In conclusion, our study suggests that the annual incidence for CKD among cirrhotic patients with HCV was determined to be about 1.0–1.5%. In addition, the annual incidence for end-stage CKD is one order of magnitude lower than that of CKD.

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# Previous Chemoembolization Response after Transcatheter Arterial Chemoembolization (TACE) Can Predict the Anti-Tumor Effect of Subsequent TACE with Miriplatin in Patients with Recurrent Hepatocellular Carcinoma

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**Key Words**
Hepatocellular carcinoma · Miriplatin · Transcatheter arterial chemoembolization

**Abstract**
***Aim:*** The purpose of this retrospective study was to evaluate the efficacy and safety of transcatheter arterial chemoembolization (TACE) with miriplatin in patients with unresectable hepatocellular carcinoma (HCC). ***Methods:*** From 2007 to 2010, 122 consecutive patients with unresectable HCC were treated by TACE with miriplatin-lipiodol suspension in our institute. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. Ninety-eight patients (80%) had a history of TACE. ***Results:*** Thirty-five of the 122 treated patients (29%) showed complete response (CR). And no serious complications were observed. Patients who had shown CR after previous TACE (pre-CR) were significantly more likely to show CR in the current study compared with patients who had shown less successful responses after previous TACE (56 vs. 20%, p = 0.003). Multivariate analysis revealed that response after previous TACE (pre-CR, risk ratio: 4.76; p = 0.035), tumor multiplicity (solitary, risk ratio: 9.69; p = 0.003), and injection artery (peripheral to segmental hepatic artery, risk ratio: 5.28; p = 0.040) were significant independent predictors associated with CR after TACE using miriplatin. ***Conclusion:*** In repetition of TACE treatment, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown a CR after previous TACE.



## Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide [1]. Since it is well known that more than 80% of HCC cases are associated with liver cirrhosis, a routine check-up including ultrasound for cirrhotic patients could potentially lead to the detection of early HCC [2–4]. Since curative therapies, including resection, liver transplantation, and percutaneous ablation (percutaneous ethanol injection and radiofrequency ablation) are applicable in only 30–40% of

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HCC patients, transcatheter arterial chemoembolization (TACE) has been recognized as an effective palliative treatment option for patients with advanced HCC [5–12].

Although many chemotherapeutic agents (e.g. doxorubicin, epirubicin, mitomycin C, and cisplatin) are used with the ethyl ester of iodized fatty acids from poppy-seed oil (Lipiodol Ultra-fluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France) in TACE, the best choices for first- and second-line drugs remain uncertain [13–15]. Miriplatin (cis-[((1R,2R)-1,2-cyclohexanediamine-N,N′)bis(myristato)]-platinum(II)monohydrate; Dainippon Sumitomo Pharma Co., Osaka, Japan) is a novel lipophilic cisplatin derivative that can be suspended in lipiodol, a lipid lymphographic agent [16–19]. When lipiodol is injected into an artery feeding HCC nodules, it selectively accumulates in the tumor. Accordingly, a miriplatin-lipiodol emulsion is deposited within the HCC nodules and gradually releases active platinum compounds into tumor tissues. Clinical trials have demonstrated that miriplatin is effective in the treatment of HCC, but the efficacy of TACE using miriplatin for patients with recurrent HCC after TACE has not been evaluated [20, 21]. The purpose of this retrospective study was to evaluate the efficacy and safety of TACE using miriplatin for patients with HCC.

## Patients and Methods

*Study Population*

From December 2007 to December 2010, 122 consecutive patients with unresectable HCC were treated by TACE with a miriplatin-lipiodol suspension at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. The study group consisted of 79 men and 43 women ranging in age from 48 to 87 years (median, 72 years). They included 11 patients (9%) positive for HBs-Ag, 103 patients (84%) positive for HCV antibody, and 8 patients (7%) negative for both. At the time of the miriplatin administration, median values were as follows: total bilirubin level = 1.1 mg/dl; serum albumin concentration = 3.3 g/dl; indocyanin-green retention rate at 15 min = 29%; prothrombin activity = 82.5%; alpha-fetoprotein (AFP) concentration = 31.2 ng/ml; and des-gamma-carboxyprothrombin (DCP) concentration = 53 AU/l. As for Child-Pugh classification, 92 patients (75%) were Class A and 30 patients (25%) were Class B. The clinical characteristics of the study group are summarized in table 1. The study protocol was approved by the ethics committee of our hospital, and written informed consent was obtained from all participating patients.

*Hepatocellular Carcinoma*

Before treatment with miriplatin, all patients underwent a comprehensive evaluation consisting of medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver-imaging studies (dynamic computerized tomography [dynamic CT], ultrasonography [US], digital-subtraction angiography [DSA]), complete blood count, and blood chemistry. Diagnosis of HCC was established based on the findings of dynamic CT, US and DSA. Patients who had extrahepatic metastasis of HCC or other malignancies were excluded.

Tumor profiles and TACE treatment history for the study group are summarized in table 2. Twenty-two patients (18%) had a solitary nodule and 100 patients (82%) had multiple nodules. The median diameter of the largest tumor was 20 mm (range 10–100 mm). Ninety-eight patients (80%) had a history of TACE. Thirty-seven patients had received cisplatin, and 80 patients had received epirubicin. Among these patients, the median number of

**Table 1.** Demographic characteristics and pretreatment assessments of 122 patients who underwent TACE using a miriplatin/lipiodol suspension for unresectable HCC

| | |
|---|---|
| Number of cases | 122 |
| Age, years | 72 (48–87) |
| Gender, male | 65% |
| Etiology, HCV/HBV/others | 103/11/8 |
| Child-Pugh Class, A/B/C | 92/30/0 |
| ICG-R15, % | 29 (4–78) |
| Albumin, g/dl | 3.3 (2.0–4.2) |
| Total bilirubin, mg/dl | 1.1 (0.4–4.9) |
| Prothrombin activity, % | 82.5 (45.7–123.1) |
| Platelet, $\times 10^3/\mu l$ | 93 (29–282) |
| AFP, ng/ml | 31.2 (1.8–152,800) |
| DCP, AU/l | 53 (6–65,290) |

HCV = Hepatitis C virus; HBV = hepatitis B virus; ICG-R15 = indocyanine-green retention rate at 15 min.
Variables are expressed as medians with ranges in parentheses.

**Table 2.** Tumor profiles, treatment history, and study drug dosages of 122 patients who underwent TACE using miriplatin for unresectable HCC

| | |
|---|---|
| Tumor size, mm | 20 (10–100) |
| Intrahepatic multiplicity, solitary | 22 (18%) |
| Number of tumors | 4 (1–100) |
| Presence of portal vein invasion | 3 (2%) |
| History of TACE | 98 (80%) |
| History of TACE with epirubicin | 80 (66%) |
| History of TACE with cisplatin | 37 (30%) |
| Median interval between previous TACE and miriplatin administration, months | 4 (1–41) |
| Dosage of miriplatin, mg | 80 (20–120) |
| Dosage of lipiodol, ml | 3 (1–6) |
| Injection from peripheral to segmental branch of the hepatic artery | 22 (18%) |

Variables are expressed as medians with ranges in parentheses or number of cases.

TACE procedures was four (range 1–13), and the median interval between previous TACE and miriplatin administration was 4 months (range 1–41 months).

*Treatment Protocol*

Patients were hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and the catheter was inserted superselectively into the hepatic artery that supplied the target tumor for injection of the miriplatin-lipiodol suspension and 1-mm gelatin cubes (Gelpart; Nippon Kayaku, Tokyo). The miriplatin-lipiodol suspension was administrated slowly under careful fluoroscopic guidance. The dose of miriplatin/lipiodol was determined according to tumor size and the degree of liver dysfunction.

