acetylation, which inhibits ubiquitination-mediated degradation. *J Biol Chem* 2004, **279(28)**:29409-29417.
39. Li G, Wang S, Gelehrter TD: **Identification of glucocorticoid receptor domains involved in transrepression of transforming growth factor-beta action.** *J Biol Chem* 2003, **278(43)**:41779-41788.
40. Mouri H, Sakaguchi K, Sawayama T, Senoh T, Ohta T, Nishimura M, Fujiwara A, Terao M, Shiratori Y, Tsuji T: **Suppressive effects of transforming growth factor-beta1 produced by hepatocellular carcinoma cell lines on interferon-gamma production by peripheral blood mononuclear cells.** *Acta Med Okayama* 2002, **56(6)**:309-315.
41. Bressac B, Kew M, Wands J, Ozturk M: **Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa.** *Nature* 1991, **350(6317)**:429-431.
42. Ozturk M: **Genetic aspects of hepatocellular carcinogenesis.** *Semin Liver Dis* 1999, **19(3)**:235-242.
43. Higashitsuji H, Itoh K, Nagao T, Dawson S, Nonoguchi K, Kido T, Mayer RJ, Arii S, Fujita J: **Reduced stability of retinoblastoma protein by gankyrin, an oncogenic ankyrin-repeat protein overexpressed in hepatomas.** *Nature medicine* 2000, **6(1)**:96-99.
44. Hsia CC, Di Bisceglie AM, Kleiner DE Jr, Farshid M, Tabor E: **RB tumor suppressor gene expression in hepatocellular carcinomas from patients infected with the hepatitis B virus.** *Journal of medical virology* 1994, **44(1)**:67-73.
45. Li DM, Sun H: **PTEN/MMAC1/TEP1 suppresses the tumorigenicity and induces G1 cell cycle arrest in human glioblastoma cells.** *Proc Natl Acad Sci USA* 1998, **95(26)**:15406-15411.

Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2407/11/3/prepub

doi:10.1186/1471-2407-11-3
Cite this article as: Nakanishi *et al.*: Loss of runt-related transcription factor 3 expression leads hepatocellular carcinoma cells to escape apoptosis. *BMC Cancer* 2011 11:3.

Dig Dis Sci
DOI 10.1007/s10620-011-1934-1

ORIGINAL ARTICLE

Effect of Previous Interferon Treatment on Outcome After Curative Treatment for Hepatitis C Virus-Related Hepatocellular Carcinoma

Hirokazu Miyatake · Yoshiyuki Kobayashi · Yoshiaki Iwasaki · Shin-ichiro Nakamura · Hideki Ohnishi · Kenji Kuwaki · Junichi Toshimori · Hiroaki Hagihara · Kazuhiro Nouso · Kazuhide Yamamoto

Received: 26 July 2011 / Accepted: 21 September 2011

Abstract
Background and Aims Treatment of chronic hepatitis C virus (HCV) infection with interferon (IFN) prevents the development of hepatocellular carcinoma (HCC). The purpose of this study was to clarify the effect of previous IFN treatment before the development of HCC on recurrence and survival in HCV-related HCC patients.
Methods Three hundred ninety-five patients who underwent curative treatment for HCV-related HCC were enrolled. Of these, 124 had received IFN treatment before the development of HCC (17 achieved sustained virological response [SVR group] and 107 did not [non-SVR group]), whereas 271 patients had never received IFN treatment (IFN-untreated group). The first and second recurrence and survival rates in these patient groups were statistically analyzed.
Results The first HCC recurrence rate was similar among patient groups. In contrast, the second HCC recurrence rate was significantly lower in the SVR group than in the non-SVR group ($p = 0.003$) and the IFN-untreated group ($p = 0.006$). In multivariate analysis, platelet count ($p = 0.033$) and number of tumors ($p = 0.001$) were associated with the first HCC recurrence, while SVR ($p = 0.002$) was the only factor associated with the second HCC recurrence. The survival rate was higher in the SVR group than in non-SVR and IFN-untreated groups, and SVR to previous IFN treatment was an independent factor associated with better survival ($p < 0.001$).
Conclusions SVR to previous IFN treatment before the development of HCV-related HCC was associated with lower risk of the second recurrence of HCC and better survival.

Keywords Hepatocellular carcinoma · Hepatitis C virus · Previous interferon therapy · Recurrence · Survival

Introduction

Chronic hepatitis and cirrhosis following hepatitis C virus (HCV) infection are major risk factors for hepatocellular carcinoma (HCC) [1–3]. Particular risk factors for developing HCV-related HCC in patients are advanced stage fibrosis, male gender, older age, heavy drinking, and high serum alanine aminotransferase (ALT) levels [4, 5]. Interferon (IFN) therapy improves hepatic inflammation and inhibits the progression of hepatic fibrosis [6]. Furthermore, treating patients with IFN with chronic HCV infection can prevent the development of HCC, particularly in patients with sustained virological response (SVR) to IFN therapy [7–13]. In contrast, HCC is liable to frequently recur even after curative therapy primarily because of its multicentric occurrence, leading to a poor prognosis [14–19]. The recurrence rate after resection of HCV-related HCC is higher in patients with HCV viremia than in those without it [20]. It has been reported that IFN therapy after resection or ablation of HCC reduces recurrence and improves prognosis in patients with HCV-related HCC [21–28]. However, no complete investigation has been performed of the possible effect of IFN therapy before HCC development on the outcome of curative treatment for

H. Miyatake · Y. Kobayashi · Y. Iwasaki (✉) · S. Nakamura · H. Ohnishi · K. Kuwaki · J. Toshimori · H. Hagihara · K. Nouso · K. Yamamoto
Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Kita-ku, Okayama 700-8558, Japan
e-mail: yiwasaki@cc.okayama-u.ac.jp

Published online: 12 October 2011

HCV-related HCC particularly in relation to the response to IFN treatment. Only a few relevant studies involving limited number of patients with previous IFN therapy are available [29–32].

The purpose of this study was to clarify the effect of previous IFN treatment before the development of HCV-related HCC on recurrence and prognosis after curative treatment of HCC in a large cohort of patients.

Patients and Methods

Patients

Between 1995 and 2006, 733 consecutive patients with HCC positive for HCV antibody and HCV RNA were diagnosed at Okayama University Hospital. Three hundred thirty-eight patients who did not receive curative treatment for HCC or undergo IFN therapy after the development of HCC were excluded from the study (Fig. 1). Inclusion criteria were as follows: (1) no evidence of HCC before consulting the Okayama University Hospital, (2) absence of hepatitis B surface antigen, (3) absence of co-existing liver diseases such as autoimmune hepatitis or primary biliary cirrhosis, and (4) absence of a history of alcohol abuse.

HCV infection was diagnosed on the basis of identification of anti-HCV antibodies using the first, second, or third enzyme-linked immunosorbent assays (Ortho Diagnostics, Tokyo, Japan). HCV RNA was identified by reverse transcription-polymerase chain reaction (RT-PCR) [33].

HCC was suspected on the basis of several imaging methods, including abdominal ultrasonography (US), dynamic computed tomography (CT), magnetic resonance imaging, and angiography. Diagnosis of HCC was confirmed by needle biopsy, by surgically resected tumor specimens, or by typical radiological findings on hepatic angiography or dynamic CT.

The study was conducted in accordance with the Helsinki Declaration and approved by the Ethical Committee of the institution.

Treatment

Of the 395 patients receiving curative treatment of HCC, 103 were treated with surgical resection and 292 with percutaneous tumor ablation (PTA) [34–37], that is, percutaneous ethanol injection therapy (PEIT) ($n = 116$), percutaneous microwave coagulation therapy (PMCT) ($n = 11$), or radiofrequency ablation (RFA) ($n = 165$). There were no patients who underwent liver transplantation or other modes of HCC treatment. The choice between surgical resection and PTA were determined according to the extent of tumor and hepatic functional reserve as assessed by Child's classification [38]. If the liver tumor consisted of fewer than three nodules that were less than 3 cm in diameter, patients were indicated

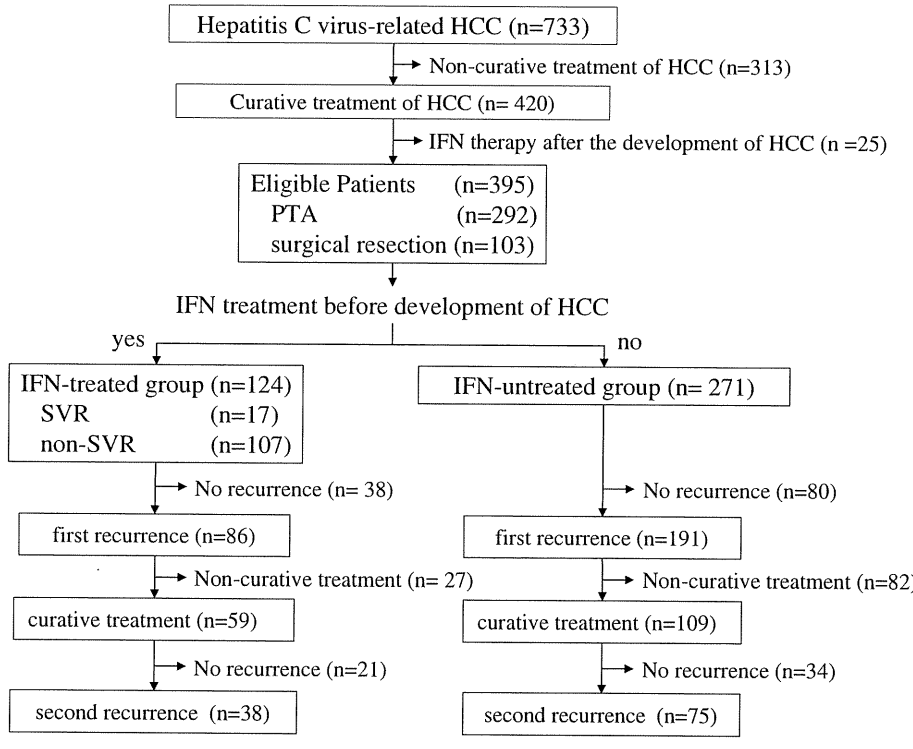

Fig. 1 Schematic presentation of patients with HCV-related hepatocellular carcinoma (HCC). Patients with HCV-related HCC who were diagnosed at Okayama University Hospital were classified into three groups according to their previous IFN treatment and response to that treatment. One hundred twenty-four patients had received IFN treatment before the development of HCC (IFN-treated group) and the remaining 271 had not (IFN-untreated group). Patients who had undergone IFN treatment before the development of HCC were further classified according to their response into a sustained virological response (SVR) group or a non-SVR group. Patients were regularly screened for HCC

for PTA. When a patient was indicated for both surgery and PTA, the modality of treatment was determined by patient choice after obtaining fully informed consent. PEIT was carried out under US guidance using a 15- or 20-cm-long needle (21 gauge) (Hakko, Chikuma, Japan) [35], PMCT was performed under US guidance using a 15-cm-long guide needle (14 gauge) according to the procedure described previously [37], and RFA was executed under US guidance using a 15- or 20-cm-long guide needle (16 gauge) (Tyco Healthcare Japan, Tokyo, Japan) [36]. PTA was repeated until complete necrosis of all HCC lesions was confirmed by dynamic CT. Treatment of HCC was considered curative, when no viable HCC lesions were detected on dynamic CT 3 months after completion of the treatment.

