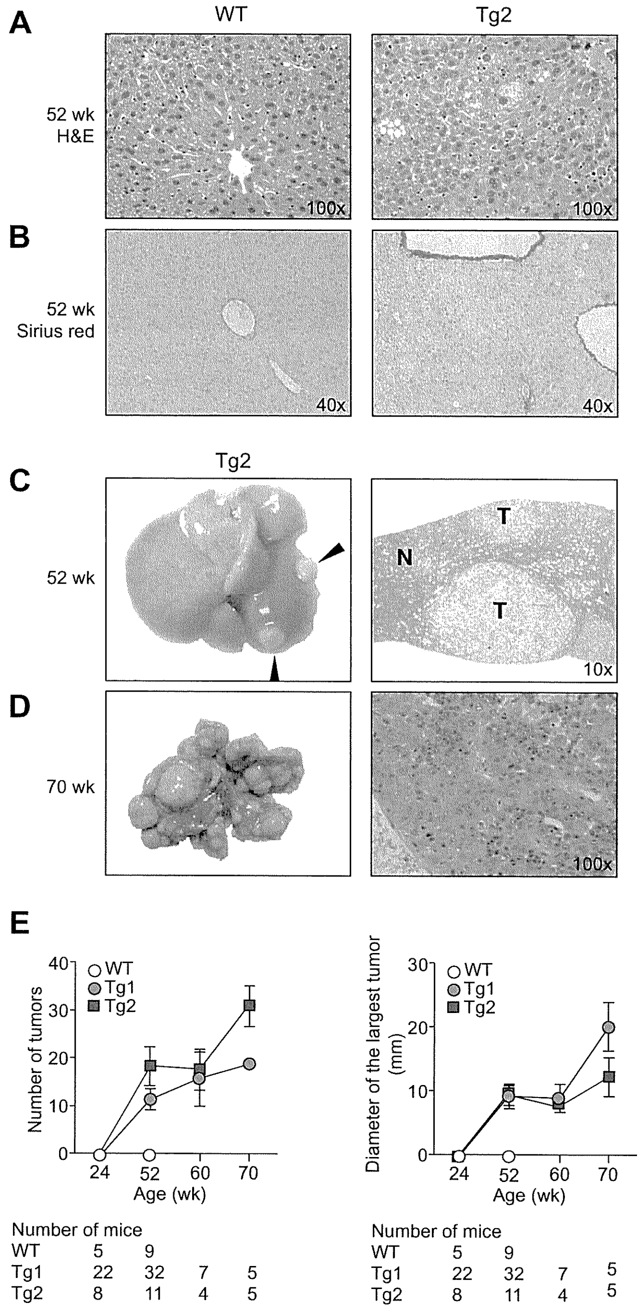

Fig. 3. Liver tumors in the *Pik3ca* Tg mice. (A) H&E and (B) Sirius red staining of livers at 52 weeks. (C) Macroscopic view (left) of the representative liver adenomas (arrowheads) at 52 weeks of age. H&E staining of an adenoma (T) and adjacent parenchyma (N) (right). (D) Tumors in *Pik3ca* Tg mice at 70 weeks (left). H&E staining of HCC (right). (E) The number (left) and size (right) of hepatic tumors. The number of mice examined is shown below the graphs.

signaling [36]. Interestingly, unsaturated FAs inhibit *Pten* expression via microRNA-21 in hepatoma [7,37,38], and the overexpression of a FA receptor (FFAR2) transformed the 3T3 fibroblasts [39], suggesting the possible relationship between FA and tumorigenesis. In the *Pik3ca* Tg liver, the tumor tissues contained higher concentrations of FAs than the non-tumor background tissues (Fig. 5A). The difference in total FA levels was largely due to the increase in levels of OA (C18:1n9) and PA (C16:0) in the tumors (Fig. 5B and C, Supplementary Fig. 12 and Table 2).