*Assessment of Therapeutic Effects*

The effect of chemotherapy was evaluated by dynamic CT 1 to 3 months after TACE with miriplatin, and was based on the change in the maximum diameter of the viable target lesions (i.e. showing enhancement in the arterial phase). Response categories, according to the criteria of Modified Response Evaluation Criteria in Solid Tumors (mRECIST) [22], are as follows: complete response (CR) = disappearance of any intratumoral arterial enhancement in all target lesions; partial response (PR) = at least a 30% decrease in the sum of diameters of viable target lesions; stable disease (SD) = any cases that do not qualify for either PR or progressive disease; and progressive disease (PD) = an increase of at least 20% in the sum of the diameters of viable target lesions.

*Toxicity Evaluation*

Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria (version 4.0). Within 2 weeks before TACE with miriplatin, and at 3 to 7 days (three times during this period) and at 1 month afterward, the following toxicity evaluations were made: hematological assessments (i.e. leukocyte and thrombocyte counts) and clinical chemistry assessments (i.e. serum aspartate aminotransferase [AST], serum alanine aminotransferase [ALT], albumin, total bilirubin, serum creatine, and prothrombin activity).

*Statistical Analysis*

The distribution of subject characteristics was assessed by the chi-square test or the Mann-Whitney's U test, as appropriate. Multivariate logistic regression analysis was used to evaluate significant factors for CR by TACE with miriplatin. All variables are expressed as mean (range). All tests were 2-sided, and p values less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS, version 13.0 (SPSS Inc., IBM, Somers, N.Y., USA).

## Results

*Dosing of Study Drugs*

Table 2 summarizes the profiles and study drug data of 122 HCC patients who were treated with miriplatin. The median dosage of miriplatin was 80 mg (range 20–120 mg), and the median dosage of lipiodol was 3 ml (range 1–6 ml). Twenty-two patients (18%) were injected with the miriplatin-lipiodol suspension from the peripheral to the segmental branch of the hepatic artery. Thirty patients (25%) were injected with the miriplatin-lipiodol suspension from the anterior or posterior segmental branch of the right hepatic artery. Sixty-six patients (54%) were injected with the miriplatin-lipiodol suspension from the right or left branch of the hepatic artery. And 4 patients (3%) were injected with the miriplatin-lipiodol suspension from the proper hepatic artery.

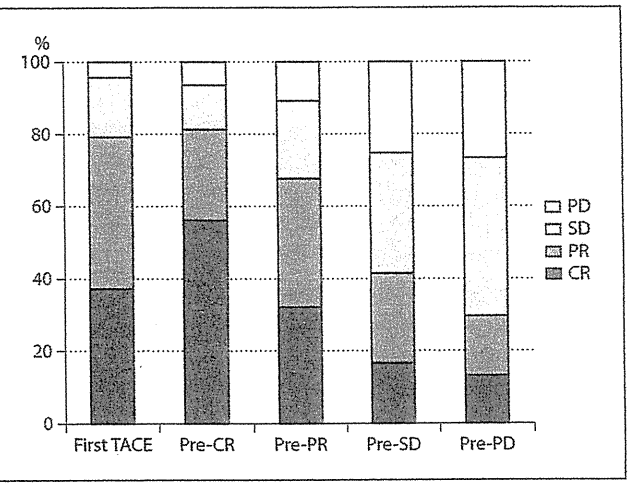


**Fig. 1.** The efficacy of TACE using miriplatin in patients with HCC according to response to previous TACE. Abbreviations used in the figure: CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. pre-CR group (n = 16): patients who showed CR after previous TACE. pre-PR group (n = 28): patients who showed PR after previous TACE. pre-SD group (n = 24): patients who showed SD after previous TACE. pre-PD group (n = 30): patients who showed PD after previous TACE.

*Treatment Effects*

Thirty-five of the 122 treated patients (29%) showed CR, 35 patients (29%) showed PR, 33 patients (27%) showed SD, and 19 patients (15%) showed PD. Overall, 58% of patients showed an objective response (i.e. CR or PR).

*Treatment Effects according to Previous TACE Effect*

The efficacy of TACE using miriplatin according to the treatment effect of previous TACE was as follows (and is illustrated in fig. 1). For the first TACE group (patients who received TACE for the first time), 9 of 24 patients (38%) showed CR; for the pre-CR group (patients who

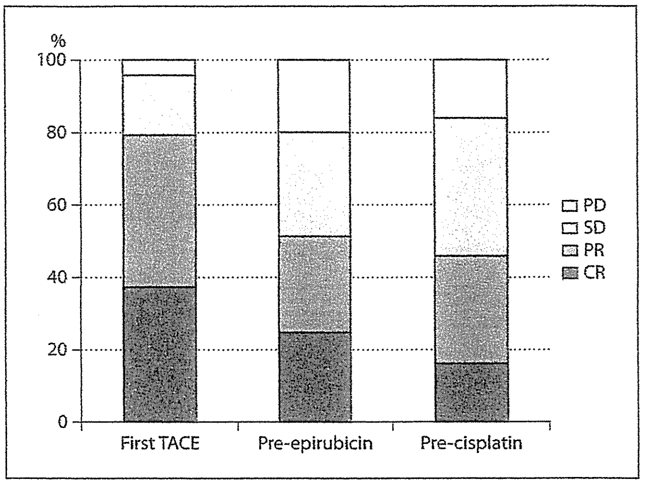


**Fig. 2.** The efficacy of TACE using miriplatin in patients with HCC according to previous TACE agent. Abbreviations used in the figure: CD = complete response; PR = partial response; SD = stable disease; PD = progressive disease. First TACE group (n = 24): patients who received TACE for the first time. Pre-cisplatin group (n = 37): patients who had received TACE using cisplatin. Pre-epirubicin group (n = 80): patients who had received TACE using epirubicin.

showed CR response after previous TACE), 9 of 16 patients (56%) showed CR; for the pre-PR group (patients who showed PR response after previous TACE), 9 of 28 patients (32%) showed CR; for the pre-SD group (patients who showed SD response after previous TACE), 4 of 24 patients (17%) showed CR; and for the pre-PD group (patients who showed PD response after previous TACE), 4 of 30 patients (13%) showed CR.

*Treatment Effects according to Previous TACE Agent*

In patients who had received TACE using epirubicin, 20 of 80 patients (25%) showed CR and 21 of 80 patients (26%) showed PR. In patients who had received TACE using cisplatin, 6 of 37 patients (16%) showed CR and 11 of 37 patients (30%) showed PR. In each of the above groups, the objective response rate (sum of CR and PR) was significantly lower than that in patients who received their first TACE ($p = 0.015$ and $p = 0.010$, respectively), as illustrated in figure 2.

Univariate analysis identified the following six factors as influencing the rate of CR: response after previous TACE (pre-CR group vs. other groups, $p = 0.005$), tumor multiplicity (solitary vs. multiple, $p < 0.0001$), gamma-GTP concentration (≤40 vs. >40 IU/l, $p = 0.037$), AFP concentration (≤40 vs. >40 ng/ml, $p = 0.042$), DCP concentration (≤50 vs. >50 AU/l, $p = 0.003$), and injection artery (peripheral to segmental hepatic artery vs. right or left hepatic artery and proper hepatic artery, $p = 0.001$). These parameters were entered into multivariate logistic regression analysis, which revealed that response after previous TACE (pre-CR group vs. other groups, risk ratio: 4.76; 95% CI: 1.11–20.37; $p = 0.035$), tumor multiplicity (solitary vs. multiple, risk ratio: 9.69; 95% CI: 2.18–42.92; $p = 0.003$), and injection artery (peripheral to segmental hepatic artery vs. right or left hepatic artery and proper hepatic artery, risk ratio: 5.28; 95% CI: 1.07–25.95; $p = 0.040$) were significant independent predictors associated with CR after TACE using miriplatin (table 3).

*Adverse Effects*

Fever, anorexia, and elevation of serum transaminase levels were observed in most patients after miriplatin administration (table 4). The following Grade 4 events were observed: decreased neutrophil count in 1 patient (1%), increased AST in 4 patients (3%), and increased ALT in 1 patient (1%); all these cases resolved within 2 weeks. In this study group, no vascular complications of the hepatic artery were observed. No other serious complications or treatment-related deaths were observed after miriplatin administration.