Of the 395 patients receiving curative treatment for HCC, 124 had received either human lymphoblastoid IFN, recombinant IFN-alpha 2a, or recombinant IFN-alpha 2b monotherapy for chronic HCV infection before the development of HCC (IFN-treated group), whereas 271 had not (IFN-untreated group) (Fig. 1). Patients received 6 million units of IFN by intramuscular injection three times weekly for 24 weeks as outpatients. If patients could not tolerate this dose, the IFN dose was reduced to 3 million units. SVR was defined as HCV RNA (as determined by RT-PCR; detection limit, 10^2 copies/ml) negativity for over 6 months after the termination of IFN therapy. SVR was achieved in 17 of the 124 patients (SVR group) and the remaining 107 were regarded as non-SVR (non-SVR group) (Fig. 1).

Follow-up of Patients

Patients attended a monthly medical consultation at the Okayama University Hospital outpatient clinic. Blood biochemical markers, including α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP), were measured every 1–2 months; US was performed every 2–3 months, and dynamic CT was performed every 6 months. If HCC recurrence was suspected, further imaging examinations including dynamic CT, magnetic resonance imaging, abdominal angiography, or US-guided tumor biopsy were performed to confirm the diagnosis.

New HCC foci as well as local recurrent nodules at tumor, node, metastasis (TNM) stage I, II, and III, were mainly treated by a second course of PTA; local recurrent nodules at TNM stage IV were treated with transarterial chemoembolization or chemotherapy. Further development of HCC and survival of patients (tumor recurrence rate and survival rate) were analyzed in relation to the time interval after treatment of HCC.

Table 1 Demographic and clinical characteristics of patients with HCV-related HCC

Groups	IFN-treated		IFN-untreated (n = 271)	p^a	p^b
	SVR (n = 17)	Non-SVR (n = 107)			
Characteristics					
Sex (men/women), n	13/4	60/47	187/84	0.049	0.112
Age (years)	63 (52–71)	65 (46–82)	67 (33–85)	0.018	0.061
Laboratory data					
Total bilirubin (mg/dl)	0.74 (0.40–1.29)	0.85 (0.36–3.28)	0.91 (0.16–4.13)	0.194	0.171
Albumin (g/dl)	4.4 (3.7–4.8)	3.7 (2.5–4.8)	3.6 (2.2–4.7)	<0.001	<0.001
Prothrombin time (%)	93 (70–121)	85 (47–142)	85 (40–145)	0.355	0.023
ALT (IU/l)	22 (10–54)	55 (12–198)	60 (14–201)	0.058	<0.001
Platelet count ($\times 10^4/\mu l$)	16.6 (8.4–30.3)	9.2 (2.8–37.2)	10.1 (3.2–31.9)	0.980	<0.001
Child–Pugh stage (A/B/C), n	17/0/0	87/20/0	213/54/4	0.236	0.049
Tumor-related variables					
Number of tumors (single/multiple), n	15/2	76/31	192/79	0.603	0.136
Size of largest tumor (mm)	20 (8–40)	18 (10–53)	20 (9–74)	0.033	0.942
AFP (ng/ml)	13 (1.9–25,716)	24 (1.7–3,480)	20 (0.6–54,535)	0.956	0.297
DCP (mAU/ml)	34 (1–35,000)	46 (10–56,000)	46 (1–66,700)	0.294	0.195
Initial treatment of HCC					
PTA/surgical resection, n	6/11	79/28	207/64	0.100	0.002

Laboratory data and tumor-related variables are at the development of HCC. Continuous variables are given as medians with ranges

HCV hepatitis C virus, *HCC* hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation

[a] IFN-treated versus IFN-untreated

[b] SVR versus non-SVR

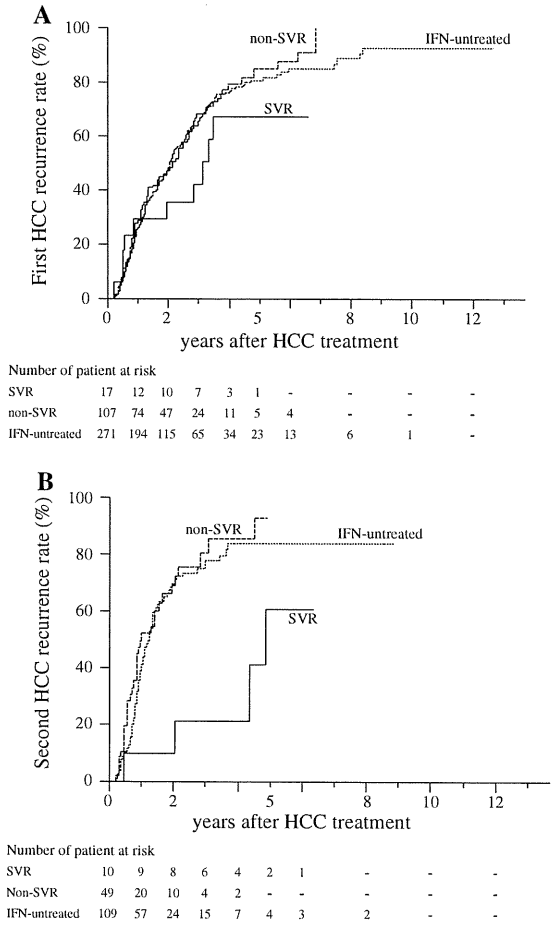

Fig. 2 Cumulative first (**a**, $n = 395$) and second (**b**, $n = 168$) HCC recurrence rates in patients with curative treatment of HCC according to the previous IFN treatment and response to the treatment. The first HCC recurrence rates were similar among SVR, non-SVR, and IFN-untreated groups (**a**). However, the second HCC recurrence rate in the SVR group at 2 years after HCC treatment was significantly lower than that in the non-SVR group (10 vs. 69%, $p = 0.003$) and the IFN-untreated group (10 vs. 70%, $p = 0.006$) (**b**)

Statistical Analysis

Statistical analysis was performed using JMP statistical discovery software, version 8.0 (SAS Institute Inc., Cary, NC). Differences between two groups were evaluated using the unpaired Student's t test or the Mann–Whitney U test. The Chi-square test or the Fisher's exact probability test was used to compare categorical data. Cumulative incidence curves were determined with the Kaplan–Meier method, and the differences between patient groups were assessed using the logrank test. Possible risk factors for recurrence of HCC and survival included both IFN-related variables and variables at the development and recurrence of HCC (age, total bilirubin level, albumin level, prothrombin time, ALT level, platelet count, number of tumors, largest tumor size, AFP level, and DCP level). Tumor associated variables, number of tumors and size of largest tumor, were transformed into categorical data consisting of two ordinal numbers by the median value. Variables exhibiting p values less than 0.10 in univariate analysis were subjected to a stepwise Cox proportional hazards regression analysis. A risk ratio with a 95% confidence interval was denoted for each analysis. p values less than 0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of patients at the development of HCC are shown in Table 1. The patient group comprised 260 men and 135 women (73 men and 51 women in the IFN-treated group), and median age was 58 years (65 years in the IFN-treated group). Of the 395 patients (80%), 317 were classified as Child–Pugh stage A. Significant differences were observed between IFN-treated and untreated patients in sex, age, albumin level, and size of largest tumor. On the other hand, significant differences were observed between IFN-treated patients with SVR and non-SVR in albumin level, prothrombin time, ALT level, platelet count, Child–Pugh stage, and initial treatment of HCC. This indicated better hepatic functional reserve in SVR patients than in non-SVR patients.

The median follow-up period after curative treatment of HCC for patients with and without IFN treatment was 3.8 years and 3.5 years, respectively. In the IFN-treated group, patients underwent IFN therapy 7.2 (0.8–17.4) (median and range) years before development of HCC. Of the 395 patients, 277 (70%) had recurrence of HCC during a median follow-up period of 2.1 (1.8–2.4) years [including 86 of 124 IFN-treated patients (69%)]. Of the 168 patients receiving curative treatment for the first recurrence of HCC, 113 (67%) had a second HCC recurrence during a median follow-up period of 1.3 (1.0–1.4) years [including 38 of 59 IFN-treated patients (64%)] (Fig. 1).

HCC Recurrence Rates

The rates of the first and second HCC recurrence after curative treatment of primary HCC in each treatment group are shown in Fig. 2. In the IFN-treated group, 86 patients (10 with SVR and 76 with non-SVR) had the first HCC recurrence and 38 (four with SVR and 34 with non-SVR) had the second HCC recurrence during the follow-up period. The average times to the first and second HCC recurrence were 632 and 1,069 days, 661 and 401 days, and 666 and 428 days in SVR, non-SVR, and IFN-untreated groups, respectively. The rates of the first recurrence at 2 years in SVR, non-SVR, and IFN-untreated groups were 36, 47, and 48%, respectively. The differences between these rates were not statistically significant

($p = 0.410$) (Fig. 2a). However, the rates of the second HCC recurrence at 2 years were significantly lower in the SVR group than in the non-SVR group (10 vs. 69%, $p = 0.003$) and in the IFN-untreated group (10 vs. 70%, $p = 0.006$) (Fig. 2b). There was no significant difference in the second HCC recurrence rates between non-SVR and IFN-untreated groups ($p = 0.441$). In multivariate analysis, platelet count ($p = 0.033$) and number of tumors ($p = 0.001$) were independent factors associated with the first recurrence of HCC (Table 2), whereas SVR to previous IFN therapy ($p = 0.002$) was the only factor associated with lower risk for the second recurrence of HCC (Table 3).

Overall Survival

Survival rates after curative treatment of primary HCC in each group are shown in Fig. 3. A tendency was observed toward a higher survival rate in the IFN-treated group than in the IFN-untreated group but it was not significant ($p = 0.053$) (Fig. 3a). In contrast, survival rates at 5 years were higher in the SVR group (100%) than in non-SVR (73%) and IFN-untreated groups (62%) ($p = 0.004$) (Fig. 3b). No significant difference was observed in the survival rates between non-SVR and IFN-untreated groups ($p = 0.450$). In multivariate analysis, SVR to previous IFN therapy ($p < 0.001$), albumin level ($p = 0.006$), number of tumors ($p = 0.007$), and AFP level ($p = 0.046$) were independent factors associated with overall death after curative treatment of primary HCC (Table 4).