OA has the potential to repress the expression of tumor suppressors and enhance colony formation in vitro

To examine the possibility that either OA or PA downregulates the expression of tumor suppressors including *Pten*, we treated BNL-CL2 cells with OA or PA. OA, but not PA, repressed the expression of *Pten*, *Arid5b*, *Xpo4*, and *Dlc1* (Fig. 6A). Moreover, BNL-CL2 cells exposed to OA formed significantly more colonies in soft agar (Fig. 6B). These findings indicate that OA potentially enhances the in *vivo* tumorigenesis in the *Pik3ca* Tg liver. As an example, it is likely that decreased PTEN expression could enhance the Akt activation by the *Pik3ca* transgene in Tg-derived tumors (Fig. 1B).

Discussion

Hepatocyte-specific overexpression of *Pik3ca* (N1068fs*4) leads to steatosis and hepatic tumor formation. This mutation was originally isolated in human HCC and gastric cancers [12], but its functional analysis has never been reported. The *in vitro* overexpression of this mutant clearly induced Akt activation, but the level of activation was comparable with that of *Pik3ca* wild type and lower than that of the oncogenic H1047R mutant, suggesting that the *Pik3ca* Tg mice provide a model for studying effects of PIK3CA overexpression rather than a gain-of-function of PIK3CA. Furthermore, the N1068fs*4 mutation was not sufficient for cellular transformation *in vitro*, different from *Pik3ca* H1047R [40]. Considering results from a previous report suggesting the pivotal role of Akt activation in cell transformation by *PIK3CA* mutation [13], the activation level of Akt induced by *Pik3ca* (N1068fs*4) expression should not be sufficient for the cell-transforming process. These data indicated that the development of hepatic tumors in Tg mice might not be always a direct effect of *Pik3ca* (N1068fs*4) but instead promoted by other *in vivo* protumorigenic factors.

We focused on FA as an additional protumorigenic factor contributing to *in vivo* hepato-tumorigenesis in Tg mice, based on recent research on their oncogenic capacity [39]. Previous studies reported that OA inhibits *PTEN* expression via the upregulation of microRNA-21 through an mTOR/NF-κB-dependent mechanism [37,38] and also that exposure to OA increases tumor growth in xenografts [7]. Here, we demonstrated the correlation between OA accumulation and downregulation of other tumor suppressors, whereas the entire molecular mechanism remains to be elucidated. At least, there is a possibility that, in the Tg-derived tumors, OA accumulation enhanced the Akt activation by the *Pik3ca* transgene, which phosphorylates Akt less strongly than other oncogenic mutants *in vitro* (Fig. 1B, Supplementary Figs. 6 and 13).

Lipogenesis is mainly mediated by two major transcription factors, PPARγ and SREBP1C [24,25]. Hepatocyte-specific *Pten* KO mice exhibited increased expression of both PPARγ and SREBP1c in the liver, whereas only PPARγ was highly expressed in the *Pik3ca* Tg liver [16]. Our *in vitro* data suggested that the PI3K signaling is upstream of the activation of PPARγ in hepatocytes. A recent study shows that levels of *PPARγ* as well as *SREBP1c* mRNA are higher in the livers of patients with steatosis

