence becomes.[35] Histological grade is relevant to the therapeutic efficiency of RFA and also plays a part in determining survival.[36]

Prognosis and possible measures to improve survival after RFA

Long-term interferon maintenance therapy improved the survival in patients with HCV related HCC after RFA.[37] On the other hand, the administration of lamivudine after RFA for hepatitis B virus (HBV)-related HCC maintained the liver function and was also safe.[38,39] The administration of vitamin K for HCV-related HCC did not produce a chmenopreventative effect.[40] The oral administration of a branched-chain amino acid after RFA made it possible to maintain the serum albumin levels and it was also useful for improving the liver function.[41]

Resection *versus* RFA

With regard to the question of whether surgical resection or RFA is superior, two randomized comparisons have been reported—all from China. In these reports, the life prognoses of single HCCs of 2 cm or less diameter were randomly compared between RFA and resection. It was reported that there would be no difference between the two, or that, for single HCC of 5 cm or less, there was no difference in terms of both disease-free survival and overall survival.[42,43] In Italy, a group of 109 patients who underwent RFA and a group of 91 patients who underwent resection were compared retrospectively; there was no difference in terms of the overall survival and disease-free survival, for HCC of 3 cm or less.[44] Likewise, a retrospective analysis conducted in Korea, compared a group of 55 patients who underwent RFA treatment for single HCC 4 cm or less and well-preserved liver function with a group of 93 patients who underwent resection; the authors concluded that there was no difference in terms of overall survival and recurrence-free survival at 1 year and 3 years after RFA.[45] When laparoscopic RFA was performed on patients with single HCC nodule with Child-Pugh A liver function, RFA and resection had similar survival rates.[46,47]

However, a case control study of resection versus RFA showed that recurrence, tumor diameter, and whether resection or RFA were performed, all affected overall survival. The authors con-

cluded that a resection provided some advantages.[48] Furthermore, with regard to cases of HCC which are not suitable candidates for liver transplantation, a Markov model was used to compare the life-adjusted survival between resection and RFA. The survival rate in the resection group was 5.33 years, while in the RFA group it was 3.91 years. It was concluded therefore, that patients treated by a resection would have a better survival rates.[49] In another study, 79 cases of resection and 79 cases of RFA treated at two different institutions were compared. The result showed that resection would be better than RFA for tumors of 3 cm and larger in diameter with Child A score, but that the overall survival would be the same for surgery and RFA in the case of Child B score.[50]

Comparison between RFA and other ablations

A comparison between microwave coagulation and RFA for HCC, 2 cm or less in tumor diameter demonstrated that RFA was superior because it created a larger necrotic area, resulting in a lower local recurrence rate; this conferred better cumulative survival, while bile duct injury and pleural effusion occurred less frequently.[51]

Another study compared percutaneous ethanol injection (PEI) and RFA. This randomized controlled trial (RCT) conducted in Taiwan demonstrated that RFA required fewer treatment sessions to achieve complete tumor necrosis, and provided better overall survival.[52] Another RCT between PEI and RFA was conducted in Japan. The 4-year survival rates were 74% for RFA versus 57% for PEI, resulting in RFA treatment being associated with a lower risk of death and recurrence. There was no difference in frequency of adverse events.[53] Although it was not RCT, another study compared PEI and RFA and found that local recurrence rates after RFA were lower.[54] An RCT conducted in Italy compared RFA with PEI and found that complete response of RFA after one year was associated with a better outcome, though no survival advantage was observed.[55]

There have been three meta-analyses, based on RCT comparing the effects and complications between RFA and PEI. Each found that RFA had better overall survival, while PEI had a higher local recurrence rate; thus RFA was superior in cancer-free survival rates.[56-58] No difference was observed in the complications between the two.

RCT was conducted to identify whether a combination of RFA and PEI would produce a better outcome than RFA alone. For tumors measuring between 3.1 cm and 5 cm in size, RFA + PEI improved patient survival, and overall recurrence was lower with combination treatment.[59]

Combined TACE and RFA treatment

The combination of transarterial embolization (TAE) and RFA or PEI was compared with TACE alone, and it was found that TACE + RFA had a better prognosis.[60] The results of a case-control comparison between RFA combined with TACE and RFA alone demonstrated that there was no difference in cases of single HCC ≤ 5 cm, but that the TACE + RFA combined treatment had a higher survival rate in cases of single HCC > 5 cm or multiple tumors.[61] The combination of TACE and RFA was technically successful in 88% of cases; such patients, complete the therapy after a single treatment session.[62] In addition, the combination of TACE and RFA produced high local control rates.[63] TACE and RFA has been performed for HCC immediately below the diaphragm, and found to be effective.[64] The combination of bland arterial embolization with RFA and a resection has also been compared; the overall survival was found to be similar in patients with single HCC measuring up to 7 cm in diameter.[65]

The extent of necrosis resulting from RFA increases when combined with hepatic arterial balloon occlusion.[66] Furthermore, combined treatment with balloon occlusion after transcatheter arterial infusion chemotherapy (TAI) is effective in expanding the necrotic area.[67] However, some researchers argue that this combination is not necessary because the effects of the combined therapy involving TACE and RFA, and that of RFA alone, for small HCC ≤ 3 cm, are the same.[68]