Discussion

In the present study, we have demonstrated that patients with SVR to previous IFN treatment before development of HCC showed lower risk for the second recurrence of HCC and better survival compared to patients with non-SVR to previous IFN treatment or IFN-untreated patients. Several studies have demonstrated that IFN therapy reduces the risk of HCC development among chronic hepatitis C patients. On the other hand, a few reports are available on the influence of previous IFN therapy before the development of HCC on patient outcomes after curative treatment of HCV-related HCC. It was initially reported that HCV-related HCC patients who received IFN therapy before development of HCC showed lower recurrence rates and better survival rates, independent of response to IFN therapy, compared to those without previous IFN therapy [29, 30]. It has recently been reported that patients showing

Table 2 Risk factors for the first recurrence of HCC ($n = 395$)

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Sex (male)	1.17 (0.91–1.51)	0.229	–	
IFN-related variables				
IFN-untreated	1			
Non-SVR	1.07 (0.82–1.39)	0.623	–	
SVR	0.68 (0.34–1.22)	0.209	–	
Variables at the development of HCC				
Age (≥60 years)	1.13 (0.84–1.56)	0.434	–	
Total bilirubin (≥1.0 mg/dl)	1.07 (0.83–1.37)	0.579	–	
Albumin (<3.5 g/dl)	1.34 (1.04–1.71)	0.022	1.24 (0.95–1.61)	0.108
Prothrombin time (<70%)	1.07 (0.79–1.43)	0.664	–	
ALT (≥40 IU/l)	1.09 (0.83–1.43)	0.542	–	
Platelet count(<10 × 10^4/μl)	1.37 (1.08–1.75)	0.009	1.34 (1.04–1.75)	0.026
Tumor-related variables				
Number of tumors (multiple vs. single)	1.66 (1.27–2.15)	<0.001	1.63 (1.24–2.14)	0.001
Size of largest tumor (≥20 mm)	1.24 (0.98–1.57)	0.074	1.22 (0.94–1.59)	0.140
AFP (≥100 ng/ml)	1.45 (1.07–1.92)	0.016	1.30 (0.96–1.74)	0.093
DCP (≥40 mAU/ml)	1.33 (1.02–1.75)	0.034	1.11 (0.85–1.44)	0.448
Initial treatment of HCC				
PTA/surgical resection	1.09 (0.84–1.43)	0.530	–	

HCC hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

Table 3 Risk factors for the second recurrence of HCC ($n = 168$)

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Sex (male)	1.07 (0.73–1.61)	0.719	–	
IFN-related variables				
IFN-untreated	1		1	
Non-SVR	1.17 (0.77–1.74)	0.447	1.09 (0.68–1.72)	0.718
SVR	0.27 (0.08–0.65)	0.002	0.10 (0.01–0.50)	0.002
Variables at the development of HCC				
Age (≥60 years)	1.50 (0.91–2.61)	0.115	–	
Total bilirubin (≥1.0 mg/dl)	1.08 (0.72–1.60)	0.701	–	
Albumin (<3.5 g/dl)	1.04 (0.68–1.57)	0.847	–	
Prothrombin time (<70%)	1.18 (0.70–1.89)	0.529	–	
ALT (≥40 IU/l)	1.30 (0.86–2.01)	0.220	–	
Platelet count (<10 × 10^4/μl)	1.00 (0.69–1.47)	0.984	–	
Number of tumors (multiple vs. single)	1.57 (1.04–2.32)	0.033	1.51 (0.93–2.42)	0.098
Size of largest tumor (≥20 mm)	0.91 (0.63–1.32)	0.613	–	
AFP (≥100 ng/ml)	0.65 (0.38–1.06)	0.084	0.77 (0.39–1.39)	0.391
DCP (≥40 mAU/ml)	0.81 (0.54–1.23)	0.331	–	
Initial treatment of HCC				
PTA/surgical resection	1.12 (0.75–1.69)	0.595	–	
Variables at the first recurrence of HCC				
Age (≥60 years)	0.97 (0.46–2.39)	0.950		
Total bilirubin (≥1.0 mg/dl)	0.94 (0.59–1.46)	0.785	–	
Albumin (<3.5 g/dl)	1.67 (1.06–2.61)	0.029	1.47 (0.90–2.36)	0.125
Prothrombin time (<70%)	1.24 (0.60–2.30)	0.531	–	
ALT (≥40 IU/l)	1.49 (0.95–2.40)	0.083	1.21 (0.75–2.01)	0.452
Platelet count (<10 × 10^4/μl)	1.13 (0.74–1.73)	0.573	–	
Number of tumors (multiple vs. single)	2.09 (1.37–3.13)	<0.001	1.47 (0.91–2.34)	0.112
Size of largest tumor (≥20 mm)	0.96 (0.62–1.45)	0.840	–	
AFP (≥100 ng/ml)	0.72 (0.32–1.41)	0.355	–	
DCP (≥40 mAU/ml)	1.05 (0.67–1.63)	0.842	–	

HCC hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

biochemical response, with or without SVR to previous IFN therapy, showed higher tumor-free survival rates after surgery than those without such a response to IFN or those without previous IFN therapy [31, 32]. In these previous reports, a biochemical response as well as SVR to previous IFN therapy was associated with favorable outcome, demonstrating the importance of response to previous IFN therapy for the outcome after surgery of HCV-related HCC.

However, in the present study, patients with non-SVR showed similar recurrence and survival rates as IFN-untreated patients. Furthermore, no difference was observed in the recurrence and survival rates among non-SVR patients with and without biochemical response to previous IFN therapy (data not shown). In fact, only patients with SVR to previous IFN therapy showed better outcome than those with non-SVR or IFN-untreated patients. Therefore, the present data indicate that SVR but not biochemical response without SVR to previous IFN treatment is a predictor of favorable outcome in patients who have developed HCC.

The reason for the difference between the present and previous studies in the outcome of non-SVR patients with biochemical response to previous IFN therapy is currently unknown. In patients with HCV-related chronic hepatitis and cirrhosis, who received IFN therapy and showed normalization of ALT levels, suppression of primary HCC development and better survival rates have been independently demonstrated of eradication of HCV infection by the IFN therapy [10, 11, 13, 39]. However, this

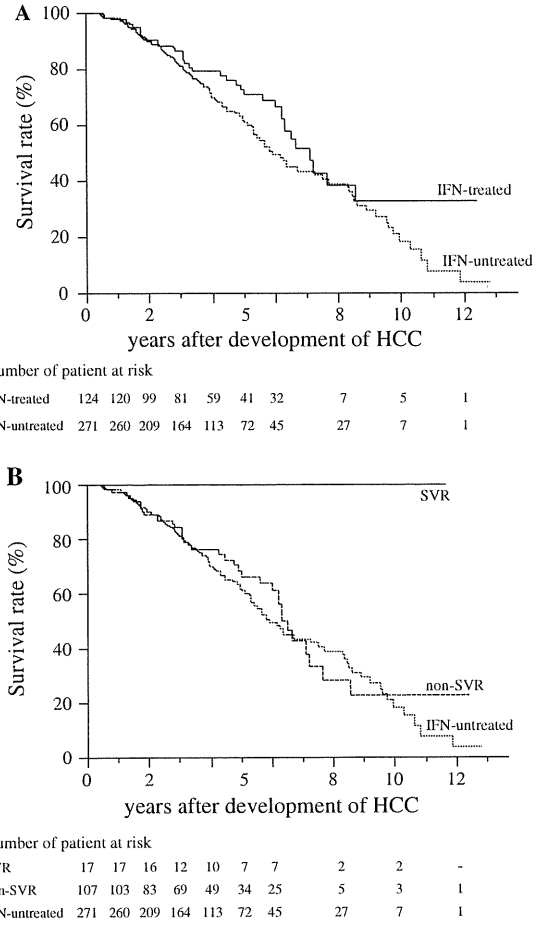

Fig. 3 Overall survival rates of HCV-related HCC patients ($n = 395$) according to their previous IFN treatment before development of HCC (**a**) and their response to the treatment (**b**). A tendency was observed toward a higher survival rate in the IFN-treated group than in the IFN-untreated group but it was not significant ($p = 0.053$) (**a**). On the other hand, the survival rate of the SVR group was significantly higher than those of non-SVR and IFN-untreated groups ($p = 0.004$) (**b**)

suppression observed for primary carcinogenesis in non-SVR patients with biochemical response to IFN therapy does not appear to be the case for secondary carcinogenesis in the present study. The period after IFN therapy was much longer in the present study than in the previous reports on primary carcinogenesis. The IFN therapy had preceded to the HCC development, that should have required long incubation after the termination of IFN treatment, and in the present study the observation of HCC recurrence and survival started with the curative treatment of the primary HCC. In patients who had sustained biochemical response but had not eradicated HCV infection, we and others demonstrated that platelet count transiently increases following IFN therapy but decrease over the following 3 years after the termination of IFN therapy. On the other hand, in patients with SVR an increase followed by persistence in platelet counts was observed [40, 41]. These observations suggest the progression of fibrosis during a longer incubation period after IFN therapy, even in the non-SVR patients with biochemical response to the therapy. Therefore, the suppressive effect of IFN therapy on development of HCC may not persist beyond the development of primary HCC particularly in these patients.

It has also been demonstrated that HCV core transgenic mice can develop HCC without apparent hepatitis [42]. Therefore, besides active hepatitis, which involves persistent hepatocyte death and regeneration, and should result in both genetic and epigenetic disorders as well as increased oxidative stress, the presence and persistence of HCV infection and viral products such as core protein may themselves play an important role in the development of HCC in non-SVR patients with biochemical response. Thus, patients with SVR who had eradicated HCV infection should have a lower incidence of HCC recurrence and higher survival rates than non-SVR patients with biochemical response.

In the present study, patients with SVR showed a better overall survival rate than other groups. However, although patients with SVR showed lower rates of the second HCC recurrence, this was not the case for the first HCC recurrence. Although both SVR and non-SVR groups have a carcinogenic background during the development of primary HCC, the carcinogenic potential in SVR patients may be gradually attenuated because of the eradication of HCV infection, whereas it may increase in those with non-SVR because of persistence of HCV infection and relapse of hepatitis, finally leading to progression of fibrosis over a longer period. However, a substantial time may be required before differences between patients with and without SVR become apparent, and these differences eventually become significant in the second recurrence of HCC.