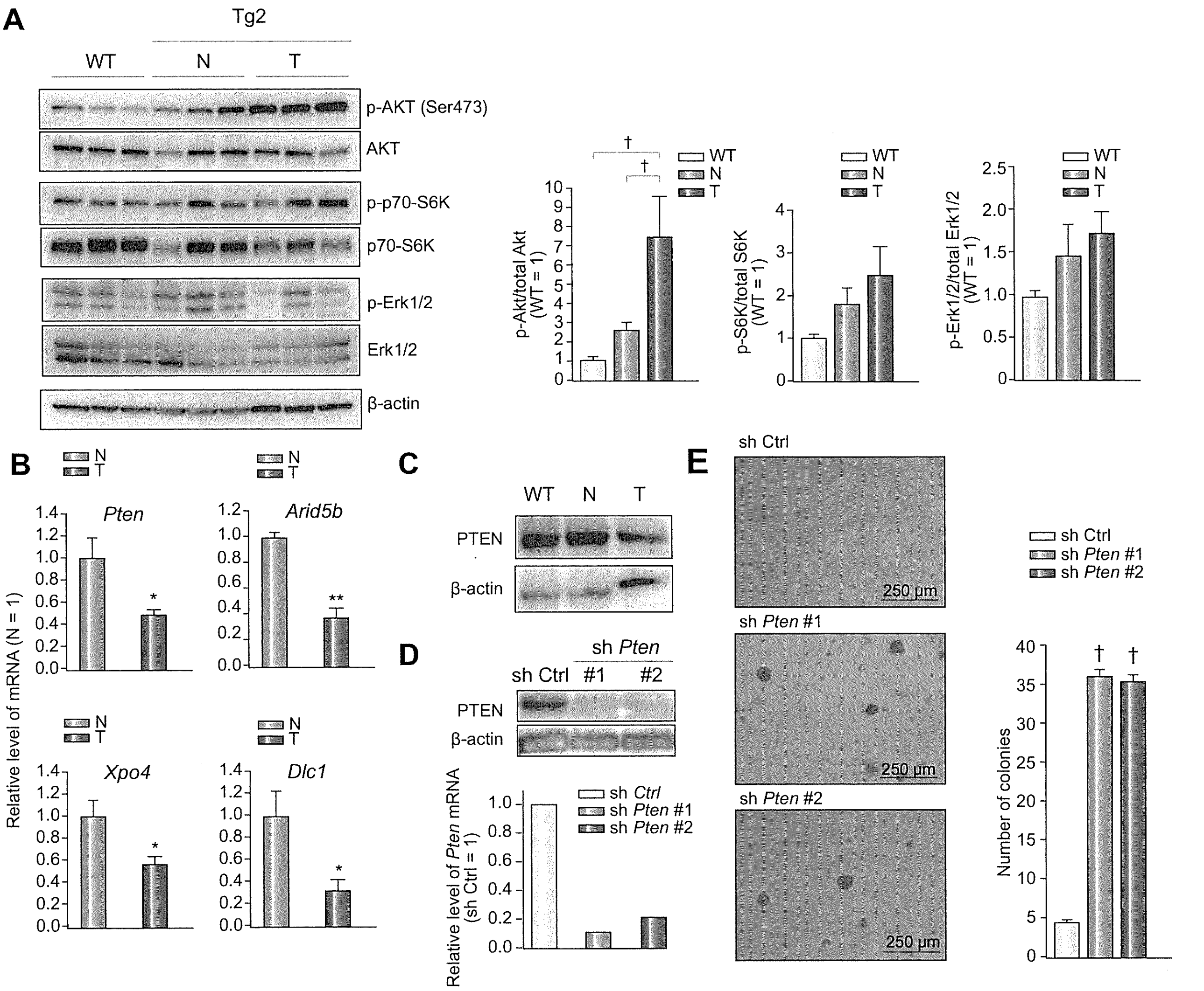

Fig. 4. *Pten* downregulation in the *Pik3ca* Tg liver. (A) Immunoblots and quantification of liver homogenates at 52 weeks (†p <0.05, ANOVA; post hoc test with WT). (B) The decreased expression of *Pten*, *Arid5b*, *Xpo4*, and *Dlc1* mRNA in the *Pik3ca* Tg liver tumors (T) relative to their expression in background liver tissues (N) (N = 5/group; *p <0.05, **p <0.01, Student's t-test). (C) Representative images of immunoblots of liver tissues from the littermates at 52 weeks. (D) Knockdown of *Pten* in BNL-CL2 cells confirmed at the protein (top) and mRNA (bottom) levels. (E) Both lines of *Pten*-depleted BNL-CL2 cells (sh*Pten* #1 and #2) formed more colonies in soft agar (N = 3/group; † p <0.05, ANOVA; post hoc test with control cells (shCtrl)).

or steatohepatitis, suggesting that the activity of PPARγ is implicated in the abnormal lipid accumulation in human livers [41] (Supplementary Fig. 13).