Complications

Data from 3891 cases were collected in a joint study conducted in Osaka, Japan. Complications were observed in 207 cases (7.9%), with 9 patients dying within 3 months. The causes of death in these cases were: liver failure in 3 cases, rapid progression in 3 cases, biliary injury in 1 case, gastrointestinal bleeding in 1 case, and myocardial infarction in 1 case.[69] Data for 255 cases in China have also been reported, with major complications observed in 31 cases (10%) as follows: 13 cases of liver failure, 10 cases of hydrothorax, 2 cases of tumor seeding, 1 case of upper gastrointestinal bleeding, and one each of intrahepatic abscess, bile duct injury, and cardiac arrest, 5 cases of hyperglycemia, and 11 cases of death due to liver failure.[70] A report from the United States noted that complications had been observed in 7 out of 91 cases as follows: 2 cases of hepatic abscess and one each of skin burn, hemorrhage, myocardial infarction, and liver failure.[71] According to the results of a multicenter survey conducted in Korea, liver abscess (0.66%), peritoneal hemorrhage (0.46%), biloma (0.20%), ground pad burn (0.20%) and pneumothorax (0.20%) were reported as complications.[72]

Liver abscess and bile duct injury

Liver abscess is the most common complication—de Raere *et al.* observed 7 cases out of 350 sessions and a high risk of this complication among patients with a revious bilioenteroc anasomosis.[73] Likewise, Choi *et al.*[74] and Elias *et al.*[75] also reported that liver abscess was seen more often in cases of biliary abnormality, as well as after TACE treatment. In one report, cholangitis and liver abscess occurred simultaneously.[76] Attention should therefore be paid to the fact that the risk for liver abscess complication is high in cases of complicated anastomosis of the bile duct to the intestinal tract.

Biliary stricture was observed in 7 cases after the RFA procedure, with liver abscess as a complication in 3 cases.[77] It was reported that intraductal chilled saline perfusion by endoscope had been effective in preventing bile duct injury.[78]

Bleeding

A total of 4133 RFA treatments were performed in 2154 cases, with hemorrhagic complications occurring in 63 treatments (1.5%)

as follows: hemoperitoneum (0.7%), hemothorax (0.3%) and hemobilia (0.5%). In addition, there were two deaths due to hemoperitoneum.[79] Poggi *et al.* reported only one case of bleeding that required surgery.[80] Attention has also been focused on bleeding which occurred in one case of subcapsular liver tumor, but there were no complications such as seeding.[81,82]

Intestinal injury

Two cases were reported in which colonic perforation occurred as a complication on the 8th day after RFA. Attention should be paid to the fact that intestinal injury was indolently present.[83,84] Another report indicated the occurrence of duodenopleural fistula formation as a complication.[85]

Hepatic infarction

Hepatic infarction has been observed after RFA; the frequency is 1.8%.[86] The use of internally cooled electrodes is a risk. In addition, portal thrombosis has also been reported to occur.[73,87]

Seeding

A report given by Llovet *et al.* in 2001 on the high rate of seeding after RFA has received much attention. The risk factors included: subcapsular tumor localization, a high degree of poor differentiation, and a high baseline AFP.[88] Since then the risk of seeding after RFA, has been attiributed to subcapsular location, poorly differentiated tumors and high AFP levels.[89,90] However, a discrepancy exists between institutions, with some arguing that, in reality, seeding is exceptionally rare.[91,92] In order to prevent seeding, tract ablation should therefore be properly performed.

Other complications

In another report, pneumothorax occurred after RFA, and so careful attention is required for tumors adjacent to the diaphragm.[93] It has been reported that myoglobinuria occurs as a complication after RFA and that the serum creatinin level rises, making it necessary for attention to be paid thereto.[94] Another case was reported in which hemolysis occurred, thus inducing hemogeobinuria as a complication.[95] There have also been reports that rapid tumor progression occurred after RFA;[96,97] however, the actual frequency was low and it is therefore necessary to investigate whether or not it was indeed a complication associated with RFA.

We have done 1440 sessions of RFA to patients with early stage HCC from July, 1999 to December 2009. The complications have been analyzed as shown in Table 2. The complication rates were 1.8% and 1.9% when the patients were treated by laparoscopic or percutaneous RFA, respectively.

Conclusion

RFA is promising for improving patient survival with early stage of HCC when performed skillfully to avoid serious complication. To prevent the recurrence of HCC is the most important issue for achieving better survival.

Table 2 Complications by laparoscopic or percutaneous RFA in Musashino Red-Cross hospital (from July, 1999 to December, 2009)

	Laparoscopic RFA (n = 107)	Percutaneous RFA (n = 1333)
Bile duct damage	0 (%)	4 (0.4%)
Liver abscess	1 (0.9%)	4 (0.3%)
Inter-costal arterial injury	0 (0%)	3 (0.3%)
Hemothorax	0 (0%)	4 (0.3%)
Hepatic infarction	0 (0%)	3 (0.3%)
Hepatic dysfunction	0 (0%)	2 (0.3%)
Skin burn	0 (0%)	2 (0.2%)
Subcutaneous hematoma	1 (0.9%)	0 (0%)
Peumothorax	0 (0%)	2 (0.1%)
Gastrointestinal perforation	0 (0)	1 (0.1%)
Total	2 (1.8%)	26 (1.9%)

References

1 Lin SM, Lin CC, Chen WT, Chen YC, Hsu CW. Radiofrequency ablation for hepatocelular carcinoma: a prospective comparison of four radiofrequency devices. *J. Vasc. Interv. Radiol.* 2007; **18**: 1118–25.