## Discussion

TACE is most widely performed in patients with HCC who are not eligible for curative therapy. The survival benefit of TACE has been confirmed by randomized controlled trials and meta-analyses. Various anti-cancer drugs, such as doxorubicin, epirubicin, mytomycin C, cisplatin, and neocarzinostatin, have been used as TACE agents for the treatment of HCC. However, the most effective and least toxic TACE protocol for HCC has yet to be identified [13–15].

Although TACE can be repeated in most patients, good therapeutic efficacy cannot be expected when the same anti-cancer drug is used more than once since various types of resistance to therapy can develop during repetition of TACE. Platinum derivatives are frequently administered to patients with advanced HCC that is unresponsive to anthracycline and antibiotic drugs [23, 24]. Miriplatin was developed as a lipophilic platinum complex in an effort to produce a superior anti-tumor effect in HCC with lower toxicity compared with cisplatin [16–

**Table 3.** Univariate and multivariate analysis of predictors of complete necrosis (logistic regression analysis)

| Category | Univariate | | Multivariate | |
|---|---|---|---|---|
| | Hazard ratio (95% CI) | p value | Hazard ratio (95% CI) | p value |
| Tumor multiplicity, solitary vs. multiple | 8.57 (3.08–23.8) | <0.0001 | 9.69 (2.19–42.9) | 0.003 |
| Response by pre-TACE, pre-CR vs. others | 4.91 (1.59–15.1) | 0.005 | 4.76 (1.11–20.3) | 0.035 |
| Injection artery, peripheral to segmental hepatic artery vs. others | 2.50 (0.96–6.48) | 0.001 | 5.28 (1.07–25.9) | 0.040 |
| DCP, ≤50 vs. >50 AU/l | 4.04 (1.61–10.13) | 0.003 | 3.55 (0.99–12.6) | 0.051 |
| gamma-GTP, ≤40 vs. >40 IU/l | 2.39 (1.05–5.44) | 0.037 | | |
| AFP, ≤40 vs. >40 ng/ml | 2.50 (1.03–6.06) | 0.042 | | |

**Table 4.** Adverse effects after miriplatin administration

| Grade: | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| White blood cell decreased | 1 (1%) | 27 (22%) | 7 (6%) | 0 |
| Neutrophil count decreased | 2 (2%) | 21 (17%) | 5 (4%) | 1 (1%) |
| Anemia | 40 (33%) | 21 (17%) | 3 (2%) | 0 |
| Platelet count decreased | 72 (59%) | 21 (17%) | 11 (9%) | 0 |
| AST increased | 55 (45%) | 23 (19%) | 30 (25%) | 4 (3%) |
| ALT increased | 54 (44%) | 12 (10%) | 19 (16%) | 1 (1%) |
| Fever | 67 (55%) | 14 (11%) | 0 | 0 |
| Anorexia | 56 (46%) | 1 (1%) | 0 | 0 |
| Nausea | 23 (19%) | 0 | 0 | 0 |
| Abdominal pain | 22 (18%) | 4 (3%) | 0 | 0 |
| Hepatic infection | 0 | 0 | 1 (1%) | 0 |

Values denote numbers of subjects. Treatment-related toxicity was assessed using the National Cancer Institute Common Terminology Criteria version 4.0.

19]. Miriplatin-lipiodol suspension is a stable colloidal emulsion that is deposited within HCC tumors, where it gradually releases active derivatives of miriplatin.

According to pharmacokinetic studies, the plasma concentration of total platinum is much lower in patients treated with miriplatin compared with that in patients treated with intra-arterial cisplatin: the Cmax is approximately 300-fold lower and the Tmax roughly 500-fold longer for miriplatin than the corresponding values for intra-arterial cisplatin.

Miriplatin/lipiodol releases 1,2-diaminocyclohexane platinum (II) dichloride (DPC) as its active platinum compound, which binds to nuclear DNA and mediates miriplatin/lipiodol cytotoxicity. In a cisplatin-resistant rat hepatoma cell-line model, cross-resistance to DPC was not observed [25].

Prior to the current study, clinical trials have shown that miriplatin is effective for the treatment of HCC, but the efficacy of switching the TACE anti-cancer drug from epirubicin or cisplatin to miriplatin for a repeat TACE had not been evaluated.

In the present study, having a low number of tumors (solitary vs. multiple), receiving the treatment injection in the peripheral to segmental hepatic artery, and having shown complete tumor necrosis after prior TACE (pre-CR group) were highly correlated with complete tumor necrosis after TACE with miriplatin. A previous CR may be a surrogate marker for other factors, such as tumor sensitivity to anti-cancer agents and intra-hepatic metastasis. Among the 54 patients in this study who had shown no change or disease progression after previous TACE (pre-SD and pre-PD groups), 19 patients (35%) showed an objective response by switching the TACE agent from epirubicin or cisplatin to miriplatin.

In repetition of TACE, vascular complications can cause development of parasitic feeding arteries for liver cancers leading to insufficient tumor embolization; rapid tumor growth may follow. In the present study, no vascular complications or other serious adverse events were observed. These results suggest that miriplatin may be used effectively and safely as a second-line TACE drug for recurrent HCC after TACE.

Previous studies reported that complete tumor necrosis after TACE offered favorable long-term survival outcomes to HCC patients [7, 26]. In the current study, miriplatin administration was associated with a beneficial tumor response even in recurrent HCC after TACE. These results suggest that miriplatin administration may offer a favorable prognosis for recurrent HCC after TACE.

## Conclusion

In repetition of TACE in HCC patients, switching the TACE agent from epirubicin or cisplatin to miriplatin offered a favorable treatment effect, especially in patients who had shown CR after previous TACE. These results suggest that miriplatin may be used effectively and safely as a second-line TACE drug for recurrent HCC after TACE.

## Disclosure Statement

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# Amino Acid Substitutions in Hepatitis C Virus Core Region Predict Hepatocarcinogenesis Following Eradication of HCV RNA by Antiviral Therapy

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Substitution of amino acid (aa) 70 and/or 91 in the core region of HCV genotype 1b (HCV-1b) is an important predictor of hepatocarcinogenesis, but its impact on the development of hepatocellular carcinoma (HCC) following eradication of HCV RNA by antiviral therapy is not clear. 1,273 patients with HCV-related chronic liver disease, with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of interferon monotherapy or interferon plus ribavirin combination therapy, were included in a follow-up study to evaluate the impact of aa substitution in the core region on hepatocarcinogenesis. Twenty six patients developed HCC during the follow-up. The cumulative rates of new HCC were 3.2%, 4.8%, and 8.6% at the end of 5, 10, and 15 years, respectively. The rates in patients infected with HCV-1b/Gln70(His70) [glutamine (histidine) at aa 70] were significantly higher than in patients infected with HCV-1b/Arg70 (arginine at aa 70) ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). The rates in patients infected with HCV-1b/Arg70 were not significantly higher than in those infected with HCV-2a/2b ($P = 0.617$; log-rank test). Multivariate analysis identified HCV-1b/Gln70(His70) (HR 10.5, $P < 0.001$), advanced fibrosis (HR 9.03, $P = 0.002$), and old age (HR 3.09, $P = 0.066$) as determinants of hepatocarcinogenesis. In conclusion, aa substitution in the core region of HCV-1b at the start of antiviral therapy is an important predictor of HCC following eradication of HCV RNA. This study emphasizes the importance of detection of aa substitutions in the core region before antiviral therapy. ***J. Med. Virol. 83:1016–1022, 2011.*** © 2011 Wiley-Liss, Inc.