It should also be noted that IFN-treated patients enrolled in the present and previous studies are a selected cohort, since the incidence rates of HCC development in patients treated with IFN should be lower than in those untreated with IFN [13]. This is particularly the case for patients with SVR to previous IFN treatment, whose risk for development of HCC is less than one fifth of that for IFN-untreated patients [13]. Reported risk factors for HCC development in patients who received IFN therapy include advanced fibrosis, lower platelet count, advanced age, male gender, and regular drinking [8, 9, 12, 13, 43]. Therefore, in the present study, HCC patients who received IFN therapy before the development of HCC may have demonstrated many of these characteristics, making them more prone to develop HCC than those not developing HCC after IFN therapy and not included in this study. Furthermore, it has been suggested that cirrhotic patients who develop primary

Table 4 Analysis of factors associated with overall death after curative treatment for primary HCC ($n = 395$)

Variable	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
Sex (male)	1.02 (0.73–1.44)	0.911	–	
IFN-related variables				
IFN-untreated	1		1	
Non-SVR	0.86 (0.59–1.24)	0.445	1.05 (0.71–1.54)	0.794
SVR	<0.01 (0–0.17)	<0.001	<0.01 (0–0.26)	<0.001
Variables at the development of HCC				
Age (≥60 years)	1.06 (0.72–1.63)	0.773	–	
Total bilirubin (≥1.0 mg/dl)	1.45 (1.04–2.01)	0.028	1.21 (0.82–1.76)	0.332
Albumin (<3.5 g/dl)	2.07 (1.49–2.89)	<0.001	1.70 (1.16–2.49)	0.007
Prothrombin time (<70%)	1.44 (0.99–2.06)	0.059	0.97 (0.65–1.43)	0.874
ALT (≥40 IU/L)	1.12 (0.78–1.67)	0.531	–	
Platelet count ($<10 \times 10^4$ μl)	1.72 (1.23–2.41)	0.001	1.35 (0.93–1.96)	0.118
Tumor-related variables				
Number of tumors (multiple vs. single)	1.59 (1.10–2.26)	0.014	1.71 (1.16–2.46)	0.007
Size of largest tumor (≥20 mm)	1.15 (0.83–1.60)	0.395	–	
AFP (≥100 ng/ml)	1.71 (1.17–2.45)	0.006	1.50 (1.00–2.18)	0.047
DCP (≥40 mAU/ml)	1.33 (0.91–1.98)	0.145	–	
Initial treatment of HCC				
PTA/surgical resection	1.69 (1.16–2.53)	0.006	1.03 (0.68–1.60)	0.882

HCC hepatocellular carcinoma, *IFN* interferon, *SVR* sustained virological response, *ALT* alanine aminotransferase, *AFP* α-fetoprotein, *DCP* des-γ-carboxy prothrombin, *PTA* percutaneous tumor ablation, *CI* confidence interval

HCC may already be at a "carcinogenic stage" and have a higher potential to develop intrahepatic multicentric carcinogenesis than those without HCC [15]. Patients who have already developed HCC may have background features such as greater age and impaired liver function because of more advanced fibrosis. Therefore, the observed recurrence and survival rates in the present study are those of selected patients who were already at the carcinogenic stage, and are thus biased in comparison to previous observations on primary prevention of HCC development in patients who had received IFN therapy. Recently, Imai et al. reported that an inhibitory effect of IFN therapy on development of HCC in older patients was limited to patients with SVR [44]. This also supports the notion that patients already at a carcinogenic stage or with risk factors associated with HCC development, such as greater age or advanced fibrosis, require eradication of HCV infection in order to achieve a significantly better prognosis.

The present observation highlights the importance of eradication of HCV in order to prevent HCC recurrence and to achieve better survival in this patient group. Plenty of reports are available that demonstrated the favorable effect of IFN therapy on the recurrence of HCC and survival particularly in patients who achieved SVR [21–28]. Therefore, re-treatment with more potent IFN therapies, such as combination therapy of PEGylated IFN plus ribavirin [45], should be recommended for patients who previously underwent IFN treatment without achieving SVR.

The present study has limitations as it is retrospective in nature, and thus, patients enrolled were biased in favor of experience of IFN treatment, and also HCC patients with previous IFN treatment were a selected population from a large cohort of patients who had undergone IFN treatment. Also, information on the histological data that may have influence on the outcome of HCC patients was not available in the present study. Further prospective studies are required to address these issues.

In conclusion, the present study demonstrated that patients with SVR to IFN treatment before the development of HCV-related HCC showed lower second HCC recurrence rates and higher survival rates than those with non-SVR to previous IFN treatment or IFN-untreated patients. Therefore, treatment with potent antiviral therapy is recommended for patients in the latter groups in order to suppress recurrence and improve survival by eradicating HCV infection.

Acknowledgments The authors thank Dr. Kenji Miyoshi and Dr. Shota Iwadou for contribution to this study.

References

1. Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. *Semin Liver Dis.* 1995;15:64–69. doi:10.1055/s-2007-1007263.
2. Shiratori Y, Shiina S, Imamura M, et al. Characteristic difference of hepatocellular carcinoma between hepatitis B- and C- viral infection in Japan. *Hepatology.* 1995;22:1027–1033.
3. Tanaka K, Hirohata T, Koga S, et al. Hepatitis C and hepatitis B in the etiology of hepatocellular carcinoma in the Japanese population. *Cancer Res.* 1991;51:2842–2847.
4. Colombo M, de Franchis R, Del Ninno E, et al. Hepatocellular carcinoma in Italian patients with cirrhosis. *N Engl J Med.* 1991;325:675–680. doi:10.1056/NEJM199109053251002.
5. Tsukuma H, Hiyama T, Tanaka S, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med.* 1993;328:1797–1801. doi:10.1056/NEJM199306243282501.
6. Shiratori Y, Imazeki F, Moriyama M, et al. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med.* 2000;132:517–524.
7. International Interferon-alpha Hepatocellular Carcinoma Study Group. Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. *Lancet.* 1998;351:1535–1539.
8. Imai Y, Kawata S, Tamura S, et al. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med.* 1998;129:94–99.
9. Kasahara A, Hayashi N, Mochizuki K, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology.* 1998;27:1394–1402. doi:10.1002/hep.510270529.
10. Nishiguchi S, Kuroki T, Nakatani S, et al. Randomised trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet.* 1995;346:1051–1055.
11. Nishiguchi S, Shiomi S, Nakatani S, et al. Prevention of hepatocellular carcinoma in patients with chronic active hepatitis C and cirrhosis. *Lancet.* 2001;357:196–197. doi:10.1016/S0140-6736(00)03595-9.
12. Okanoue T, Itoh Y, Minami M, et al. Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in an advanced stage: a retrospective study in 1148 patients. Viral Hepatitis Therapy Study Group. *J Hepatol.* 1999;30:653–659.
13. Yoshida H, Shiratori Y, Moriyama M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med.* 1999;131:174–181.
14. Belghiti J, Panis Y, Farges O, Benhamou JP, Fekete F. Intrahepatic recurrence after resection of hepatocellular carcinoma complicating cirrhosis. *Ann Surg.* 1991;214:114–117.
15. Ikeda K, Arase Y, Kobayashi M, et al. Significance of multicentric cancer recurrence after potentially curative ablation of hepatocellular carcinoma: a long-term cohort study of 892 patients with viral cirrhosis. *J Gastroenterol.* 2003;38:865–876. doi:10.1007/s00535-003-1163-2.
16. Lam VWT, Ng KKC, Chok KSH, et al. Risk factors and prognostic factors of local recurrence after radiofrequency ablation of hepatocellular carcinoma. *J Am Coll Surg.* 2008;207:20–29. doi:10.1016/j.jamcollsurg.2008.01.020.
17. N'Kontchou G, Mahamoudi A, Aout M, et al. Radiofrequency ablation of hepatocellular carcinoma: long-term results and prognostic factors in 235 Western patients with cirrhosis. *Hepatology.* 2009;50:1475–1483. doi:10.1002/hep.23181.
18. Okada S, Shimada K, Yamamoto J, et al. Predictive factors for postoperative recurrence of hepatocellular carcinoma. *Gastroenterology.* 1994;106:1618–1624.
19. Shiina S, Tagawa K, Niwa Y, et al. Percutaneous ethanol injection therapy for hepatocellular carcinoma: results in 146 patients. *AJR Am J Roentgenol.* 1993;160:1023–1028.
20. Kubo S, Nishiguchi S, Shuto T, et al. Effects of continuous hepatitis with persistent hepatitis C viremia on outcome after resection of hepatocellular carcinoma. *Jpn J Cancer Res.* 1999;90:162–170.
21. Breitenstein S, Dimitroulis D, Petrowsky H, Puhan MA, Mullhaupt B, Clavien PA. Systematic review and meta-analysis of interferon after curative treatment of hepatocellular carcinoma in patients with viral hepatitis. *Br J Surg.* 2009;96:975–981. doi:10.1002/bjs.6731.
22. Ikeda K, Arase Y, Saitoh S, et al. Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor-A prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology.* 2000;32:228–232. doi:10.1053/jhep.2000.9409.
23. Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Kinoshita H. Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg.* 2002;89:418–422. doi:10.1046/j.0007-1323.2001.02054.x.
24. Kubo S, Nishiguchi S, Hirohashi K, et al. Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. *Ann Intern Med.* 2001;134:963–967.
25. Mazzaferro V, Romito R, Schiavo M, et al. Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology.* 2006;44:1543–1554. doi:10.1002/hep.21415.
26. Shen YC, Hsu C, Chen LT, Cheng CC, Hu FC, Cheng AL. Adjuvant interferon therapy after curative therapy for hepatocellular carcinoma (HCC): a meta-regression approach. *J Hepatol.* 2010;52:889–894. doi:10.1016/j.jhep.2009.12.041.
27. Shiratori Y, Shiina S, Teratani T, et al. Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. *Ann Intern Med.* 2003;138:299–306.
28. Singal AK, Freeman DH Jr, Anand BS. Meta-analysis: interferon improves outcomes following ablation or resection of hepatocellular carcinoma. *Aliment Pharmacol Ther.* 2010;32:851–858. doi:10.1111/j.1365-2036.2010.04414.x.
29. Komorizono Y, Sako K, Yamasaki N, et al. Outcome of patients with hepatitis C virus-related hepatocellular carcinoma occurring after interferon therapy. *Anticancer Res.* 2002;22:3573–3578.
30. Kubo S, Nishiguchi S, Hirohashi K, et al. Influence of previous interferon therapy on recurrence after resection of hepatitis c virus-related hepatocellular carcinoma. *Jpn J Cancer Res.* 2001;92:59–66.
31. Uenishi T, Kubo S, Hirohashi K, et al. Relationship between response to previous interferon therapy and postoperative recurrence of hepatitis C virus-related hepatocellular carcinoma. *Hepatol Res.* 2002;24:404–412.
32. Uenishi T, Nishiguchi S, Tamori A, et al. Influence of interferon therapy on outcome after surgery for hepatitis C virus-related hepatocellular carcinoma. *Hepatol Res.* 2006;36:195–200. doi:10.1016/j.hepres.2006.07.011.