2 Tateishi R, Shiina S, Teratani T *et al.* Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. *Cancer* 2005; **103**: 1201–9.

3 Yan K, Chen MH, Yang W *et al.* Radiofrequency ablation of hepatocellular carcinoma: long-term outcome and prognostic factors. *Eur. J. Radiol.* 2008; **67**: 334–47.

4 Teratani T, Yoshida H, Shiina S *et al.* Radiofrequency ablation for hepatocellular carcinoma in so-called high-risk locations. *Hepatology* 2006; **43**: 1101–8.

5 Song I, Rhim H, Lim HK, Kim YS, Choi D. Percutaneous radiofrequency ablation of hepatocellular carcinoma abutting the diaphragm and gastrointestinal tract with the use of artificial ascites: safety and technical efficacy in 143 patients. *Eur. Radiol.* 2009; **19**: 2630–40.

6 De Baere T, Rehim MA, Teriitheau C *et al.* Usefulness of guiding needles for radiofrequency ablative treatment of liver tumors. *Cardiovasc. Intervent. Radiol.* 2006; **29**: 650–4.

7 Hirooka M, Kisaka Y, Uehara T *et al.* Efficacy of laparoscopic radiofrequency ablation for hepatocellular carcinoma to percutaneous radiofrequency ablation with artificial ascites. *Dig. Endosc.* 2009; **21**: 82–6.

8 Asahina Y, Nakanishi Y, Izumi N. Laparoscopic radiofrequency ablation for hepatocellular carcinoma. *Dig. Endosc.* 2009; **21**: 67–72.

9 Norimiya T, Seo Y, Yano Y *et al.* Evaluation of the therapeutic effects using MD-CT immediately after RFA for HCC. *Hepatogastroenterology* 2006; **53**: 558–60.

10 Gallotti A, D'Onofrio M, Ruzzenente A *et al.* Contrast-enhanced ultrasonography (CEUS) immediately after percutaneous ablation of hepatocellular carcinoma. *Radiol. Med.* 2009; **114**: 1094–105.

11 Kisaka Y, Hirooka M, Koizumi Y *et al.* Contrast-enhanced sonography with abdominal virtual sonography in monitoring radiofrequency ablation of hepatocellular carcinoma. *J. Clin. Ultrasound* 2010; **38**: 138–44.

12 N'Kontchou G, Mahamoudi A, Aout M *et al.* Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognoistic factors in 235 Western patients with cirrhosis. *Hepatology* 2009; **50**: 1475–83.