**KEY WORDS:** HCV; genotype; sustained virological response; hepatocellular carcinoma; core region; glutamine



## INTRODUCTION

Infection with hepatitis C virus (HCV) is often persistent and can progress to chronic hepatitis, cirrhosis of the liver, and hepatocellular carcinoma (HCC) [Niederau et al., 1998; Kenny-Walsh, 1999]. At present, interferon (IFN), in combination with ribavirin, is the mainstay for treatment of HCV infection. In Japan, HCV genotype 1b (HCV-1b) and high viral loads account for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C [Tsubota et al., 2005].

Despite numerous lines of epidemiological evidence of an association between HCV infection and the development of HCC, it remains controversial whether the virus itself plays a direct role or an indirect role in the pathogenesis of HCC [Koike, 2005]. It has become evident that the HCV core region is potentially oncogenic in transgenic mice, but the clinical impact of the core region on hepatocarcinogenesis is still unclear [Moriya et al., 1998]. Previous reports indicated that amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with HCV-1b are pretreatment predictors of poor virological response to pegylated IFN (PEG-IFN)/ribavirin combination therapy and triple therapy of

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telaprevir/PEG-IFN/ribavirin [Akuta et al., 2005, 2007a, 2010; Donlin et al., 2007], and also affect hepatocarcinogenesis [Akuta et al., 2007b; Fishman et al., 2009; Hu et al., 2009; Nakamoto et al., 2010]. These reports support the oncogenic potential of the core region from the clinical aspect. However, hepatocarcinogenesis still occurs even after eradication of HCV RNA by antiviral therapy [Ikeda et al., 2003, 2005; Tokita et al., 2005; Kobayashi et al., 2007; Hirakawa et al., 2008], though whether substitutions of aa 70 and/or 91 in the core region also affect hepatocarcinogenesis following eradication of HCV RNA await further investigation.

The present study included 1,273 patients with HCV-related chronic liver disease, with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy (IFN monotherapy or IFN plus ribavirin combination therapy). The aims of this study were to evaluate the impact of aa substitutions in the core region detected at the start of antiviral therapy on hepatocarcinogenesis following eradication of HCV RNA.

## PATIENTS AND METHODS

### Patients

Among 4,570 consecutive patients infected with HCV, in whom antiviral therapy (IFN monotherapy or IFN plus ribavirin combination therapy) was initiated between February 1987 and June 2010 at the Toranomon Hospital, 1,273 were selected for the present study. We included patients who fulfilled the following criteria: (1) Patients positive for anti-HCV (by a third-generation enzyme immunoassay, Chiron Corp., Emerville, CA) and for HCV RNA by qualitative or quantitative analysis, before antiviral therapy. (2) Patients with sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy, based on HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manheim, Germany) or by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (3) Patients without HCC, before and during IFN therapy. (4) Patients infected with a single genotype of HCV-1b, 2a, or 2b. (5) Patients negative for hepatitis B surface antigen (by radioimmunoassay, Dainabot, Tokyo). (6) Patients free of coinfection with the human immunodeficiency virus. (7) Lifetime cumulative alcohol intake <500 kg (mild to moderate alcohol intake). (8) Patients free of other types of hepatitis, and without hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, and autoimmune liver disease. (9) Each signed a consent form of the study protocol that had been approved by the human ethics review committee.

Table I summarizes the profile and laboratory data at the start of antiviral therapy of 1,273 patients with sustained virological response. They included 783 males and 490 females, aged 15–83 years (median, 53 years). The median follow-up time, from the end of antiviral therapy until the last visit, was 1.1 years (range, 0.0–18.0 years).

### Laboratory Investigations

Blood samples were frozen at −80°C within 4 hr of collection and were not thawed until used for testing. HCV genotype was determined by PCR using a mixed primer set derived from nucleotide sequences of the NS5 region [Chayama et al., 1993]. HCV RNA was quantitated by branched DNA assay version 2.0 (Chiron Corp.), AMPLICOR GT HCV Monitor version 2.0 using the 10-fold dilution method (Roche Molecular Systems, Inc., Pleasanton, CA), or COBAS TaqMan HCV test (Roche Diagnostics). A high viral load was defined as branched DNA assay value of

TABLE I. Clinical Profile and Laboratory Data at the Start of Antiviral Therapy

| | |
|---|---|
| Demographic data | |
| Number of patients | 1,273 |
| Sex (male/female) | 783/490 |
| Age (years)* | 53 (15–83) |
| Body mass index (kg/m$^2$)* | 22.7 (14.4–38.0) |
| Laboratory data | |
| Serum aspartate aminotransferase (IU/L)* | 48 (11–1,386) |
| Serum alanine aminotransferase (IU/L)* | 68 (10–2,009) |
| Total cholesterol (mg/dl)* | 168 (79–328) |
| Fasting plasma glucose (mg/dl)* | 93 (69–290) |
| HCV genotype (1b/2a/2b)* | 664/433/176 |
| Level of viremia (high viral load/low viral load) | 838/415 |
| Treatment regimen | |
| IFN monotherapy/IFN plus ribavirin | 545/728 |
| Histological findings | |
| Stage of fibrosis (F1/F2/F3/F4) | 508/224/62/47 |
| Amino acid substitutions in the HCV genotype 1b | |
| Core aa 70 [arginine/glutamine (histidine)] | 348/127 |
| Core aa 91 (leucine/methionine) | 321/156 |

The enrolled patients had sustained virological response, defined as negative HCV RNA at 24 weeks after cessation of antiviral therapy.
Data are numbers and percentages of patients, except those denoted by asterisk (*), which represent the median (range) values.

≥1.0 Meq/ml, AMPLICOR GT HCV Monitor ≥100 × $10^3$ IU/ml, or COBAS TaqMan HCV test ≥5.0 log IU/ml. Low viral load was defined as branched DNA assay value of <1.0 Meq/ml, AMPLICOR GT HCV Monitor <100 × $10^3$ IU/ml, or COBAS TaqMan HCV test <5.0 log IU/ml. The lower limit of HCV RNA qualitative analysis (Amplicor, Roche Diagnostics, Manheim) was 100 copies/ml, and that of COBAS TaqMan HCV test was 1.2 log IU/ml. Samples with undetectable HCV RNA at 24 weeks after cessation of antiviral therapy by qualitative analysis or COBAS TaqMan HCV test were defined as HCV RNA-negative.

### Detection of Amino Acid Substitutions in the Core Regions of HCV-1b

In the present study, aa substitutions in the core region of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of antiviral therapy and reverse transcribed with a random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo, Japan). Nucleic acids of the core region were amplified by nested PCR using the following primers. The first-round PCR was performed with CE1 (sense, 5′-GTC TGC GGA ACC GGT GAG TA-3′, nucleotides: 134–153) and CE2 (antisense, 5′-GAC GTG GCG TCG TAT TGT CG-3′, nucleotides: 1096–1115) primers, and the second-round PCR with CC9 (sense, 5′-ACT GCT AGC CGA GTA GTG TT-3′, nucleotides: 234–253) and CE6 (antisense, 5′-GGA GCA GTC GTT CGT GAC AT-3′, nucleotides: 934–953) primers. All samples were initially denatured at 95°C for 2 min. The 35 cycles of amplification were set as follows: denaturation for 30 sec at 95°C, annealing of primers for 30 sec at 55°C, and extension for 1 min at 72°C with an additional 7 min for extension. Then, 1 μl of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo, Japan) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (Perkin-Elmer, Tokyo, Japan).

Using HCV-J (accession no. D90208) as a reference [Kato et al., 1990], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed using 50 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [Akuta et al., 2005]. Thus, patients were classified into three HCV subgroups according to the HCV genotype and aa substitutions in the HCV-1b core region: (1) HCV-1b with Arg70, (2) HCV-1b with Gln70(His70), and (3) HCV-2a/2b.

### Liver Histopathological Examination

Liver biopsy specimens were obtained percutaneously or at peritoneoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan). The samples were fixed in 10% formalin and then stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. Each specimen submitted for examination contained ≥6 portal areas. Histopathological diagnosis was made by an experienced liver pathologist (HK) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on the scoring system of Desmet et al. [1994] for histopathological assessment.