Unlike the hepatocyte-specific *Pten* KO mice [16], cellular infiltration and fibrosis were not observed in the *Pik3ca* Tg liver. One explanation is the possibility that *Pten* deficiency induces certain pathological mechanisms independently of PI3K-Akt activation, as previously reported for mammary tumorigenesis [18–20,42–45]. Indeed, although genetic changes in PTEN result in potent Akt phosphorylation, *in vivo* studies have suggested that they show distinct phenotypes [42]. The conditional knockout of PTEN enhanced tumorigenesis in the mammary gland [43]; however, transgenic mice expressing constitutively active Akt in the mammary gland did not show tumor formation [44]. PTEN directly associates with p53, thereby increasing its stability, protein level, and transcriptional activity [18,19]. PTEN induces apoptosis and cell cycle arrest through PI3K/Akt-independent pathways [20]. PTEN also has important roles in integrin signaling and has the ability to dephosphorylate focal adhesion kinase, reducing cell adhesion and enhancing migration [46]. These findings support an alternative mechanism of PTEN-mediated tumorigenesis independent on PI3K/Akt pathway. As a second reason for the difference from *Pten* KO mice, it is possible that PI3K catalytic beta has a distinct role with PIK3CA in the phenotype of *Pten* deficiency [47].

The discrepancy between the scarce inflammatory levels in the *Pik3ca* Tg liver and the strong increase in serum ALT levels indicative of severe liver injury is to be solved in the near future. We found that inflammatory cytokine IL-1α and Fas ligand were more highly expressed in the *Pik3ca* Tg liver than in the WT liver (Supplementary Fig. 4). Taking into account reports demonstrating that these factors can lead to liver damage [28,29], it can be suggested that their abnormal upregulation in Tg livers is in part responsible for liver damage, whereas the entire molecular process inducing them remains unknown (Supplementary Fig. 13).