13 Livraghi T, Meloni F, Di Stasi M *et al.* Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: is resection still the treatment of choice? *Hepatology* 2008; **47**: 82–9.
14 Peng ZW, Zhang YJ, Chen MS, Liang HH, Li JQ, Lau WY. Risk factors of survival after percutaneous radiofrequency ablation of hepatocellular carcinoma. *Surg. Oncol.* 2008; **17**: 23–31.
15 Choi D, Lim HK, Rhim H *et al.* Percutaneous radiofrequency ablation for early-stage hepatocellular carcinoma as a first-line treatment: long-term results and prognostic factors in a large single-institution series. *Eur. Radiol.* 2007; **17**: 684–92.
16 Kobayashi M, Ikeda K, Kawamura Y *et al.* High serum des-gamma-carboxy prothrombin level predicts poor prognosis after radiofrequency ablation of hepatocellular carcinoma. *Cancer* 2009; **115**: 571–80.
17 Takahashi S, Kudo M, Chung H *et al.* PIVKA-II is the best prognostic predictor in patients with hepatocellular carcinoma after radiofrequency ablation therapy. *Oncology* 2008; **75S**: 91–8.
18 Ogawa C, Kudo M, Minami Y, Chung H, Kawasaki T. Tumor markers after radiofrequency ablation therapy for hepatocellular carcinoma. *Hepatogastroenterology* 2008; **55**: 1454–7.
19 Du XL, Ma QJ, Wu T, Baqo GQ, Lu JG, Chu YK. Significance of alpha-fetoprotein mRNA level in hepatocellular carcinoma patients treated with radiofrequency ablation. *Hepatobiliary Pancreat. Dis. Int.* 2007; **6**: 172–5.
20 Poon RT, Lau C, Pang R, Ng KK, Yuen J, Fan ST. 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.* 2007; **14**: 1835–45.
21 Grieco A, Pompili M, Camitiniti G *et al.* Prognostic factors for survival in patients with early-intermediate hepatocellular carcinoma undergoing non-surgical therapy: comparison of Okuda, CLIP, and BCLC staging systems in a single Italian centre. *Gut* 2005; **54**: 411–8.
22 Yamagiwa K, Shiraki K, Yamamoto K *et al.* Survival rates according to the cancer of the Italian program score of 345 hepatocellular carcinoma patients after multimodality treatments during a 10-year period in a retrospective study. *J. Gastroenterol. Hepatol.* 2008; **23**: 482–90.
23 Kudo M, Chung H, Osaki Y. Prognostic staging system for hepatocellular carcinoma (CLIP score): its value and limitations, and a proposal for a new staging system, the Japan Integrated Staging Score (JIS score). *J. Gastroenterol.* 2003; **38**: 207–15.
24 Kudo M, Chung H, Haji S *et al.* Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. *Hepatology* 2004; **40**: 1396–405.
25 Lee JH, Han SY, Jo JH *et al.* Prognostic factors for survival in patients with hepatocellular carcinoma after radiofrequency ablation. *Korean J. Gastroenterol.* 2007; **49**: 17–23.
26 Takahashi S, Kudo M, Chung H *et al.* Initial treatment response is essential to improve survival in patients with hepatocllular carcinoma who underwent curative radiofrequency ablation therapy. *Oncology* 2007; **72S**: 98–103.
27 Liu CH, Arellano RS, Uppot RN, Samir AE, Gervais DA, Mueller PR. Radiofrequency ablation of hepatic tumours: effect of post-ablation margin on local tumour progression. *Eur. Radiol.* 2010; **20**: 877–85.
28 Lam VW, Ng KK, Chok KS *et al.* Risk factors and prognostic factors of local recurrence after radiofrequency ablation of hepatocellular carcinoma. *J. Am. Coll. Surg.* 2008; **207**: 20–9.
29 Lam VW, Ng KK, Chok KS *et al.* Imcomplete ablation after radiofrequency ablation of hepatocellular carcinoma: analysis of risk factors and prognostic factors. *Ann. Surg. Oncol.* 2008; **15**: 782–90.
30 Pitton MB, Herber S, Raab P *et al.* Percutaneous radiofrequency ablation of liver tumors using the LeVeen 4 cm array probe. *Rofo* 2003; **175**: 1525–31.
31 Okuwaki Y, Nakazawa T, Shibuya A *et al.* Intrahepatic distant recurrence after radiofrequency ablation for a single small hepatocellular carcinoma: risk factors and patterns. *J. Gastroenterol.* 2008; **43**: 71–8.
32 Izumi N, Asahina Y, Noguchi O *et al.* Risk factors for distant recurrence of hepatocellular carcinoma in the liver after complete coagulation by microwave or radiofrequency ablation. *Cancer* 2001; **91**: 949–56.
33 Ng KK, Poon RT, Lo CM, Yuen J, Tso WK, Fan ST. Analysis of recurrence pattern and its influence on survival outcome after radiofrequency ablation of hepatocellular carcinoma. *J. Gstrointest. Surg.* 2009; **12**: 183–91.
34 Izumi N, Asahina Y, Tsuchiya K *et al.* Repeated radiofrequency ablation for the distant recurrence in the liver in patients with chronic hepatitis C virus infection achieving long-term survival. *Hepatol. Res.* 2007; **37S2**: S254–63.
35 Yamashiki N, Yoshida H, Tateishi R *et al.* Recurrent hepatocellular carcinoma has an increased risk of subsequent recurrence after curative treatment. *J. Gastroenterol. Heapatol.* 2007; **22**: 2155–60.
36 Kim SH, Lim HK, Choi D *et al.* Percutaneous radiofrequency ablation of hepatocellular carcinoma: effect of histologic grade on therapeutic results. *Am. J. Roentgenol.* 2006; **186**: S327–33.
37 Kudo M, Sakaguchi Y, Chung H *et al.* Long-term interferon maintenance therapy improves survival in patients with HCV-related hepatocellular carcinoma after curative radiofrequency ablation. A matched case-control study. *Oncology* 2007; **72S**: 132–8.
38 Kuzuya T, Katano Y, Kumada T *et al.* Efficacy of antiviral therapy with lamivudine after initial treatment for hepatitis B virus-related hepatocellular carcinoma. *J. Gastroenterol. Hepatol.* 2007; **22**: 1929–35.
39 Yoshida H, Yoshida H, Goto E *et al.* Safety and efficacy of lamivudine after radiofrequency ablation in patients with hepatits B viru-related hepatocellular carcinoma. *Hepatol. Int.* 2008; **2**: 89–94.
40 Kakizaki S, Sohara N, Sato K *et al.* Preventive effects of vitamin K on recurrent disease in patients with hepatocellular carcinoma arising from hepatitis C viral infection. *J. Gastroenterol. Hepatol.* 2007; **22**: 518–22.
41 Ishikawa T, Michitaka I, Kamimura H *et al.* Oral branched-chain amino acids administration improves impaired liver dysfunction after radiofrequency ablation therapy for hepatocellular carcinoma. *Hepatogastroenterology* 2009; **56**: 1491–5.
42 Lu MD, Kuang M, Liang LJ *et al.* Surgical resection versus percutaneous thermal ablation for early-stage hepatocellular carcinoma: a randomized clinical trail. *Zhonghua Yi Xue Za Zhi* 2006; **28**: 801–5.
43 Chen MS, Li JQ, Zheng Y *et al.* A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann. Surg.* 2006; **243**: 321–8.
44 Guglielmi A, Ruzzenente A, Valdgegamberi A *et al.* Radiofrequency ablation versus surgical resection for the treatment of hepatocellular carcinoma in cirrhosis. *J. Gastrointest. Surg.* 2008; **12**: 192–8.
45 Hong SN, Lee SY, Choi MS *et al.* Comparing the outcomes of radiofrequency ablation and surgery in patients with a single small hepatocellular carcinoma and well-preserved hepatic function. *J. Clin. Gastroenterol.* 2005; **39**: 247–52.
46 Santambrogio R, Opocher E, Zuin M *et al.* Surgical resection versus laparoscopic radiofrequency ablation in patients with hepatocelllar carcinoma and child-pugh class A liver cirrhosis. *Ann. Surg. Oncol.* 2009; **16**: 3289–98.