### Follow-Up and Diagnosis of Hepatocellular Carcinoma

Hematological, biochemical, and virological tests were performed at least once every month until the virological response was determined. When sustained virological response was confirmed, blood tests and imaging studies (computed tomography or ultrasonography) were conducted once or twice per year in the majority of patients, except those lost to follow-up. When HCC was suspected, additional procedures, such as magnetic resonance imaging, abdominal angiography, and ultrasonography-guided tumor biopsy when necessary, were used to confirm the diagnosis.

### Statistical Analysis

The cumulative rate of new cases of HCC was calculated using the Kaplan–Meier technique, and differences between the curves were tested using the log-rank test. Differences in the proportion of new cases of HCC according to groups were analyzed according to the period between the end of antiviral therapy and appearance of HCC. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with the development of HCC. The hazard ratio (HR) and 95% confidence interval (95%CI) were also calculated. Potential predictive factors associated with the development of HCC included the following variables: sex, age, body mass index, AST, ALT, total cholesterol, fasting plasma glucose, HCV genotype, level of viremia, treatment regimen, stage of fibrosis, and HCV subgroup according to HCV genotype in combination with aa substitutions in the core region. Variables that achieved statistical significance ($P < 0.05$) on univariate analysis were entered into a multivariate Cox proportional hazard model to identify significant independent factors. Statistical comparisons were performed using The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL). All $P$ values of less than 0.05 by the two-tailed test were considered significant.

## RESULTS

### Rate of New Cases of HCC in Patients With Sustained Virological Response

During the follow-up, 26 patients (2.0%) developed HCC. The median interval between the end of antiviral therapy and detection of HCC (latency to HCC) was 2.5 years (range, 0.0–15.9 years). The cumulative rates of new cases of HCC were 3.2%, 4.8%, and 8.6% at the end of 5, 10, and 15 years, respectively.

### HCC Rate According to HCV Genotype and Amino Acid Substitutions in the Core Region of HCV-1b

During the follow-up, 7 (5.5%), 5 (1.4%), and 12 (2.0%) patients developed HCC in the HCV-1b with Gln70(His70), HCV-1b with Arg70, and HCV-2a/2b groups, respectively. The median latency to HCC was 1.1 years (range, 0.0–14.0 years), 3.9 (range, 0.0–15.9), and 2.8 (range, 0.0–12.9), respectively, and the cumulative rates of new cases of HCC were 10.6%, 3.6%, 3.0% at the end of 5 years; 10.6%, 6.3%, 5.2% at the end of 10 years; and 62.7%, 6.3%, 7.2% at the end of 15 years, respectively. The rates were significantly different among the three HCV subgroups ($P < 0.001$; log-rank test; Fig. 1). Especially, the rates for HCV-1b with Gln70(His70) were significantly higher than those for HCV-1b with Arg70 ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). However, the rates for the HCV-1b with Arg70 group were not significantly higher than those for the HCV-2a/2b group ($P = 0.617$; log-rank test).

During the follow-up, 4 (2.6%) and 7 (2.2%) patients with HCV-1b/Met91, and HCV-1b/Leu91 developed HCC, respectively. In these two subgroups, the respective median latency to HCC was 3.4 years (range, 0.0–14.0 years) and 1.1 (range, 0.0–12.4), and the cumulative rates of new cases of HCC were 1.3%, 8.6% at the end of 5 years; 5.4%, 8.6% at the end of 10 years; and 36.9%, 14.7% at the end of 15 years. The rates for the HCV-1b/Met91 group were not significantly different from those for the HCV-1b/Leu91 group ($P = 0.908$; log-rank test).

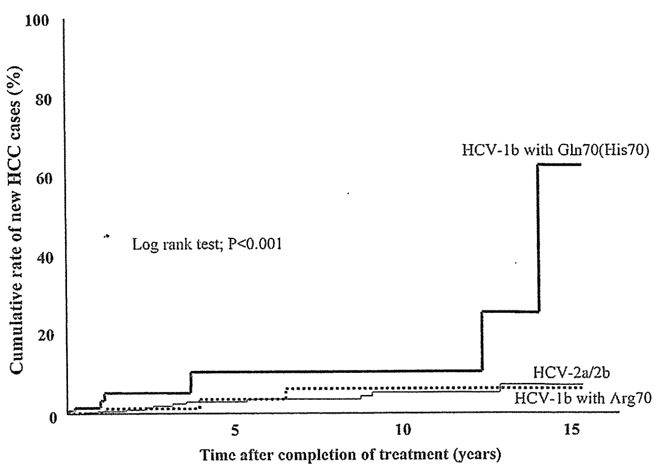


Fig. 1. Cumulative rates of new cases of HCC according to HCV genotype and amino acid substitutions in the core region of HCV-1b. The rates were significantly different among the three HCV groups ($P < 0.001$; log-rank test). Especially, the rate in patients with HCV-1b/Gln70(His70) was significantly higher than those of patients with HCV-1b/Arg70 ($P = 0.007$; log-rank test) and HCV-2a/2b ($P < 0.001$; log-rank test). Furthermore, the rate in patients with HCV-1b/Arg70 was not significantly higher than that in HCV-2a/2b ($P = 0.617$; log-rank test).

### Predictive Factors Associated With the Development of HCC in Patients of Sustained Virological Response

Next, we analyzed the predictor of HCC using data of the entire group. There were significant relationships between the rate of new cases of HCC and male sex ($P = 0.003$), severe fibrosis (F3,4) ($P < 0.001$), old age (≥55 years) ($P = 0.002$), high levels of AST (≥39 IU/L) ($P = 0.023$), and HCV-1b/Gln70(His70) (log-rank test). These five factors were entered into multivariate analysis, which then identified three parameters that independently tended to or significantly influenced the development of HCC; HCV-1b/Gln70(His70) (HR 10.5, $P < 0.001$), advanced stage of fibrosis (F3,4; HR 9.03, $P = 0.002$), and old age (≥55 years; HR 3.09, $P = 0.066$; Table II).

### Predictors of HCC in HCV-1b Patients With Sustained Virological Response

Finally we analyzed the data of 664 patients with HCV-1b to determine the predictors of HCC with sustained virological response. Univariate analysis identified three parameters that significantly correlated with the development of HCC: male sex ($P = 0.005$), old age ($P = 0.020$), and HCV-1b with Gln70(His70) ($P = 0.007$; log-rank test). These three factors were entered into multivariate analysis, which then identified HCV-1b with Gln70(His70) as the single parameter that significantly influenced the development of HCC (HR 8.19, $P = 0.034$).

## DISCUSSION

Previous studies reported that the risk factors for hepatocarcinogenesis after elimination of HCV RNA

TABLE II. Results of Multivariate Analysis (Cox Proportional Hazard Model) for Factors Associated With Hepatocarcinogenesis in Patients With Sustained Virological Response

| Factors and categories | Hazard ratio (95%CI) | P-Value |
|---|---|---|
| HCV group | | |
| HCV-2a/2b | 1 | |
| HCV-1b with Arg70 | 1.15 (0.24–5.56) | 0.863 |
| HCV-1b with Gln70(His70) | 10.5 (2.89–38.2) | <0.001 |
| Fibrosis stage | | |
| F1,2 | 1 | |
| F3,4 | 9.03 (2.32–35.2) | 0.002 |
| Age (years) | | |
| <55 | 1 | |
| ≥55 | 3.09 (0.93–10.3) | 0.066 |

were severe fibrosis, male sex, and old age at the start of IFN treatment [Ikeda et al., 2003, 2005; Tokita et al., 2005; Kobayashi et al., 2007; Hirakawa et al., 2008]. In the present study, multivariate analysis identified HCV-1b with Gln70(His70), advanced fibrosis stage, and old age as determinants of HCC in patients with a sustained virological response. The present study is the first report to indicate that aa substitution in the core region at the start of antiviral therapy also influences hepatocarcinogenesis following eradication of HCV RNA. This result should be interpreted with caution since races other than the Japanese and patients infected with HCV-1a were not included. Any generalization of the results should await confirmation by studies of patients of other races and those infected with HCV-1a.