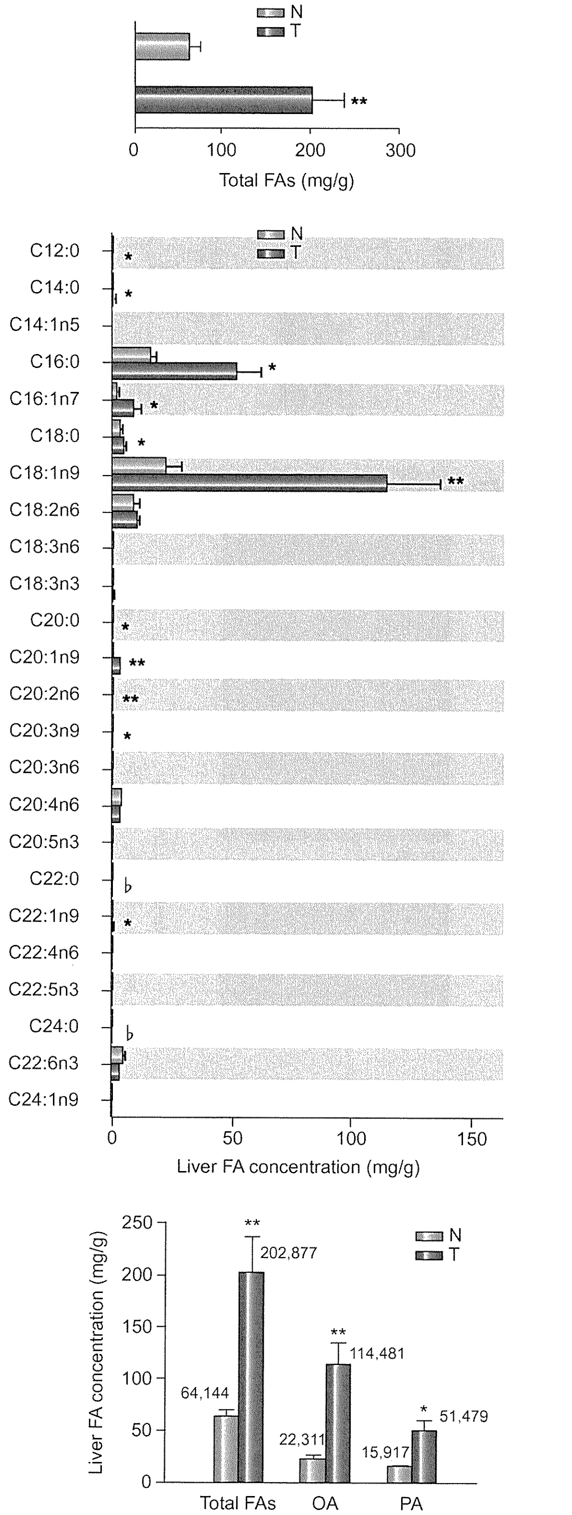

Fig. 5. The total FA composition in the *Pik3ca* Tg liver tissues and tumors. (A) The levels of FAs in the tumor (T) and non-tumor background tissue (N) in *Pik3ca* Tg mice at 52 weeks (N = 4/group; $^{**}p < 0.01$, Student's *t*-test). (B) FA composition in background (N) and tumor tissues (T) (N = 4/group; statistically increased FA levels in the tumors are shown with asterisks ($^{*}p < 0.05$, $^{**}p < 0.01$) and significantly decreased levels are shown with flat (♭, $p < 0.05$), Student's *t*-test). (C) The concentration of total FAs, OA, and PA in background (N) and tumor tissues (T) (N = 4/group; $^{*}p < 0.05$, $^{**}p < 0.01$, Student's *t*-test).

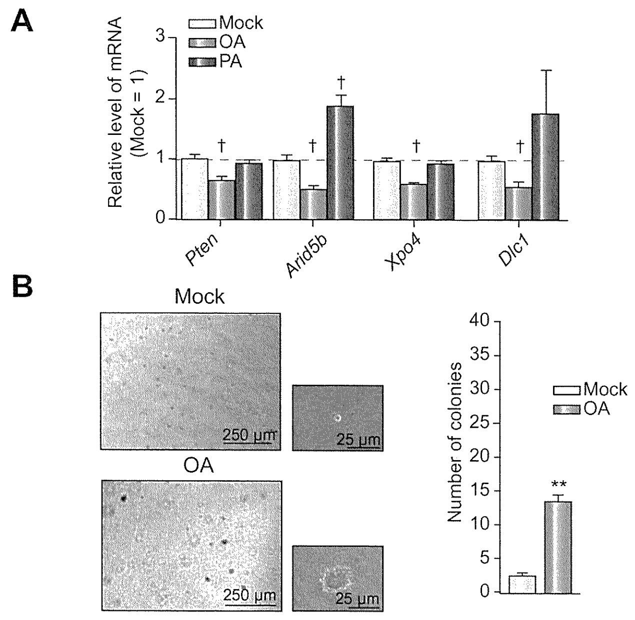

Fig. 6. OA enhances the colony-forming activity of immortalized hepatocytes. (A) OA but not PA decreased *Pten*, *Arid5b*, *Xpo4*, and *Dlc1* mRNA *in vitro* (N = 3/group; $^{\dagger}p < 0.05$, ANOVA; post hoc test with Mock group). (B) Colony formation assay of BNL-CL2 cells with or without 50 μmol/L OA in 10% or 0.5% FBS media (N = 3/group; $^{**}p < 0.01$, Student's *t*-test).