47 Montorsi M, Santambrogio R, Bianchi P *et al.* Survival and recurrences after hepatic resection or radiofrequency for hepatocellular carcinoma in cirrhotic patients: a multivariate analysis. *J. Gastrointest. Surg.* 2005; **9**: 62–7.
48 Abu-Hilal M, Primrose JN, Casaril A, McPhail MJ, Pearce NW, Nicoli N. Surgical resection versus radiofrequency ablation in the treatment of small unifocal hepatocellular carcinoma. *J. Gastroenterol. Surg.* 2008; **12**: 1521–6.
49 Molinari M, Helton S. Hepatic resection versus radiofrequency ablation for hepatocellular carcinoma in cirrhotic individuals not candidates for liver transplantation: a Markov model decision analysis. *Am. J. Surg.* 2009; **198**: 396–406.
50 Vivarelli M, Guglielmi A, Ruzzenente A *et al.* Surgical resection versus percutaneous radiofrequency ablation in the treatment of hepatocellular carcinoma on cirrhotic liver. *Ann. Surg.* 2004; **240**: 102–7.
51 Ohmoto K, Yoshioka N, Tomiyama Y *et al.* Radiofrequency ablation versus percutaneous microwave coagulation therapy for small hepatocellular carcinomas: a retrospective comparative study. *Hepatogastroenteology* 2007; **54**: 985–9.
52 Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma ≤ 4 cm. *Gastroenterology* 2004; **127**: 1714–23.
53 Shiina S, Teratani T, Obi S *et al.* A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005; **129**: 122–30.
54 Iwata K, Sohda T, Nishizawa S *et al.* Postoperative recurrence in hepatocellular carcinoma: comparison between percutanepus ethanol injection and radiofrequency ablation. *Hepatol. Res.* 2006; **36**: 143–8.
55 Brunello F, Veltri A, Carucci P *et al.* Radiofrequency ablation versusu ethanol injection for early hepatoclellular carcinoma: a randomized controlled trial. *Scand. J. Gastroenterol.* 2008; **43**: 727–35.
56 Cho YK, Kim JK, Kim MY, Rhim H, Han JK. Systemic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. *Hepatology* 2009; **49**: 453–9.
57 Bouza C, Lopez-Cuadrado T, Alcazar R, Saz-Parkinson Z, Amate JM. Meta-analysis of percutaneous radiofrequency ablation versus ethanol injection in hepatocellular carcinoma. *BMC Gastroenterol.* 2009; **9**: 31.
58 Orlando A, Leandro G, Olivo M, Andriulli A, Cottone M. Radiofrequency thermal ablation vs. Percutaneous ethanol injection for small hepatocellular carcinoma in cirrhosis: meta-analysis of randomized controlled trials. *Am. J. Gastroenterol.* 2009; **104**: 514–24.
59 Zhang YJ, Liang HH, Chen MS *et al.* Hepatocellular carcinoma treated with radiofrequency ablation with or without ethanol injection: a prospective randomized trial. *Radiology* 2007; **244**: 599–607.
60 Kirikoshi H, Saito S, Yoneda M *et al.* Outcome of transarterial chemoembolization monotherapy, and in combination with percutaneous ethanol injection, or radiofrequency ablation therapy for hepatocelllar carcinoma. *Hepatol. Res.* 2009; **39**: 553–62.
61 Peng ZW, Chen MS, Liang HH *et al.* A case-control study comparing percutaneous radiofrequency ablation alone or combined with transcatheter arterial chemoembolization for hepatocellular carcinoma. *Eur. J. Surg. Oncol.* 2010; **36**: 257–63.
62 Gadaleta C, Catino A, Ranieri G *et al.* Single-step therapy-feasibility and safety of simultaneous transarterial chemoembolization and radiofrequency ablation for hepatic malignancies. *In Vivo* 2009; **23**: 813–20.
63 Veltri A, Moretto P, Doriguzzi A, Pagano E, Carrara G, Gandini G. Radiofrequency thermal ablation (RFA) after transarterial chemoembolization (TACE) as a combined therapy for unresectable non-early hepatocellular carcinoma (HCC). *Eur. Radiol.* 2006; **16**: 661–9.
64 Yamakado K, Nakatsuka A, Takaki H *et al.* Subphrenic versus nonsubphrenic hepatocellular carcinoma: combined therapy with chemoembolization and radiofreqeuency ablation. *Am. J. Roentgenol.* 2010; **194**: 530–5.
65 Maluccio M, Covey AM, Gandhi R *et al.* Comparison of survival rates after bland arterilal embolization and ablation versus surgical resection for treating solitary hepatocellular carcinoma up to 7 cm. *J. Vasc. Interv. Radiol.* 2005; **16**: 955–61.
66 Yamazaki T, Kimura T, Kurokawa F *et al.* Percutaneous radiofrequency ablation with cooled electrodes combined with hepatic arterial balloon occlusion in hepatocellular carcinoma. *J. Gastroenterol.* 2005; **40**: 171–8.
67 Shiraishi R, Yamasaki T, Saeki I *et al.* Pilot study of combination therapy with transcatheter arterial infusion chemotherapy using iodized oil and percutaneous radiofrequency ablation during occlusion of hepatic blood flow for hepatocelluar carcinoma. *Am. J. Clin. Oncol.* 2008; **31**: 311–6.
68 Shibata T, Isoda H, Hirokawa Y, Arizono S, Shimada K, Togashi K. Small hepatocellular carcinoma: is radiofrequency ablation combined with transcatheter arterial chemoembolization more effective than radiofrequency ablation alone for treatment? *Radiology* 2009; **252**: 905–13.
69 Kasugai H, Osaki Y, Oka H, Kudo M, Seki T. Severe complications of radiofrequency ablation therapy for hepatocellular carcinoma: an analysis of 3891 ablations in 2614 patients. *Oncology* 2007; **72** (Suppl. 1): 72–5.
70 Kong WT, Zhang WW, Qui YD *et al.* Major complications after radiofrequency ablation for liver tumors: analysis of 255 patients. *World J. Gastroenterol.* 2009; **15**: 2651–6.
71 Wood TF, Rose DM, Chung M, Allegra DP, Foshag LJ, Bilchik AJ. Radiofrequency ablation of 231 unresectable hepatic tumors: indications, limitations, and complications. *Ann. Surg. Oncol.* 2000; **7**: 593–600.
72 Rhim H, Yoon KH, Lee JM *et al.* Major complications after radio-freqeuncy thermal ablation of hepatic tumors: a spectrum of imaging findings. *Radiographics* 2003; **23**: 123–34.
73 De Baere T, Risse O, Kuoch V *et al.* Adverse events during radiofrequency treatment of 582 hepatic tumors. *Am. J. Roentgenol.* 2003; **181**: 695–700.
74 Choi D, Lim HK, Kim MJ *et al.* Liver abscess after percutaneous radiofrequency ablation for hepatocellular carcinomas: frequency and risk factors. *Am. J. Roentgenol.* 2005; **184**: 1860–7.
75 Elias D, Pietroantonio D, Gachot B, Menegon P, Hakime A, De Baere T. Liver abscess after radiofrequency ablation of tumors in patients with a biliary tract procedure. *Gastroenterol. Clin. Biol.* 2006; **30**: 823–7.
76 Shibata T, Yamamoto Y, Yamamoto N *et al.* Cholangitis and liver abscess after percutaneous ablation therapy for liver tumors: incidence and risk factors. *J. Vasc. Interv. Radiol.* 2003; **14**: 1535–42.
77 Jensen MC, van Duijnhoven FH, van Hellegersberg R *et al.* Adverse effects of radiofrequency ablation of liver tumors in the Netherlands. *Br. J. Surg.* 2005; **92**: 1248–54.
78 Ohnishi T, Yasuda I, Nishigaki Y *et al.* Intraductal chilled saline perfusion to prevent bile duct injury during percutaneous radiofrequency ablation for hepatocellular carcinoma. *J. Gastrenterol. Hepatol.* 2008; **23**: 410–5.
79 Goto E, Tateishi R, Shiina S *et al.* Hemorrhagic complications of percutaneous radiofrequency ablation for liver tumors. *J. Clin. Gastroenterol.* 2010; **44**: 374–80.