33. Kato N, Yokosuka O, Omata M, Hosoda K, Ohto M. Detection of hepatitis C virus ribonucleic acid in the serum by amplification with polymerase chain reaction. *J Clin Invest*. 1990;86: 1764–1767. doi:10.1172/JCI114903.
34. Farmer DG, Rosove MH, Shaked A, Busuttil RW. Current treatment modalities for hepatocellular carcinoma. *Ann Surg*. 1994;219:236–247.
35. Lee MJ, Mueller PR, Dawson SL, et al. Percutaneous ethanol injection for the treatment of hepatic tumors: indications, mechanism of action, technique, and efficacy. *AJR Am J Roentgenol*. 1995;164:215–220.
36. Livraghi T, Goldberg SN, Lazzaroni S, Meloni F, Solbiati L, Gazelle GS. Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. *Radiology*. 1999;210:655–661.
37. Seki T, Wakabayashi M, Nakagawa T, et al. Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma. *Cancer*. 1994;74:817–825.
38. Arii S, Yamaoka Y, Futagawa S, et al. Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinomas: a retrospective and nationwide survey in Japan. The Liver Cancer Study Group of Japan. *Hepatology*. 2000;32:1224–1229. doi: 10.1053/jhep.2000.20456.
39. Yoshida H, Arakawa Y, Sata M, et al. Interferon therapy prolonged life expectancy among chronic hepatitis C patients. *Gastroenterology*. 2002;123:483–491.
40. Moriyama M, Matsumura H, Aoki H, et al. Long-term outcome, with monitoring of platelet counts, in patients with chronic hepatitis C and liver cirrhosis after interferon therapy. *Intervirology*. 2003;46:296–307. doi:10.1159/000073209.
41. Taniguchi H, Iwasaki Y, Fujiwara A, et al. Long-term monitoring of platelet count, as a non-invasive marker of hepatic fibrosis progression and/or regression in patients with chronic hepatitis C after interferon therapy. *J Gastroenterol Hepatol*. 2006;21: 281–287. doi:10.1111/j.1440-1746.2006.04201.x.
42. Moriya K, Fujie H, Shintani Y, et al. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. *Nat Med*. 1998;4:1065–1067. doi:10.1038/2053.
43. Iwasaki Y, Takaguchi K, Ikeda H, et al. Risk factors for hepatocellular carcinoma in Hepatitis C patients with sustained virologic response to interferon therapy. *Liver Int*. 2004;24:603–610. doi:10.1111/j.1478-3231.2004.0956.x.
44. Imai Y, Tamura S, Tanaka H, et al. Reduced risk of hepatocellular carcinoma after interferon therapy in aged patients with chronic hepatitis C is limited to sustained virological responders. *J Viral Hepat*. 2010;17:185–191. doi:10.1111/j.1365-2893.2009.01163.x.
45. Huang JF, Yu ML, Huang CF, et al. The efficacy and safety of pegylated interferon plus ribavirin combination therapy in chronic hepatitis c patients with hepatocellular carcinoma post curative therapies—a multicenter prospective trial. *J Hepatol*. 2011;54:219–226. doi:10.1016/j.jhep.2010.07.011.

Int J Clin Oncol (2011) 16:210–220
DOI 10.1007/s10147-010-0150-x

ORIGINAL ARTICLE

Effect of pegylated interferon therapy on intrahepatic recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma

Hiroaki Hagihara · Kazuhiro Nouso · Yoshiyuki Kobayashi · Yoshiaki Iwasaki · Shinichiro Nakamura · Kenji Kuwaki · Junichi Toshimori · Hirokazu Miyatake · Hideki Ohnishi · Hidenori Shiraha · Kazuhide Yamamoto

Received: 7 July 2010 / Accepted: 2 November 2010 / Published online: 9 December 2010

Abstract
Background We wished to determine whether pegylated interferon (PEG-IFN) therapy after curative treatment of hepatocellular carcinoma (HCC) prevents a recurrence of HCC.
Methods Thirty-seven HCC patients with hepatitis C virus (HCV) infection who were treated with PEG-IFN after curative treatment (PEG-IFN group) and 145 controls without IFN therapy (non-IFN group) were enrolled. The overall survival and recurrence-free survival rates were compared between the groups, and the predisposing factors for recurrence and survival were analyzed. The rates were also examined by propensity score (PS) matched analysis that could minimize selection biases.
Results The median follow-up period was 3.7 years. The 5-year survival rate in the PEG-IFN group (91%) was significantly higher than that in the non-IFN group (56%; $P < 0.01$). The rate of the second recurrence but not that of the first recurrence of HCC in the sustained virological responder (SVR) group was lower than that in the non-IFN group ($P = 0.03$). Improvement of survival by PEG-IFN and low rate of second recurrence in the SVR group were also observed in PS matched analysis. Multivariate analysis revealed that PEG-IFN therapy and high serum albumin were good prognostic factors for survival. Although low serum albumin and large and multiple tumors were risk factors for the first recurrence, non-SVR and low serum albumin were risk factors for the second recurrence.
Conclusion PEG-IFN-therapy after curative treatment of HCC improved the rate of survival, and SVR was found to be closely correlated with the prevention of recurrence.

Keywords Hepatitis C virus · Hepatocellular carcinoma · Recurrence · Survival · PEG-IFN

H. Hagihara (✉) · K. Nouso · Y. Kobayashi · Y. Iwasaki · S. Nakamura · K. Kuwaki · J. Toshimori · H. Miyatake · H. Ohnishi · H. Shiraha · K. Yamamoto
Department of Gastroenterology and Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Kita-ku, Okayama 700-8558, Japan
e-mail: h2mof1977@gmail.com

K. Nouso
Department of Molecular Hepatology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

H. Miyatake
Department of Gastroenterology and Hepatology, Mitoyo General Hospital, Kagawa, Japan

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. Chronic infection with hepatitis C virus (HCV) is one of the major causes of HCC [1–3], and the percentage of HCC patients with HCV infection is about 70% in Japan. Recent advances in imaging and treatment modalities have improved the prognosis of patients with HCV-related HCC, but outcomes are still unsatisfactory. The 5-year survival rate is only 50–70%, even after curative treatment [4, 5], such as surgical resection and percutaneous ablation [percutaneous ethanol injection therapy (PEIT), microwave coagulation therapy (MCT), and radiofrequency thermal ablation (RFA)] [6, 7]. This unfavorable prognosis is caused by high intrahepatic tumor recurrence rates and sustained hepatic damage, both correlated with sustained viral infection [8]. The rate of intrahepatic tumor recurrence within 1 year is

20–40%, rising to about 80% by 5 years [9–11]. Thus, alleviation of the effect of HCV is a high priority for improving the prognosis of patients with HCV-related HCC.

Interferon (IFN) therapy is effective in reducing serum alanine transaminase (ALT) activity and in eradicating HCV [12, 13]. Thus, IFN could have value in minimizing hepatic necrosis, inflammation, and fibrosis, as well as reducing the incidence of HCC. In 1995, a small randomized controlled trial (RCT) showed a reduction in the incidence of HCC in cirrhotic patients with HCV infection by IFN treatment [14]. Yu et al. [15] reported that the cumulative incidences of HCC were 12.2% and 35.2% in IFN-treated and untreated chronic hepatitis C patients, respectively ($P = 0.001$). Tanaka et al. [16] also reported that interferon therapy decreased the risk of developing HCC by 48% compared with that in a control group ($P = 0.064$). In addition, several recent studies have shown that IFN therapy, even after curative treatment of HCV-related HCC, could prevent recurrence and improve the rate of survival [17–30]. Because these studies used different IFN regimens and the background characteristics of patients were diverse, the results varied, and no standard IFN regimen has been established for patients after curative treatment of HCV-related HCC.

Recently, the administration of pegylated interferon (PEG-IFN) has become the standard treatment for patients with chronic HCV infection. Treatment with PEG-IFN and oral ribavirin produces a virological response in more than 50% of patients, which is better than that in conventional α-IFN therapy [31, 32]. However, there are few reports that demonstrate the effect of PEG-IFN therapy after curative treatment of HCV-related HCC.

The present study involves analysis of the efficacy of PEG-IFN after the curative treatment of HCC for the prevention of HCC recurrence and for improving the rate of survival.

Patients and methods

Patients

From January 1997 until March 2009, 358 consecutive patients with HCV-related HCC underwent curative treatment as an initial treatment at Okayama University Hospital. Here, curative treatment is defined as surgical operation (resection; $n = 86$), RFA ($n = 228$), PEIT ($n = 30$), or MCT ($n = 14$). Among the patients, 176 patients were excluded because 163 patients had previously received IFN therapy and, for 13 patients, information was lacking on whether they had previously received IFN treatment. The remaining 182 patients were enrolled in the study. Informed consent was obtained from all patients for use of their clinical data. The study protocol conformed to the ethical guidelines of the World Medical Association Declaration of Helsinki, and was approved by the ethical committees of the institute. This study is a retrospective cohort study.

Diagnosis

HCC was diagnosed on the basis of typical findings by ultrasonography, computed tomography (CT) scans, and magnetic resonance imaging (MRI) scans (hyperattenuation in the arterial phase and hypoattenuation in the portal-venous phase). The imaging diagnoses were confirmed by at least two imaging modalities. The diagnosis of HCC was confirmed histopathologically with ultrasound-guided biopsy in nine patients because no typical findings were identified in imaging modalities.

IFN therapy

After curative treatment of primary HCC and confirmed that no residual tumor was existed by imaging modalities, 37 of the 182 patients were assigned to PEG-IFN therapy (PEG-IFN group). The remaining 145 patients did not receive any IFN treatment (non-IFN group). IFN treatment was performed on patients who agreed to use IFN after receiving a full explanation regarding the benefits and side effects of the treatment and who met the following inclusion criteria: (1) tumor–node–metastasis (TNM) stage of I, II, or III; (2) detectable serum HCV-RNA; (3) seronegative for hepatitis B virus surface antigen; (4) Child-Pugh class A or B; (5) platelet count above 80,000/mm^3; and (6) age less than 75 years. In the PEG-IFN group, 15 patients received 90–180 μg pegylated interferon alpha-2a (Pegasys; F-Hoffmann-La Roche, Basel, Switzerland) subcutaneously once per week for 24–48 weeks, and 22 patients received 60–100 μg pegylated interferon alpha-2b (PegIntron; Schering-Plough, Kenilworth, NJ, USA) plus ribavirin (Rebetol; Schering-Plough) at 600–800 mg/body for 24–48 weeks, according to the guideline on medical care for chronic hepatitis C prepared by the Ministry of Health, Labor and Welfare of Japan [33]. The median period between the day of curative treatment and PEG-IFN therapy was 242 days.

Patients stopped posttreatment PEG-IFN therapy when HCC recurrence was detected or if the hemoglobin level was <8.5 g/dl, the leukocyte count was <1,000/mm^3, the neutrophil count was <500/mm^3, or the platelet count was <50,000/mm^3, and then restarted the therapy after the treatment of HCC whenever possible.