Mechanisms involved in the pathogenesis of non-alcoholic steatohepatitis (NASH) remain unclear, but the "two-hit theory" is widely accepted [48]. That is, in the first hit, insulin-resistance is followed by lipid accumulation in the liver, and the second hit, possibly involving inflammatory cytokines or oxidative stress, results in hepatic injury and fibrosis. It has been reported that ROS has certain roles in *in vivo* carcinogenesis [35], and the concentration of ROS is upregulated in the liver suffering NASH or NASH-derived HCC [49]. Regardless of the obvious fatty liver, our model mice have not shown impaired glucose tolerance. The concentration of ROS in the *Pik3ca* Tg mice was comparable with that of WT mice (Supplementary Fig. 11), which can be partly explained by the lower expression of fat-oxidative genes (Fig. 2F) and lack of inflammatory cell infiltration. These findings indicate that *Pik3ca* Tg mice do not always mimic the entire pathological mechanisms causing NASH, while they might be useful as a prototype to determine which pathological processes are required for the progression from the fatty liver to NASH. In addition, given the low rate of HCC development in these mice, they can be potentially useful for discovering tumor-promoting factors in hepatic steatosis. For example, although it was unlikely that ROS is involved in the initiation of hepatic tmor in the *Pik3ca* Tg liver, we can examine the pathological significance of ROS in tumor progression as well as hepatitis induction by applying the *Pik3ca* Tg liver to the condition producing high levels of ROS.

Recent clinical findings have advocated the relationship between volume of visceral fat and tumor progression [1–4]. While there is no direct molecular evidence to address the notion that abnormal body fat accumulation accelerates tumor growth, our data might provide new insights into the mechanisms of the "lipotoxicity-related" tumorigenesis. Future researches are needed to unravel how OA affects gene expression.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2011.03.025.

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ORIGINAL ARTICLE

Safety and tolerance of sorafenib in Japanese patients with advanced hepatocellular carcinoma

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Abstract
Purpose Sorafenib provides a survival benefit for patients with advanced hepatocellular carcinoma (HCC). However, there has been little experience with it in Japan. This study evaluated the safety and tolerance of sorafenib in Japanese patients with HCC.
Methods Clinical data for patients given sorafenib for advanced HCC were captured from eight institutions. All patients were classified as Child-Pugh A and the treatment was started at 400 mg twice daily. We recorded adverse events, treatment duration, and survival retrospectively. Adverse events were graded using Common Terminology Criteria, version 3.0; tumor response was assessed according to Response Evaluation Criteria in Solid Tumor, version 1.1.
Results Of the 54 patients treated, their median age was 69 years (range 48–82), 91% were males, 52% had HCV infection, and 22% had HBV infection. The most common drug-related adverse events were hand–foot skin reactions (HFSR) (72%), aspartate transaminase elevation (55%), alanine aminotransferase elevation (52%), rash (50%), fatigue (41%), and diarrhea (32%). Liver failure occurred in 19%. The median time to treatment failure was 2 months. Dose reduction was required in 83% of the patients, and this occurred within 2 weeks in 44%. The median overall survival was 6.9 months.
Conclusions These data suggest that sorafenib is generally tolerated in Japanese patients with HCC. Nevertheless, the majority needed a dose reduction. Adverse events including HFSR, rash, and liver failure occurred more frequently in our patients than those reported elsewhere. Careful attention must be paid to these adverse events during sorafenib administration.

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Keywords Hepatocellular carcinoma · Sorafenib · Safety · Tolerance · Japanese

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide [1]. HCC develops mostly in patients with liver cirrhosis, which is typically caused by hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection, or alcohol [2]. The annual incidence of HCC in HCV-positive liver cirrhosis and chronic hepatitis is 6–7% and 1–2%, respectively [2]. The risk of cancer developing from chronic hepatitis or cirrhosis depends on the degree of fibrosis [3]. The hepatocarcinogenesis in the patients with hepatitis viruses differs between HCV and HBV. HCC occurs frequently in the cirrhotic livers of patients with HCV-positive liver disease. By contrast, HCC often develops in chronic HBV infection in the absence of cirrhosis. HCC developing from HBV infection has a lower cirrhosis complication rate than does HCC developing from HCV infection.

The etiology of HCC varies regionally [4]. In the Asia-Pacific region, except Japan, 70% of HCC is HBV-related and 20% is HCV-related [5]. In contrast, in Japan, 71–75% of HCC is HCV-related [2, 6]. The incidence of HCV infection is also increasing in the USA and Europe, as is the incidence of HCC [7].