80 Poggi G, Riccardi A, Quaretti P *et al.* Complications of percutaneous radiofrequency thermal ablation of primary and secondary lesions of the liver. *Anticancer Res.* 2007; **27**: 2911–6.
81 Sartori S, Tombesi P, Macario F *et al.* Subcapsular liver tumors treated with percutaneous radiofrequency ablation: a prospective comparison with nonsubcapsular liver tumors for safety and effectiveness. *Radiology* 2008; **248**: 670–9.
82 Kim YJ, Raman SS, Yu NC, Busuttil RW, Tong M, Lu DS. Radiofrequency ablation of hepatocellular carcinoma: can subcapsular tumors be safely ablated? *Am. J. Roentgenol.* 2008; **190**: 1029–34.
83 Yeung YP, Hui J, Yip WC. Delayed colonic perforation after percutaneous radiofrequency ablation of hepatocellular carcinoma. *Surg. Laparosc. Endosc. Percutan. Tech.* 2007; **17**: 342–4.
84 Meloni MF, Goldberg SN, Moser V, Piazza G, Livraghi T. Colonic perforation and abscess following radiofrequency ablation of hepatoma. *Eur. J. Ultrasound* 2002; **15**: 73–6.
85 Chan FS, Ng KK, Poon RT, Yuen J, Tso WK, Fan ST. Duodenopleural fistula formation after percutaneous radiofrequency ablation for recurrent hepatocellular carcinoma. *Asian J. Surg.* 2007; **30**: 278–82.
86 Kim YS, Rhim H, Lim HK, Choi D, Lee WJ, Kim SH. Hepatic infarction after radiofrequency ablation of hepatocellular carcinoma with an internally cooled electrode. *J. Vasc. Interv. Radiol.* 2007; **18**: 1126–33.
87 Zheng RQ, Kudo M, Inui K *et al.* Transient portal vein thrombosis caused by radiofrequency ablation for hepatocellular carcinoma. *J. Gastroenterol.* 2003; **38**: 101–3.
88 Llovet JM, Vilana R, Bru C *et al.* Increased risk of tumor seeding after percutaneous radiofrequency ablation for single hepatocellular carcinoma. *Hepatology* 2001; **33**: 1124–9.
89 Livraghi T, Lazzaroni S, Meloni F, Solbiati L. Risk of tumour seeding after percutaneous radiofrequency ablation for hepatocellular carcinoma. *Br. J. Surg.* 2005; **92**: 856–8.
90 Imamura J, Tateishi R, Shiina S *et al.* Neoplastic seeding after radiofrequency ablation for hepatocellular carcinoma. *Am. J. Gastroenterol.* 2008; **103**: 3057–62.
91 Giorgia A, Tarantino L, de Stafano G, Coppola C, Ferraioli G. Complications after percutaneous saline-enhanced radiofrequency ablation of liver tumors: 3-year experience with 336 patients at a sigle center. *Am. J. Roentgenol.* 2005; **184**: 207–11.
92 Latteri F, Sandonato L, Di Marco V *et al.* Seeding after radiofreqeuncy ablation of hepatocellular carcinoma in patients with cirrhosis: a prospective study. *Dig. Liver Dis.* 2008; **40**: 684–9.
93 Shankar S, van Sonnenberg E, Silverman SG, Tuncali K, Morrison PR. Management of pneumothorax during percutaneous radiofrequency ablation of a lung tumor: technical note. *J. Thorac. Imaging* 2003; **18**: 106–9.
94 Rodriguez J, Tellioglu G, Siperstein A, Berber E. Myoglobinuria after laparoscopic radiofrequency ablation of liver tumors. *J. Gastrointest. Surg.* 2010; **14**: 664–7.
95 Tsui SL, Lee AK, Lui SK, Poon RT, Fan ST. Acute intraoperative hemolysis and hemoglobinuria during radiofrequency ablation of hepatocellular carcinoma. *Hepatogastroenterology* 2003; **50**: 526–9.
96 Seki T, Tamai T, Ikeda K *et al.* Rapid progression of hepatocellular carcinoma after transcatheter arterial chemoembolization and percutaneous radiofrequency ablation in the primary tumour region. *Eur. J. Gastroenterol. Hepatol.* 2001; **13**: 291–4.
97 Zavaglia C, Corso R, Rampoldi A *et al.* Is percutaneous radiofrequency thermal ablation of hepatocellular carcinoma a safe procedure? *Eur. J. Gastroenterol. Hepatol.* 2008; **20**: 196–201.