In the control group (non-IFN group), the patients were prescribed ursodeoxycholic acid (UDCA) and the stronger neo-minophagen C (SNMC).

A sustained virological response (SVR) was defined as HCV-RNA negativity, determined by reverse transcription-polymerase chain reaction, more than 6 months after the termination of IFN therapy. The rest of the patients were considered to have exhibited a nonsustained virological response (non-SVR).

Follow-up of the patients

After curative treatment of primary HCC, all patients underwent liver function tests every 1–2 months, and ultrasonography or three-phase dynamic CT scanning every 3 months. The serum levels of alpha-fetoprotein (AFP), AFP-L3, and des-γ-carboxy prothrombin (DCP) were also determined every 2–3 months. The recurrence of HCC was diagnosed using the same criteria as for the initial development of HCC.

Statistical analysis

Statistical analysis was performed using SAS version 9.1 package and JMP software, version 8.0 (SAS Institute, Cary, NC, USA). Differences between two groups were evaluated using the unpaired Student's *t* test. The χ^2 test or Fisher's exact probability test was used to compare categorical data. Cumulative incidence curves were determined with the Kaplan–Meier method, and the differences between groups were assessed using the log-rank test. Possible risk factors for survival and HCC recurrence were examined by the Cox proportional hazards regression model with the following 12 variables: interferon-related variables (application of interferon therapy, response to interferon therapy, and HCV genotype), background, liver function, and tumor factors at the first treatment and at recurrence of HCC [age, alanine aminotransferase (ALT), albumin (ALB), total bilirubin (T.Bil), platelet counts (PLT), prothrombin time (PT), AFP, DCP, maximum tumor size, and tumor number]. Parameters that proved to be significant in the univariate analysis were tested by the multivariate Cox proportional hazards regression model.

We also conducted propensity score (PS) matched analysis that can adjust the clinical background of the patients in each group. To calculate PS, we used seven covariates: sex of patients, and variables at the time of development of HCC (age at the time of development of HCC, ALT, ALB, T.Bil, PLT, maximum tumor size, and tumor numbers). The propensity score of choosing the IFN treatment was calculated, followed by matching IFN group and non-IFN group according to a greedy matching technique [34]. The survival and recurrence rates of matched patients were compared by the Kaplan–Meier method and the differences were evaluated by the log-rank test. A *P* value less than 0.05 was considered statistically significant.

Results

Characteristics of the patients

Table 1 shows the clinical features of the patients in the PEG-IFN and non-IFN (control) groups at the first treatment of HCC, and Table 2 shows their data at the first recurrence of HCC. Clinical and laboratory characteristics were similar in both groups, but those in the PEG-IFN group were slightly younger (63 vs. 67 years old), and

Table 1 Profiles and laboratory tests of the patients

Variables	PEG-IFN	Non-IFN	*P* value
Number of patients	37	145	
Age (years)	63 (48–77)	67 (43–85)	<0.01*
Sex (male)	29 (78%)	95 (65%)	0.10
HCV genotype (1b high/others/unknown)	23/14/0	55/30/60	0.83
Response to IFN therapy (SVR/non-SVR)	19/18		
Observation period (years)	4.5 (0.8–12.7)	3.3 (0.3–10.8)	0.01*
T.Bil (mg/dl)	0.7 (0.3–2.7)	0.9 (0.2–2.9)	0.04*
ALB (g/dl)	3.9 (2.5–4.7)	3.7 (2.2–4.6)	<0.01*
ALT (IU/l)	75 (17–168)	54 (14–183)	<0.01*
PLT (×1,000/mm^3)	141 (31–307)	96 (34–281)	<0.01*
PT (%)	94 (62–118)	85 (48–145)	0.01*
AFP (ng/ml)	12 (1.6–1,729)	16.9 (0.6–54,535)	0.49
DCP (mAU/ml)	26 (0–5,230)	34 (0–66,700)	0.52
Number of tumors (solitary)	27 (72%)	105 (72%)	0.34
Size of main tumor (mm)	18 (7–55)	20 (9–74)	0.11
Disease stage (I/II/III/IVA)	16/15/6/0	47/48/44/6	0.88

All variables are shown as the median (range in parentheses) unless otherwise noted

IFN interferon, *PEG-IFN* pegylated interferon, *HCV* hepatitis C virus, *SVR* sustained virological response, *ALB* albumin, *T.Bil* total bilirubin, *ALT* alanine aminotransferase, *PLT* platelet, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

* *P* values less than 0.05 were considered statistically significant

Table 2 Profiles and laboratory tests of the patients at first recurrence

Variables	PEG-IFN	Non-IFN	*P* value
Number of patients	18	63	
Sex (male)	14 (78%)	40 (63%)	0.24
HCV genotype (1b high/others/unknown)	12/6/0	26/13/24	0.89
Response to IFN therapy (SVR/non-SVR)	8/10		
Treatment method (RFA/ope/PEIT/MCT/other)	15/0/0/1/2	50/4/5/2/2	0.20
T.Bil (mg/dl)	0.7 (0.4–1.4)	0.9 (0.3–2.6)	0.18
ALB (g/dl)	3.7 (2.9–5.0)	3.2 (2.8–4.6)	0.20
ALT (IU/l)	38 (9–295)	50 (16–137)	0.70
PLT ($\times$1,000/mm^3)	105 (39–250)	97 (43–31.2)	0.48
PT (%)	89 (65–117)	83 (35–124)	0.16
AFP (ng/ml)	12 (2.6–144)	11 (1.1–835)	0.40
DCP (mAU/ml)	23 (10–661)	41 (10–28,132)	0.51
Number of tumors (solitary)	11 (61%)	40 (63%)	0.71
Size of main tumor (mm)	13 (6–20)	15 (9–29)	0.16
Disease stage (I/II/III/IVA)	11/5/2/0	36/22/4/1	0.54

All variables are shown as the median (range) unless otherwise noted

IFN interferon, *PEG-IFN* pegylated interferon, *HCV* hepatitis C virus, *RFA* radiofrequency thermal ablation, *ope* operation, *PEIT* percutaneous ethanol injection therapy, *MCT* microwave coagulation therapy, *SVR* sustained virological response, *ALB* albumin, *T.Bil* total bilirubin, *ALT* alanine aminotransferase, *PLT* platelet, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

exhibited higher levels of ALB (3.9 vs. 3.7 g/dl), ALT (78 vs. 54 IU/l), and PLT (141 vs. 96 $\times$ 1,000/mm^3) than those in the non-IFN group. The median follow-up was 4.6 years for patients receiving PEG-IFN and 3.6 years for the controls. In the PEG-IFN group, 19 patients exhibited an SVR (12 monotherapy and 7 combination therapy), 2 were biochemical responders, and the other 17 patients were nonresponders.

Adherence and side effects of IFN therapy

Life-threatening adverse events were not observed in this study. In 11 cases of mild to moderate toxicity (5 thrombocytopenia, 3 anemia, and 3 neutropenia), IFN dose was reduced by 50%. Three patients eventually discontinued treatment with the drug because of adverse events: depression and severe malaise ($n = 1$), hemolytic anemia ($n = 1$), and IFN retinopathy ($n = 1$). In 8 cases with moderate toxicity, IFN treatment could be continued.

Cumulative survival rates of hepatocellular carcinoma

In this study, 2 patients in the PEG-IFN group and 39 patients in the non-IFN group died. All the patients who died had recurrence of HCC. The overall survival rate of PEG-IFN patients was higher than that of non-IFN patients (Fig. 1). Five-year survival rates of the PEG-IFN and non-IFN groups were 91% and 65%, respectively ($P < 0.01$).

Recurrence of hepatocellular carcinoma

At the end of the study, recurrence of HCC had occurred in 8 patients (42%) in the SVR group, 10 (55%) in the non-SVR group, and 63 (43%) in the non-IFN group.

The rate of first HCC recurrence after curative therapy of HCC in SVR patients tended to be lower than that in non-IFN patients (48 vs. 70% at 5 years, respectively, $P = 0.05$; Fig. 2); however, there was no significant difference between non-SVR patients and non-IFN patients (72 vs. 70% at 5 years, respectively; $P = 0.73$). In addition, there was no significant difference between the PEG-IFN group and the non-IFN group (58 vs. 70% at 5 years, respectively; $P = 0.17$). At first HCC recurrence, there was no significant difference in tumor number or liver function between the PEG-IFN and non-IFN groups; however, maximum tumor size in the PEG-IFN group was smaller than that in the non-IFN group (13 vs. 16 mm, respectively; $P = 0.03$). Fifteen of the 17 patients in the PEG-IFN group underwent curative treatment at the first recurrence of HCC.

The rate of second recurrence was not significantly different between the PEG-IFN and non-IFN groups (78 vs. 83% at 3 years, respectively; $P = 0.26$). However, the rate in the SVR group was significantly lower than that in the non-IFN group (65 vs. 83% at 3 years, respectively, $P = 0.03$; Fig. 3). At second HCC recurrence, in the PEG-IFN group, maximum tumor size was smaller (12 vs.

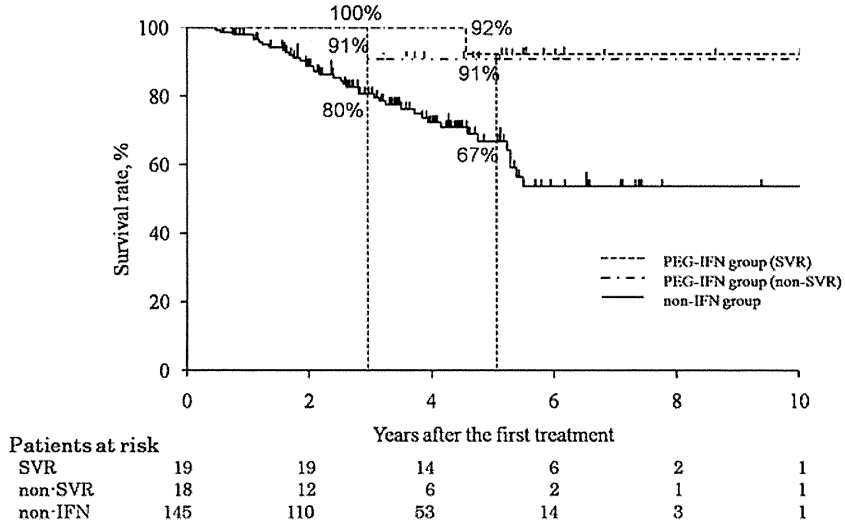

Fig. 1 Cumulative survival rates of pegylated interferon (PEG-IFN) group and non-interferon (non-IFN) group. Two patients in the PEG-IFN group died during the observation period. The survival rate was significantly different between the three groups ($P = 0.01$). *SVR* sustained virological response