Both surgical resection and local ablation therapy, including radiofrequency ablation, are considered curative for HCC [8–10]. Transarterial chemoembolization (TACE) has been applied to patients with advanced incurable HCC [11, 12]. However, the majority of patients experience recurrence or metastasis after these treatments. Although systemic therapy is available for advanced HCC, the prognosis remains poor. No standard systemic therapy that prolongs survival had been indentified before sorafenib was approved.

Sorafenib, an oral multikinase inhibitor, blocks tumor cell proliferation by targeting Raf/MEK/ERK signaling at the level of Raf kinase, and exerts an antiangiogenic effect by targeting vascular endothelial growth factor receptor-beta (VEGFR-β, PDGF-β) tyrosine kinases [13]. The Sorafenib HCC Assessment Randomized Protocol (SHARP) and Asia-Pacific studies demonstrated a significant survival benefit and good tolerance in patients with advanced HCC, making sorafenib the new reference standard for systemic therapy of patients with advanced HCC [14, 15]. In the SHARP study, approximately 90% of the patients were enrolled from Europe [14], and the Asia-Pacific study was conducted in China, Taiwan, and South Korea [15], but not Japan. The sorafenib groups in the SHARP and Asia-Pacific studies reflected the geographic patient pools, including HCV infection (29 vs. 10.7%) and HBV infection (19 vs. 70.7%) [14, 15]. In both studies, baseline disease characters differed from those of Japanese HCC patients. HCV-related HCC is most common in Japan, as mentioned above, and most of these patients have hepatitis or cirrhosis due to HCV.

In Japan, a phase I study evaluated the pharmacokinetics, safety, and preliminary efficacy of sorafenib in HCC patients [16]. Then, based on the results of the SHARP and Asia-Pacific studies, together with the phase I study in Japanese HCC, the use of sorafenib to treat HCC patients was approved by the Japanese Ministry of Health, Labour, and Welfare in May 2009 [14–16]. However, the phase I study included few patients (six Child-Pugh A patients and eight Child-Pugh B patients receiving 400 mg twice daily) [16]. Thus, little is, in fact, known about the safety and tolerance profile of sorafenib in Japanese HCC patients. In this study, we evaluated the safety and tolerance of sorafenib in Japanese HCC patients.

Materials and methods

HCC patients treated with sorafenib between May 2009 and December 2009 at eight medical centers in Japan were analyzed retrospectively. Patients were required to meet the following criteria at baseline: (1) diagnosis of HCC based on the European Association for the Study of Liver Disease/American Association for Liver Disease criteria or liver histology [8]; (2) Eastern Cooperative Oncology Group Performance Status (ECOG-PS) 0, 1, or 2; (3) classified as Child-Pugh A; (4) required to have adequate renal, hematological, and hepatic function (platelet count $\geq 50 \times 10^9$/L, hemoglobin concentration ≥ 8.5 g/L, albumin concentration ≥ 2.8 g/L, total bilirubin concentration ≤ 3.0 mg/dL, alanine aminotransferase (ALT) concentration ≤ 5 times the upper limit of normal (ULN), serum creatinine concentration ≤ 1.5 times the ULN, and prothrombin time-international normalized rate (INR) ≤ 2.3. Patients who received 400 mg sorafenib twice daily as an initial dose were selected, and treatment interruptions and dose reductions (first to 400 mg once daily, and then to 400 mg once every other day) were allowed for the toxicity study. Dose reduction and treatment discontinuation were based on the package insert and were required for drug-related toxicities. For grade 3/4 toxicities, patients received a lower dose when the toxicity improved to grade 2 or better, but therapy was discontinued if the recovery time was 30 days or longer. Dose reduction was introduced for grade 3 non-hematologic toxicities until the toxicity was grade 2 or better; patients were then treated at one dose level lower, and therapy was discontinued if the recovery time was 30 days or longer. Treatment was discontinued for patients with drug-related grade 4 non-hematologic toxicities. However, a modified scale resulting from a phase II trial was used for skin toxicity [17].