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Effect of Aging on Risk for Hepatocellular Carcinoma in Chronic Hepatitis C Virus Infection

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An increase in the aging population is an impending problem. A large cohort study was carried out to determine the influence of aging and other factors on hepatocarcinogenesis in patients treated with interferon. Biopsy-proven 2547 chronic hepatitis C patients registered at our referral center since 1992 were included. Of these, 2166 were treated with interferon-based therapy. Incidences of hepatocellular carcinoma (HCC) associated with interferon were analyzed by Kaplan-Meier and person-years methods for an average follow-up of 7.5 years. Factors associated with HCC risk were determined by Cox proportional hazard analysis. HCC developed in 177 interferon-treated patients. The risk for HCC depended on age at primary biopsy and increased more than 15-fold after 65 years of age. Even when stratified by stage of fibrosis, the cumulative and annual incidences of HCC were significantly higher in older patients than in younger patients ($P < 0.001$) at the same stage of fibrosis, except for cirrhosis. Progression of fibrosis over time was significantly accelerated in older patients. The impact of viral eradication on HCC prevention was less significant in older patients than in younger patients. Multivariate analysis confirmed that age, gender, liver fibrosis, liver steatosis, total cholesterol level, fasting blood sugar level, baseline and postinterferon alpha-fetoprotein level, and virological response to interferon were independent risk factors associated with HCC. Aging was the strongest risk factor for a nonvirological response to interferon-based antiviral therapy. *Conclusion:* Elderly patients are at a higher risk for HCC. Hepatitis C viral eradication had a smaller effect on hepatocarcinogenesis in older patients. Patients should therefore be identified at an earlier age and treatment should be initiated. (HEPATOLOGY 2010;52:518-527)

Primary liver cancer is the third most common cause of cancer mortality worldwide,[1] and hepatocellular carcinoma (HCC) is one of the most frequent primary liver cancers.[2,3] Infection with hepatitis C virus (HCV) is a common cause of chronic hepatitis, which progresses to HCC in many patients.[4] The prevalence of older patients has been increasing in Japan, and this is an impending problem in other countries where viral spread has occurred more recently.[5] The number of Americans older than 65 years is expected to double by the year 2030.[6] In Western Europe, people older than 65 years already constitute 15%-18% of the population[7]; thus, aging patient who is chronically infected with HCV is

Abbreviations: AFP, alpha-fetoprotein; HBc, hepatitis B core; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; SVR, sustained virological response.