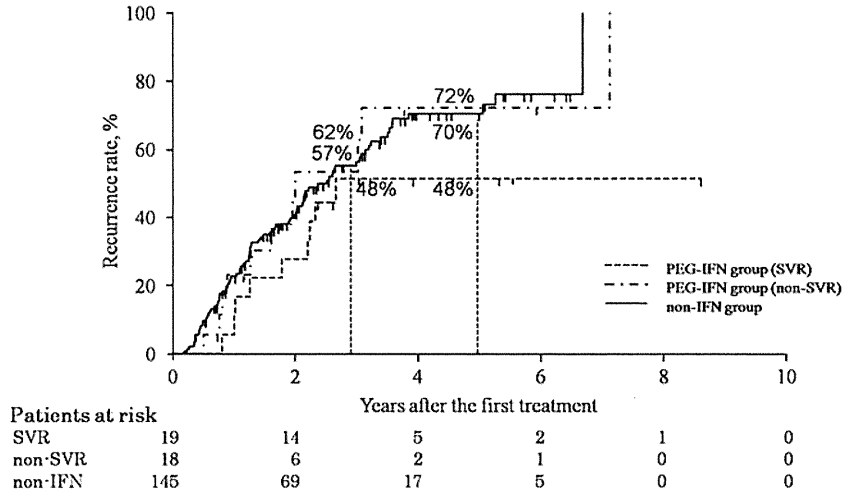

Fig. 2 The rates of first hepatocellular carcinoma (HCC) recurrence. The recurrence rate in SVR patients tended to be lower than that in non-IFN patients (48 vs. 70% at 5 years, respectively; $P = 0.05$); however, there was no significant difference between non-SVR patients and non-IFN patients (72 vs. 70% at 5 years, respectively; $P = 0.73$). *SVR* sustained virological response

15 mm, respectively; $P = 0.02$) and serum ALB was higher (3.3 vs. 3.1 g/dl, respectively; $P = 0.04$) than that in the non-IFN group.

Propensity score matched analysis

To minimize the biases of the PEG-IFN group and non-IFN group, we conducted a propensity score (PS) matched analysis. Thirty-four matched pairs were selected from the PEG-IFN group and non-IFN group by PS. No significant difference in clinical characteristics was observed between the groups (Table 3). Eighteen patients exhibited an SVR [11 monotherapy and 7 combination therapy, 9 (43%) genotype 1b high and 9 (69%) others]. Overall survival rate of the PEG-IFN group was higher than that of the non-IFN group ($P = 0.04$; Fig. 4). Although no significant difference in the first and second HCC recurrence ($P = 0.55$ and 0.62, respectively) was observed between the IFN group and non-IFN group, the rate of second recurrence in the SVR group was significantly lower than that in the non-IFN group (65 vs. 79% at 3 years, respectively, $P = 0.01$; Figs. 5, 6).

Prognostic factors and risk factors of HCC recurrence

To identify the factors that contributed to survival and the recurrence of HCC, a Cox proportional hazard analysis was performed.

Univariate analysis showed that PEG-IFN therapy, low T.Bil, and high serum ALB were independent factors favorably associated with long survival. Among the factors that were significant in the analysis, PEG-IFN therapy [risk ratio = 2.72; 95% confidence interval (CI), 1.29–9.04] and a serum ALB level >3.5 g/dl (risk ratio = 2.51; 95% CI, 1.29–4.98) were shown to be significantly associated with better survival in the multivariate analysis (Table 4).

On the other hand, non-SVR, low ALB, and large and multiple tumors at the initial treatment were significantly

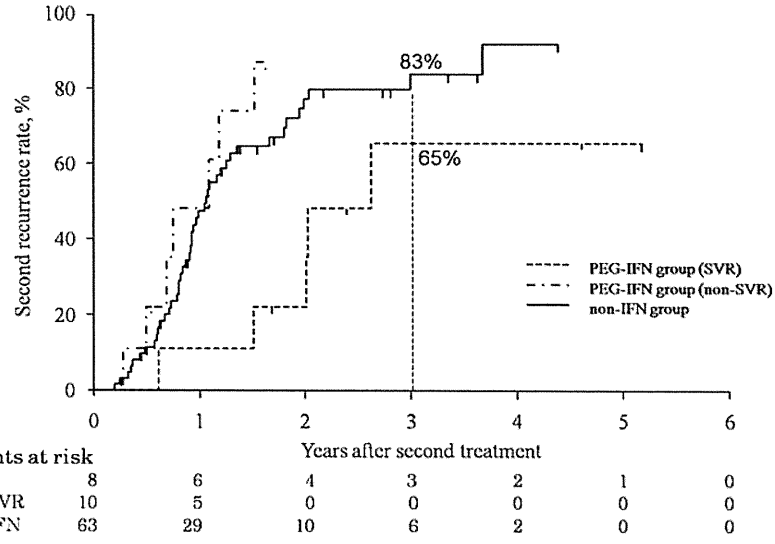

Fig. 3 Rates of second HCC recurrence. The second recurrence rate in the SVR group was significantly lower than that in the non-IFN group (65 vs. 83% at 3 years, respectively; $P = 0.03$. *SVR* sustained virological response

Table 3 Profiles and laboratory tests of the patients (propensity score matched cases)

Variables	PEG-IFN	Non-IFN	*P* value
Number of patients	34	34	
Age (years)	64 (48–77)	64 (43–85)	0.97
Sex (male)	26 (76%)	29 (85%)	0.48
HCV genotype (1b high/others/unknown)	21/13/0	17/8/9	0.62
Response to IFN therapy (SVR/non-SVR)	18/16		
Observation period (years)	4.6 (0.8–12.7)	3.4 (0.8–10.8)	0.22
T.Bil (mg/dl)	0.7 (0.3–2.7)	0.7 (0.43–1.8)	0.77
ALB (g/dl)	3.9 (2.5–4.7)	3.6 (3.1–4.7)	0.83
ALT (IU/l)	69 (17–168)	61 (17–183)	0.43
PLT ($\times$1,000/mm^3)	147 (31–307)	137 (42–216)	0.49
PT (%)	95 (62–118)	85 (52–110)	0.07
AFP (ng/ml)	11 (1.6–1,729)	10.8 (1.3–11,006)	0.38
DCP (mAU/ml)	29 (0–5,230)	27 (0–66,700)	0.34
Number of tumors (solitary)	25 (74%)	27 (79%)	0.81
Size of main tumor (mm)	19 (7–55)	21 (9–50)	0.06
Disease stage (I/II/III/IVA)	14/14/6/0	12/11/9/2	0.27

All variables are shown as the median (range) unless otherwise noted

IFN interferon, *PEG-IFN* pegylated interferon, *HCV* hepatitis C virus, *SVR* sustained virological response, *ALB* albumin, *T.Bil* total bilirubin, *ALT* alanine aminotransferase, *PLT* platelet, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

associated with first recurrence of HCC in univariate analysis. Multivariate analysis showed that low ALB (risk ratio = 1.70; 95% CI, 1.11–2.56) and large (risk ratio = 1.65; 95% CI, 1.02–2.59) and multiple (risk ratio = 1.66; 95% CI, 1.05–2.56) tumors were independent risk factors; however, response to PEG-IFN therapy was not determined to be a significant factor for the first recurrence of HCC (risk ratio = 1.60; 95% CI, 0.83–3.48; Table 5).

Regarding the second recurrence of HCC, non-SVR (risk ratio = 2.51; 95% CI, 1.06–7.40) and low ALB at the first recurrence of HCC (risk ratio = 2.56; 95% CI, 1.46–4.83) were found to be independent risk factors in multivariate analysis as well as univariate analysis (Table 6).

Discussion

Persistent active hepatitis is common in the advanced stage of chronic HCV infection and is a risk factor for the development of HCC. Several reports have shown the inhibitory effects of IFN therapy on the development of HCC. In these reports, the inhibitory effect was considered to be the result of the remission of inflammation, necrosis, and fibrosis in addition to the direct action of IFN on tumor cells [35–39]. Recently, several studies were conducted to show the effect of IFN therapy after curative treatment of HCC, which reduced the risk for recurrence and improved the rate of survival. To date, reports on eight randomized control trials (RCTs) [17–24] and six non-RCTs [25–30] on this effect have been published.

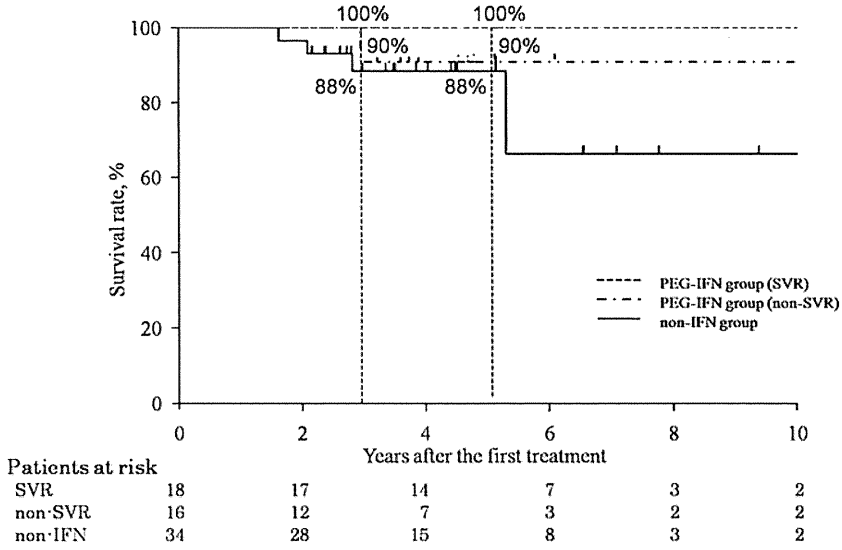

Fig. 4 Cumulative survival rates of PEG-IFN group and non-IFN group after propensity score (PS) matching. Overall-survival rate of the PEG-IFN group was higher than that of non-IFN group ($P = 0.04$). *SVR* sustained virological response

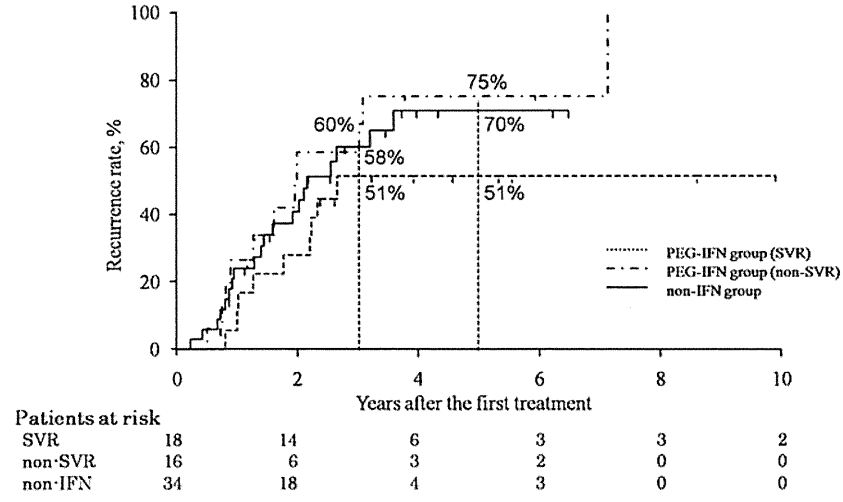

Fig. 5 Rates of first HCC recurrence after PS matching. We found no significant differences between the two groups with respect to first HCC recurrence ($P = 0.55$). *SVR* sustained virological response