Despite numerous lines of epidemiological evidence linking HCV infection to the development of HCC, it remains controversial whether HCV itself plays a direct or indirect role in the pathogenesis of HCC [Koike, 2005]. Evidence suggests that the HCV core region is potentially oncogenic in the transgenic mice [Moriya et al., 1998], though the clinical impact of the core region on hepatocarcinogenesis remains unclear. Previous reports indicated that aa substitutions in the core region of HCV-1b are pretreatment predictors of poor virological response to antiviral therapy [Akuta et al., 2005, 2007a, 2010; Donlin et al., 2007], and also are etiological factors in HCC [Akuta et al., 2007b; Fishman et al., 2009; Hu et al., 2009; Nakamoto et al., 2010]. Importantly, the present study indicated that aa substitution in the core region at the start of antiviral therapy also affects the development of HCC even after the eradication of HCV RNA, and this is the first report to suggest the persistent oncogenic potential of the core region regardless of HCV RNA persistence. Previous reports identified the PA28γ-dependent pathway as one of the mechanisms of HCV-associated hepatocarcinogenesis. Moriishi et al. [2003, 2007] reported that knockout of the PA28γ gene induces accumulation of HCV core protein in the nuclei of hepatocytes of HCV core gene transgenic mice and disrupts the development of both hepatic steatosis and HCC. Furthermore, the HCV core protein also enhances the binding of liver X receptor α (LXRα) and retinoid X receptor α (RXRα) to the LXR-response element in the presence of PA28γ [Moriishi et al., 2007]. Thus, it seems that PA28γ plays a crucial role in the development of HCV-associated steatosis and HCC. However, these basic studies were performed under the state of HCV RNA persistence [Moriya et al., 1998; Moriishi et al., 2003, 2007; Koike, 2005], and further studies should be performed to investigate the oncogenic potential of aa substitution in the core region detected at the start of antiviral therapy on hepatocarcinogenesis following eradication of HCV RNA.

The association between HCV genotype and the risk of HCC is not clear. A study of Italian cohort indicated that the rate of HCC in patients infected with HCV-1b was significantly higher than that of patients infected with HCV-2a/2c [Bruno et al., 2007]. On the other hand, the present study of Japanese patients indicated that the rates in patients infected with HCV-1b were not significantly higher than those in those infected with HCV-2a/2b. The discrepancy between the present result and the above Italian study may be explained by differences in host factors [Montes-Cano et al., 2010], and/or differences in viral factors, such as the distribution of HCV-1b with Arg70 or Gln70(His70), and geographic diversities of HCV-1b [Nakano et al., 1999].

Previous studies showed that the 12- and 24-week regimen of telaprevir/PEG-IFN/ribavirin achieved sustained virological response rates of 35–60% and 61–69% in patients infected with HCV-1, respectively [Hézode et al., 2009; McHutchison et al., 2009; Akuta et al., 2010]. Furthermore, the PROVE3 study also showed that the 24- and 48-week regimen of triple therapy achieved sustained virological response rates of 51% and 53%, respectively, in patients infected with HCV-1 who had been unsuccessfully treated with PEG-IFN/ribavirin [McHutchison et al., 2010]. While it is anticipated that larger numbers of HCV-1 patients will achieve sustained virological response in response to telaprevir/PEG-IFN/ribavirin, a larger proportion of patients could develop HCC following eradication of HCV RNA by antiviral therapy. Hence, our study indicated that aa substitutions in the core region of HCV-1b should be detected before eradication of HCV RNA by antiviral therapy. Especially, even if patients of HCV-1b with Gln70(His70) could achieve sustained virological response, blood tests and imaging studies should be conducted at regular intervals in this high risk group for early detection and treatment of HCC.

Genetic variations near the IL28B gene are pretreatment predictors of poor virological response to the combination therapy of PEG-IFN/ribavirin and triple therapy of telaprevir/PEG-IFN/ribavirin [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; Akuta et al., 2010; Rauch et al., 2010], but their impact on hepatocarcinogenesis are unknown at this stage. In this study, 387 of 1,273 patients were evaluated for HCC according to genetic variation in rs8099917 (data not shown). A preliminary study based on a small number of patients showed that the HCC rate in genotype TT of treatment sensitive type (2.2%) was not significantly different from that in genotype non-TT of treatment resistant type (1.6%). Unfortunately, we could analyze the effect of rs8099917 on HCC following eradication of HCV RNA by antiviral therapy. Further studies of larger patient populations should be performed to investigate the relationship between genetic variations near the IL28B gene and HCC.

The limitations of the present study were that viral factors associated with hepatocarcinogenesis were incompletely investigated. Ogata et al. [2003] reported that HCV-1b strains might be associated with HCC on the basis of the secondary structure of the amino-terminal portion of the HCV NS3 protein. Giménez-Barcons et al. [2001] reported that high amino acid variability within the NS5A of HCV might be associated with HCC in patients with HCV-1b-related cirrhosis. In the present study, the clinical impact of other regions on hepatocarcinogenesis could not be investigated, except for aa 70 and 91 in the HCV core region. The results should also be interpreted with caution since patients infected with HCV-1a were not included. Other limitations include lack of analysis of the effects of life-style related diseases (such as diabetes, insulin resistance or non-alcoholic steatohepatitis) on hepatocarcinogenesis, except for fasting plasma glucose and total cholesterol [Sumida et al., 2010a,b]. The impact of viral factors and life-style related diseases on hepatocarcinogenesis should also be investigated in future studies.

In conclusion, aa substitution in the core region of HCV-1b at the start of antiviral therapy is an important predictor of hepatocarcinogenesis following eradication of HCV RNA. This study emphasizes the importance of detection of aa substitutions in the core region before antiviral therapy.

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CLINICAL STUDIES

# Stage progression of small hepatocellular carcinoma after radical therapy: comparisons of radiofrequency ablation and surgery using the Markov model

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hepatocellular carcinoma – Markov model – radiofrequency ablation – recurrence – surgery – survival

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## Abstract

*Background:* Stage progression of 374 small hepatocellular carcinomas (HCC) was retrospectively analysed. *Patients and methods:* During 8 years, 236 patients with the early stage of HCC received radiofrequency ablation (RFA), and 138 underwent surgery as an initial therapy. More patients of young age and with better liver function tended to undergo surgical treatment. Based on 1892 patient-year data, the Markov model analysed the stepwise progression of early stage (multiple up to three nodules, 3 cm or less each) to intermediate stage (four nodules or more, or larger than 3 cm), to advanced stage (portal invasion, extrahepatic metastasis or Child–Pugh C) and to death. *Results:* The recurrence rates after RFA and surgery were 53.3 and 40.6% in the third year. The annual progression rates from the early stage to the intermediate stage, advanced stage and death were 5.40, 1.63 and 1.73% in the RFA group and 3.90, 1.87 and 0.62% in the surgery group respectively. The progression rate from the early to the intermediate stage was significantly lower (2.34% annually) in the younger patient group (< 60 years) than that in the older group (≥60 years, 5.70%, $P=0.0053$). In contrast, the progression rate from the intermediate to the advanced stage was significantly higher in the younger patient group (< 60 years, 37.50% annually) than that in the older groups (60–69 years, 30.30%, 70 years or older 22.09%, $P=0.0011$). Multivariate hazard analysis showed that initial treatment did not significantly affect the stage progression rate (hazard ratio of RFA 1.09, $P=0.70$) and the survival rate (hazard ratio of RFA 1.09, $P=0.73$). *Conclusion:* Although the recurrence rate was slightly higher in the RFA group, additional ablation procedures could control the progression of HCC, with a rate comparable to the surgical group.