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one of the most important issues confronted by physicians.

Viral eradication with interferon-based therapy for chronic hepatitis C has been shown to prevent HCC by studies conducted in Japan and Italy.[8-11] However, this finding is controversial according to another study conducted in Europe and Canada,[12] in which viral eradication did not significantly reduce the risk for HCC in 479 consecutively treated patients. The likelihood of development of HCC among interferon-treated patients is difficult to determine because of the paucity of adequate long-term cohort studies. Moreover, in patients who are treated with interferon the effect of certain factors, including aging, on the risk for HCC remains unclear. Furthermore, the benefit of viral eradication with interferon-based therapy, including pegylated interferon and ribavirin combination therapy, in older patients remains unknown. To further clarify this, we conducted a large-scale, long-term cohort study and analyzed the influence of aging and other host and virological factors in patients treated with interferon.

Patients and Methods

Patients. Consecutive patients (n = 2547) chronically infected with HCV who underwent liver biopsy between 1992 and January 2008 at our referral center were enrolled. Of these, 2166 patients were treated with interferon-based antiviral therapy, whereas 381 patients did not receive interferon treatment (Fig. 1). All patients had histologically proven chronic hepatitis or cirrhosis. HCV infection was proven in all patients by identification of HCV RNA. Patients with a history of HCC, autoimmune hepatitis, or primary biliary cirrhosis were excluded. We also excluded patients who had a history of excessive alcohol consumption (50 g/day) and confirmed alcohol abstinence during follow-up. No patient was positive for hepatitis B surface antigen or antihuman immunodeficiency virus antibody. Written informed consent was obtained from all patients. The study was approved by the Ethical Committee of Musashino Red Cross Hospital in accordance with the Declaration of Helsinki.

Histological Evaluation. A liver biopsy specimen was obtained laparoscopically using 13G needles. When laparoscopy was impossible, ultrasound-guided liver biopsy was performed with 15G needles (n = 254). The mean length of the specimen was 18 mm (range 12-40 mm), and the mean number of portal tracts was 17 (range 8-34). Liver biopsy specimens were scored by board-certified pathologists for stage of fibrosis and grade of inflammatory activity according to the classification of Desmet et al.[13] Additional macroscopic pathological information was obtained from laparoscopic findings. The percentage of steatosis was quantified by determining the average proportion of hepatocytes affected by steatosis. In this study, superimposed nonalcoholic steatohepatitis (NASH) was defined as a central pattern of colocalization of hepatic steatosis and hepatocyte ballooning with pericellular/perisinusoidal fibrosis or Mallory hyaline.

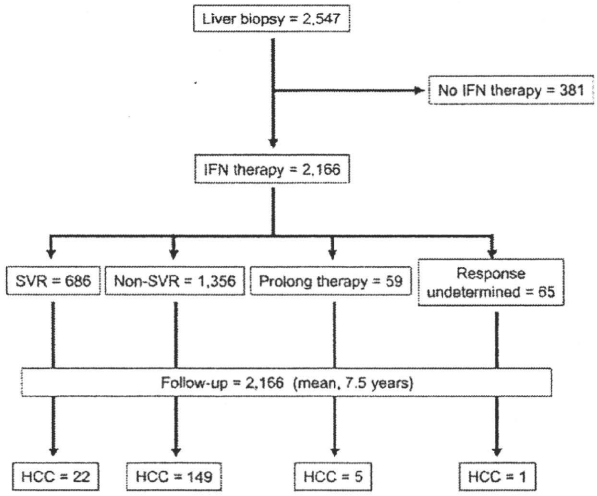

Fig. 1. Clinical outcomes of the patients enrolled in the present study. HCC, hepatocellular carcinoma; SVR, sustained virological response.

Interferon Treatment. Among the 2166 patients treated with interferon-based antiviral therapy, 1062 patients received interferon-alpha or beta monotherapy either for 24 weeks (n = 1003) or for 2 to 5 years (n = 59); 386 patients received interferon-alpha and ribavirin combination therapy for 24 weeks; 306 received pegylated interferon-alpha monotherapy for 48 weeks; and 412 received pegylated interferon-alpha and ribavirin combination therapy for 48 weeks. All interferon treatment was initiated within 48 weeks after liver biopsy.

Definitions of Response to Interferon Therapy. A patient negative for serum HCV RNA after the first 6 months of completion of interferon-based therapy was defined as a sustained viral responder. HCV RNA was determined by the qualitative Amplicor or TaqMan HCV assay (Roche Molecular Diagnostics, Tokyo, Japan).

Data Collection and Patient Follow-up. Data on patient characteristics, biochemical data, hematological