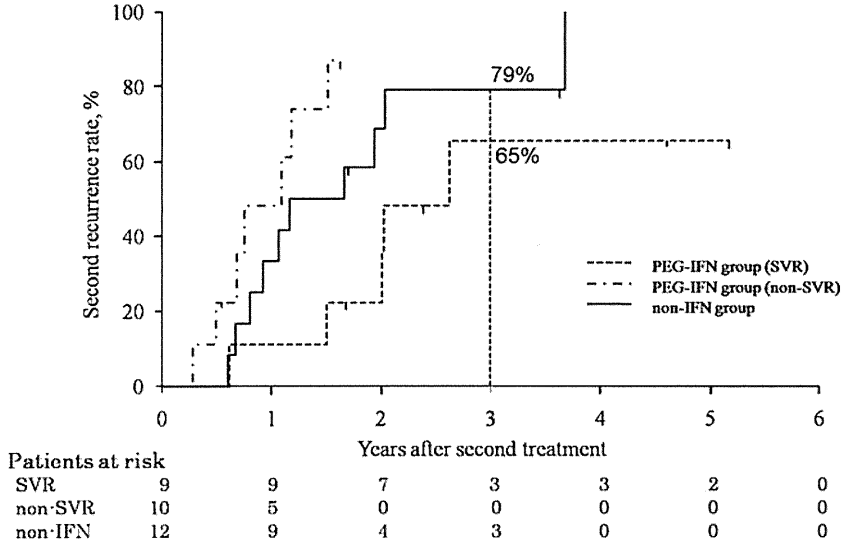

Fig. 6 Rates of second HCC recurrence after PS matching. The second recurrence rate in the SVR group was significantly lower than that in the non-IFN group (65 vs. 79% at 3 years, respectively; $P = 0.01$), although no statistical difference was observed between the IFN group and non-IFN group ($P = 0.62$). *SVR* sustained virological response

Table 4 Factors contributing to survival after HCC development

	Univariate analysis		Multivariate analysis	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
Interferon-related variables				
Application of interferon therapy	3.24 (1.52–11.0)	<0.01*	2.72 (1.29–9.04)	<0.01*
Response to interferon therapy (SVR vs. non-SVR + non-IFN)	10.5 (2.33–121)	<0.01*	–	
Variables at the first treatment of HCC				
Age (<60 years)	0.59 (0.29–1.32)	0.19		
T.Bil (<1.0 mg/dl)	2.68 (1.45–5.02)	<0.01*	1.69 (0.87–3.31)	0.11
ALB (≧3.5 g/dl)	3.45 (1.86–6.55)	<0.01*	2.51 (1.29–4.98)	<0.01*
ALT (<80 IU/l)	0.74 (0.35–1.45)	0.40		
PT (≧70%)	1.48 (0.63–3.06)	0.33		
PLT (≧10 × 10^4/mm^3)	1.63 (0.88–3.07)	0.11		
AFP (<100 ng/ml)	1.42 (0.66–2.81)	0.34		
DCP (<40 mAU/ml)	1.06 (0.56–1.99)	0.84		
Maximum tumor size (<30 mm)	1.48 (0.70–2.87)	0.28		
Number of tumors (single)	0.98 (0.45–1.94)	0.97		

RR risk ratio, *CI* confidence interval, *IFN* interferon, *PEG-IFN* pegylated interferon, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma, *SVR* sustained virological response, *ALB* albumin, *T.Bil* total bilirubin, *ALT* alanine aminotransferase, *PLT* platelet, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

* *P* values less than 0.05 were considered statistically significant

Table 5 Risk factors contributing to first recurrence of hepatocellular carcinoma (HCC)

	Univariate analysis		Multivariate analysis	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
Interferon-related variables				
Application of interferon therapy	1.31 (0.97–1.84)	0.07		
Response to interferon therapy (non-SVR + non-IFN vs. SVR)	1.92 (1.01–4.15)	0.04*	1.60 (0.83–3.48)	0.16
Variables at the first treatment of HCC				
Age (≧60 years)	1.29 (0.76–2.37)	0.35		
T.Bil (≧1.0 mg/dl)	1.15 (0.75–1.72)	0.50		
ALB (<3.5 g/dl)	1.55 (1.03–2.29)	0.03*	1.70 (1.11–2.56)	0.01*
ALT (≧80 IU/l)	0.97 (0.63–1.46)	0.91		
PT (<70%)	0.74 (0.41–1.27)	0.30		
PLT (<10 × 10^4/mm^3)	1.26 (0.85–1.85)	0.23		
AFP (≧100 ng/ml)	1.50 (0.91–2.36)	0.11		
DCP (≧40 mAU/ml)	1.45 (0.97–2.17)	0.06		
Maximum tumor size (≧30 mm)	1.71 (1.07–2.65)	0.02*	1.65 (1.02–2.59)	0.04*
Number of tumors (multiple)	1.60 (1.02–2.43)	0.03*	1.66 (1.05–2.56)	0.02*

RR risk ratio, *CI* confidence interval, *IFN* interferon, *PEG-IFN* pegylated interferon, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma, *SVR* sustained virological response, *ALB* albumin, *T.Bil* total bilirubin, *ALT* alanine aminotransferase, *PLT* platelet, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

* *P* values less than 0.05 were considered statistically significant

However, there have been few trials involving PEG-IFN therapy.

In this study, the overall survival rate of PEG-IFN-treated patients was higher than that of non-IFN patients, and the HCC recurrence rate after curative therapy for HCC in SVR patients was significantly lower than that in non-IFN patients. The survival rates are not different, although the rates of first and second recurrence of the PEG-IFN group (SVR) and PEG-IFN group (non-SVR) were different. The main reason for this discrepancy is that

Table 6 Risk factors contributing to second recurrence of HCC

	Univariate analysis		Multivariate analysis	
	RR (95% CI)	*P* value	RR (95% CI)	*P* value
Interferon-related variables				
Application of interferon therapy	1.97 (0.97–2.15)	0.06		
Response to interferon therapy (non-SVR + non-IFN vs. SVR)	2.77 (1.20–8.05)	0.01*	2.51 (1.06–7.40)	0.03*
Variables at the time of first recurrence of HCC				
Age (≧60 years)	0.81 (0.41–1.77)	0.57		
T.Bil (≧1.0 mg/dl)	1.70 (0.89–3.12)	0.10		
ALB (<3.5 g/dl)	2.81 (1.55–5.09)	<0.01*	2.65 (1.46–4.83)	<0.01*
ALT (≧80 IU/l)	1.36 (0.72–2.69)	0.34		
PT (<70%)	2.47 (0.98–5.46)	0.05		
PLT (<10 × 10^4/mm^3)	0.94 (0.52–1.70)	0.86		
AFP (≧100 ng/ml)	2.13 (0.86–4.54)	0.09		
DCP (≧40 mAU/ml)	1.46 (0.78–2.76)	0.23		
Maximum tumor size (≧30 mm)	1.26 (0.64–2.31)	0.47		
Number of tumors (multiple)	1.21 (0.67–2.13)	0.51		

RR risk ratio, *CI* confidence interval, *IFN* interferon, *PEG-IFN* pegylated interferon, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma, *SVR* sustained virological response, *ALB* albumin, *T.Bil* total bilirubin, *ALT* alanine aminotransferase, *PLT* platelet, *PT* prothrombin time, *AFP* alpha-fetoprotein, *DCP* des-γ-carboxy prothrombin

* *P* values less than 0.05 were considered statistically significant

few patients died during follow up in both groups. In addition, we observed a significant effect of PEG-IFN (SVR) in the prevention of recurrence by two different analyses (PS score matched analysis and multivariate analysis), although the effect was limited to the prevention of second recurrence, and the term of surveillance was relatively short because PEG-IFN was only available in Japan after 2004. The results were quite similar to those of reports on conventional non-PEG-IFN therapy [17].

We conducted propensity score (PS) matched analysis to adjust the clinical background of the patients in each group. PS in this analysis is a probability of choosing PEG-IFN treatment among the patients that was calculated using seven covariates. By matching the score of the patients in the PEG-IFN group and non-IFN group, we could reconstruct a situation similar to randomization.

PEG-IFN is considered to be more beneficial than non-PEG because it results in the SVR rate being higher and the IFN concentration being maintained at a high level for a longer period [40, 41], which is favorable for its action as a direct anticancer agent. However, there was no difference between conventional IFN and PEG-IFN with regard to the prevention of only late (second) recurrence. We did not compare the effect of PEG-IFN with that of non-PEG-IFN directly, but our results that non-SVR was an independent risk factor for second recurrence but not for first recurrence suggested that IFN treatment after curative treatment of HCC is more beneficial for the suppression of de novo HCC than for preventing the progression of preexisting very small HCC or intrahepatic metastasis, regardless of the type of interferon used.

In the PEG-IFN group, tumor size at HCC recurrence was smaller (13 vs. 16 mm, respectively; $P = 0.03$) and liver function tended to be better (T.Bil, ALB, PLT, PT) than in the non-IFN group. These results suggested that PEG-IFN might inhibit the growth of recurrent tumors as well as preserve liver function, although the inhibitory effect does not appear to be sufficient for complete prevention of recurrence.

PEG-IFN therapy after curative treatment of HCC was generally well tolerated in our study. Among the 37 patients, the PEG-IFN dose had to be reduced for 8 patients (21%); however, only 3 (8%) discontinued treatment with the drug because of adverse events. This rate was similar to that of the non-PEG-IFN group after HCC treatment (8–15%) [17–24]. However, PEG-IFN therapy has fewer side effects than non-PEG-IFN therapy, such as high-grade fever and general fatigue. The good adherence of patients to treatment should be noted, with a low rate of withdrawal as a consequence of adverse events [32]. The number of elderly patients with HCC will increase in the future. Because of fewer side effects and a higher rate of SVR, HCV-related HCC treatment with PEG-IFN should be considered for these elderly patients.

The weak point of this study is that it is a retrospective study and it is difficult to eliminate biases completely even with PS analysis, although no statistical difference was observed between the PEG-IFN group and non-IFN group.