Hepatocellular carcinoma (HCC) is one of the most common neoplasms in the world today (1). Although routine imaging check-ups can often detect a small HCC at an early stage in high-risk patients with chronic hepatitis and cirrhosis, surgical resection is performed only in 20% or less of the cases because of the association of cirrhosis and tumour multiplicity (2–5). In the management of patients with HCC associated with cirrhosis, treatment repetition is common and inevitable for newly appearing multicentric tumours (6–8), and many practitioners hope each ablation procedure to be less invasive, less expensive and with a shorter hospitalization period.

Radiofrequency ablation (RFA) is currently considered the most effective percutaneous therapy for small HCCs, and certain centres now use it as a first-line treatment option (9), even in patients suitable for surgery. Indeed, RFA is sometimes considered as a less radical therapy compared with surgical resection because of the relatively high rate of local recurrence (10–12), but most of the local tumour progression can be completely treated through an additional RFA procedure. Surgical therapy, on the other hand, is an invasive mode of treatment with a higher cost (10), but achieves a lower recurrence rate. Only a few studies have evaluated the long-term outcome and prognostic factors of percutaneous RFA in comparison with surgical therapy (12–14).

When a recurrent tumour shows relatively advanced characteristics at an intermediate stage with a large tumour or multiples of four or more, transcatheter arterial chemoembolization (TACE) is preferred to surgical therapy or local ablation (15). We introduced the

Markov model to simulate the steps of stage progression of patients with small HCC under an intensive medical intervention. Here, we retrospectively evaluated the progression of HCC and the long-term prognosis of patients who had undergone RFA or surgical resection as the initial therapy for small HCCs, and assessed the prognostic factors of those patients.

The purposes of this study were, therefore, (i) to compare the recurrence rates, progression of tumour stage and survival rates between those patients who received percutaneous RFA and those who underwent surgery and (ii) to elucidate the significance of the selection of initial therapy for small HCCs from the viewpoints of stage progression and prognosis.

## Patients and methods

### Patients

A total of 468 patients were diagnosed as having a small HCC 3 cm or less in diameter, from March 1999 to April 2006, at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these 468 patients, 236 patients (50.4%) underwent percutaneous RFA therapy as a curative mode of treatment and the remaining 138 patients (29.5%) received surgical resection, 52 had TACE and the remaining 42 patients were treated with ethanol injection, microwave coagulation or other palliative methods of treatment.

A total of 374 consecutive patients with a small HCC, who underwent either RFA or surgery, were analysed in this study. None had been treated previously for HCC, and all had single or multinodular (up to three) HCCs 3 cm or less in diameter each, absence of portal venous thrombosis and known extrahepatic metastases, and Child–Pugh class A or B liver function.

The patients included 246 men and 128 women, and ranging in age from 29 to 87 years, with a median age of 65 years. The demography, laboratory data and features of cancer were compared between the two therapy groups (Table 1). Patients' age was lower in the surgery group by 4.5 years. The rate of HBV-positive disease was significantly higher in the surgery group, and liver function tests were also significantly better in the surgery group.

### Hepatocellular carcinoma

Patients were required to have HCC with a definitive diagnosis by either typical hypervascular radiological features or histology through needle biopsy. Tumours had to be measurable by ultrasonography (US), computerized tomography (CT) and digital subtraction angiography. In order to elucidate the detailed characteristics of the HCC, CT during arterial portography and CT hepatic arteriography were performed in all the patients. Among 374 patients, HCC was confirmed by a resected specimen in 138 patients, by typical hypervascular characteristics on at least two modalities of imagings in 219 and by a fine-needle biopsy in 17.

Most patients (82.2%, 309 of 376) had a single tumour, and the median tumour diameter was 19 mm, ranging from 5 to 30 mm. The characteristics of the tumour in the subgroup of RFA and surgery are given in Table 1. The median size of the largest tumour was 18 mm in the RFA group and 20 mm in the surgery group ($P < 0.001$).

**Table 1.** Clinical features of the patients with small liver cancer

| Initial therapy | Radiofrequency ablation ($n = 236$) | Hepatic resection ($n = 138$) | $P$ |
|---|---|---|---|
| Demography | | | |
| Men:women | 145:91 (38.6%) | 101:37 (26.8%) | 0.0021 |
| Age (median, range) | 67 (38–87) | 62.5 (29–80) | < 0.001 |
| Decompensated cirrhosis | 16 (6.8%) | 5 (3.6%) | 0.20 |
| HBsAg | 24 (10.2%) | 46 (33.3%) | < 0.001 |
| Antibody to HCV | 197 (83.5%) | 84 (60.9%) | < 0.001 |
| History of alcohol intake > 500 kg | 21 (8.9%) | 16 (11.6%) | 0.40 |
| Observation period (year) | 3.7 (0.1–9.9) | 4.5 (0.1–10.0) | 0.041 |
| Laboratory data (median, range) | | | |
| ICG R15 (%)* | 28 (1–100) | 21 (3–68) | < 0.001 |
| Bilirubin (mg/dl) | 1.0 (0.2–3.1) | 1.0 (0.3–2.2) | 0.003 |
| Albumin (g/dl) | 3.5 (2.2–4.2) | 3.6 (2.8–4.4) | < 0.001 |
| Aspartic transaminase (IU) | 55 (17–311) | 45 (17–386) | 0.006 |
| Platelet count ($\times 10^3/mm^3$) | 97 (19–253) | 127 (38–272) | < 0.001 |
| Prothrombin time (%) | 84 (31–125) | 91 (59–115) | 0.001 |
| Liver cancer | | | |
| Median size (mm) | 18 (8–30) | 20 (5–30) | < 0.001 |
| Single/multiple | 195/41 (17.4%) | 114/24 (17.4%) 1.00 | |
| α-fetoprotein (ng/ml) | 19 (1–2080) | 17 (1–2610) | 0.84 |
| PIVKA-II (AU/L)† | 17 (7–1470) | 20 (9–1650) | 0.008 |

*ICG R15, indocyanine green retention rate at 15 min.
†PIVKA-II, protein induced by vitamin K antagonist-II.
HCV, hepatitis C virus.

### Treatment for initial hepatocellular carcinoma

Physicians and surgeons usually held a conference about the choice of therapy in individual patients. RFA or surgical therapy were selected considering the site, size and number of tumours, liver function and the patient's general status. Both RFA and the surgical procedure were explained fully to all the patients, and informed consent was obtained. Despite the feasibility and availability of surgery, some patients voluntarily preferred RFA under informed consent.

Radiofrequency ablation therapy was performed percutaneously under US or CT guidance, under conscious sedation with fentanyl citrate (0.1–0.2 mg, Fentanyl; Daiichi-Sankyo, Tokyo, Japan) or pethidine hydrochloride (35–70 mg, Opystan; Tanabe-Mitsubishi, Osaka, Japan) administered intravenously. RFA was performed using three kinds of apparatus: a radiofrequency interstitial tumour ablation system (RITA, RITA Medical Systems Inc., Mountain View, CA, USA), a cool-tip system (Tyco Healthcare Group LP, Burlington, VT, USA) and a radiofrequency tumour coagulation system (RTC system, Boston-Scientific Japan Co., Tokyo, Japan).

Hepatic resection was performed under intra-operative US monitoring and guidance. In the cases of small and superficial HCC, arterial and portal vein clumping at the hepatic hilum was not usually performed for maintenance of liver perfusion.

### Evaluation of the therapeutic effect

To evaluate the efficacy of local ablation, a dynamic CT was performed at 2–7 days after treatment with RFA, and 8–21 days after surgery. CT findings were confirmed by consensus among at least two hepatologists and radiologists. On dynamic CT images, the non-enhancing area was measured as the ablated area. When the diameter of the non-enhancing area was greater than that of the ablated nodule, RFA was considered to have had a complete effect, and the treatment was terminated. When patients had a smaller ablated area or a positively enhanced area in the original tumour based on CT results after RFA therapy, they usually underwent an additional RFA within several days.

### Follow-up of patients

Physicians observed the patients every 4–8 weeks after the first treatment. Liver function test, haematology and tumour markers were measured every 1–2 months. After the completion of eradication of HCC, recurrence was surveyed with CT or magnetic resonance imagings (MRI) every 3–4 months. Serum $\alpha$-fetoprotein (AFP) and des-$\gamma$-carboxy prothrombin were also measured every 1–2 months to detect recurrence as early as possible.

During a median observation period of 4.2 years, four patients (1.1%) were lost to follow-up.

### Statistical analysis and the Markov model

Standard statistical measures and procedures were used. The $\chi^2$-test, Fisher's exact test and Mann–Whitney's *U*-test were used to analyse the differences in the demography, laboratory findings and tumour characteristics between the RFA group and the surgery group. The recurrence rate, progression rates and survival rate were analysed using the Kaplan–Meier technique (16) with the log-rank test. Cox's proportional hazard analysis was performed to evaluate independent predictors of the outcomes.

The Markov model (17) was adopted to analyse the transition rates from the early stage to the intermediate stage of HCC, intermediate to advanced stage and advanced stage to death. A homologous Markov chain consisted of four states (Fig. 1). These were the early stage of HCC (solitary or multiple up to three nodules, 3 cm or less each), the intermediate stage (four nodules or more, or larger than 3 cm), the advanced stage (portal vein

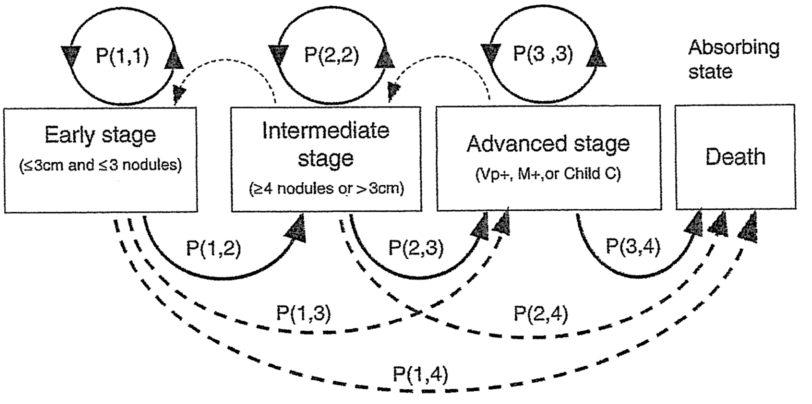


**Fig. 1.** The Markov state transition diagram of hepatocellular carcinoma. Four states were defined: early stage (solitary or multiple up to three nodules, 3 cm or less in diameter each), intermediate stage (multiple nodules of four or more, or 3.1 cm or more), advanced stage (main portal vein invasion, extrahepatic metastasis or Child–Pugh C) and death. Of these, death was the absorbing state from which no transitions to the other states occurred. The transition in one cycle (1 year) is shown. Arrows connecting two different states indicate the transitions observed.

invasion, extrahepatic metastasis or Child–Pugh score C) and death as an absorbing state from where no transitions to the other states occurred. The model was based on the following principles: (i) the four states are mutually exclusive and collectively exhaustive; (ii) the Markov assumption for the current state without any memories of prior states; (iii) time intervals are uniform; and (iv) transition probabilities are constant and time independent. Items (i) and (ii) define a Markov chain, whereas items (iii) and (iv) characterize a homogenous Markov chain (18).

A $P$-value of $< 0.05$ in a two-tailed test was considered significant. Data analysis was performed using the computer program IBM SPSS STATISTICS ver. 18 (19).

## Results

### Effect of initial treatment

After the initial session of RFA or surgery, complete ablation for entire tumour nodules was obtained in 232 patients (98.3%) in the RFA group and in 138 patients (100%) in the surgery group. Among four patients (1.7%) with incomplete ablation after the initial session of RFA, two achieved complete necrosis by re-RFA performed after a few months, and the other two underwent TACE for the residual tumour nodules.

### Complications of treatment (Table 2)

After the initial therapy with RFA or surgery, 12 patients developed major complications after treatment: seven in the RFA group and five in the surgery group. There was no treatment-related death within 6 months after therapy in any of the patients in the RFA and surgery groups. Although abdominal pain, mild aggravation of liver function test, low-grade fever, transient elevation of aminotransferases and bilirubin values were often found after RFA therapy, significant deterioration of performance status and prolonged stay in the hospital were not observed.

### Cumulative recurrence rates and treatment for recurrent hepatocellular carcinoma

The initial recurrence rates were compared between the two groups according to the initial therapy. The initial recurrence rates after treatment in the RFA and the surgery group were 11.3 and 14.2% at the end of the first year, 40.4 and 29.3% in the second year, 53.3 and 40.6% in the third year, 65.0 and 48.8% in the fourth year and 69.5 and 53.7% in the fifth year respectively. The recurrence rate in the RFA group was significantly higher than that of the surgery group (log-rank test, $P = 0.015$) (Fig. 2).

For the treatment of a recurrent tumour, we fundamentally adopted RFA or surgical treatment when patients had an early stage of HCC with sufficient liver function. Although initial therapy included surgery, patients with a recurrent tumour tended to receive RFA therapy more frequently. When a tumour progressed to the intermediate stage with a large tumour and/or multiple nodules, TACE was usually performed using anti-tumour agents, iodinated poppy seed oil fatty acid (Lipiodol Ultra-Fluide$^{TM}$, Guerbet Japan, Tokyo) and gelatin sponge particles. When the tumour progressed to the advanced stage (portal invasion, extrahepatic metastasis, or Child–Pugh C) during repeated local ablation or TACE therapy, anti-tumour therapy was usually not performed, except for systemic or intra-arterial chemotherapy. Anti-molecular targeted agents were not available during the study period in Japan.

**Table 2.** Complications after the initial treatment

| Complication | Initial therapy: Radiofrequency ablation ($n = 236$) | Hepatic resection ($n = 138$) |
|---|---|---|
| Perforation of jejunum | 2 | 0 |
| Biloma and/or biliary infection | 3 | 1 |
| Prolonged ascites | 1 | 2 |
| Jaundice | 0 | 1 |
| Haemorrhage requiring transfusion | 1 | 1 |

### Cumulative progression rates from the early to the intermediate stage

A total of 98 (26.2%) developed to the intermediate stage during the observation: 65 (27.5%) in the RFA group and 33 (23.9%) in the surgery group.

Crude development rates to the intermediate stage in the RFA and surgery groups were 18.2 and 13.0% in the third year, 33.1 and 22.1% in the fifth year, and 40.9 and 31.8% in the fifth year respectively. The development rate of the RFA group was slightly higher ($P = 0.14$) (Fig. 3a).

Independent factors associated with the stage development rate were explored in the patients. Multivariate hazard analysis showed that the rate is independently associated with positive HBsAg ($P = 0.041$) and a high platelet count ($P = 0.032$). The factor of initial therapy

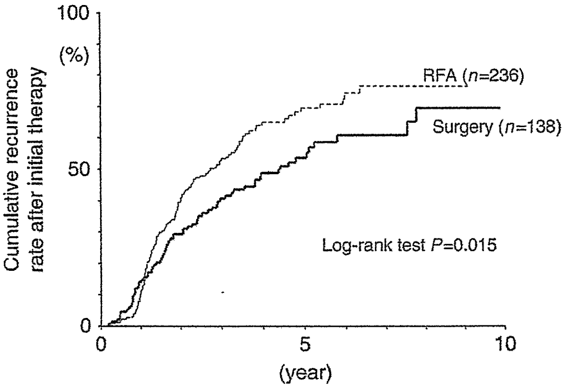


**Fig. 2.** Cumulative recurrence rates after therapy in patients with an early stage of hepatocellular carcinoma, according to initial therapy. RFA, radiofrequency ablation.