We recorded demographics, prior therapy, plasma α-fetoprotein (AFP) level, existence of microvascular invasion, or extrahepatic spread of HCC, Barcelona Clinic Liver Cancer (BCLC) score, tumor response, survival data, and relevant toxicities.

Adverse events were recorded according to the Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE v3.0). Based on contrast-enhanced computed tomography (CT) or contrast-enhanced magnetic resonance imaging (MRI), performed at baseline and 1–3 months after treatment, the tumor response was evaluated using the Response Evaluation Criteria in Solid Tumors criteria version 1.1 (RECIST v1.1). The duration of treatment and survival were estimated using the Kaplan–Meier method.

Results

Patient baseline characteristics

In total, 54 patients were included in this retrospective study. Their median age was 69 years (range 48–82), and 49 patients (91%) were males. Most had good performance status (ECOG-PS was 0 in 81% and 1 in 15% of patients). At baseline, 28 patients (52%) had HCV infection and 12 patients (22%) had HBV infection. Of the patients, 38 (70%) were classified as BCLC stage C and 28 patients (52%) had extrahepatic metastases. Before receiving sorafenib therapy, 50 patients (93%) had been treated with surgery, local ablation, or TACE (Table 1).

Safety and tolerability

The overall incidence of drug-related adverse events of any grade was 98% and 36 patients (68%) experienced grade 3/4 adverse events (Table 2). HFSR occurred in 39 patients (72%) and was grade 3/4 in 14 patients (26%). Rash occurred in 27 patients (50%) and was grade 3/4 in 7 patients (13%). Fatigue, diarrhea, and hypertension occurred in 22 (41%), 17 (32%), and 14 patients (26%), respectively; none of these toxicities was grade 3/4. Liver failure under treatment, defined as encephalopathy, massive ascites, or jaundice, occurred in ten patients (19%). The median average daily dose was 450 mg (range 182–800 mg). Dose reduction was required in 45 patients (83%) (Table 3). The most common adverse events leading to dose reduction were HFSR ($n = 21$, 38%), aspartate transaminase (AST)/ALT elevation ($n = 8$, 15%), rash ($n = 7$, 13%), and liver failure ($n = 4$, 7%). Treatment was discontinued in 17 patients (31%) for sorafenib intolerance (Table 4). The most frequent adverse events leading to

Table 1 Baseline demographics and disease characteristics of the enrolled patients

Number of patients	54
Sex, no. (%)	
Male	49 (91)
Female	5 (9)
Age (years)	
Median (range)	69 (48–82)
Body weight (kg)	
Median (range)	60.8 (43.6–81.3)
Body surface area ($m^{2)}$	
Median (range)	1.66 (1.32–1.93)
ECOG PS, no. (%)	
0	44 (81)
1	8 (15)
2	2 (4)
Child-Pugh score, no. (%)	
5	36 (67)
6	18 (33)
Hepatitis virus status, no. (%)	
HCV infection	28 (52)
HBV infection	12 (22)
Alcohol	8 (15)
Other	6 (11)
BCLC stage, no. (%)	
B (intermediate)	16 (30)
C (advanced)	38 (70)
Macroscopic vascular invasion, no. (%)	12 (22)
Extrahepatic spread, no. (%)	
Any	28 (52)
Lymph nodes	8 (15)
Lung	14 (26)
Bone	6 (11)
Prior treatment, no. (%)	
Any	50 (93)
Surgery	27 (50)
Local ablation	25 (46)
Transarterial chemoembolization	43 (80)
Biochemical analysis, median (range)	
Platelets/mm^3	133,500 (50,000–296,000)
Albumin (g/dL)	3.7 (2.8–4.9)
Total bilirubin (mg/dL)	0.8 (0.2–1.9)
Aspartate aminotransferase (AST) (IU/L)	51 (18–176)
Alanine aminotransferase (ALT) (IU/L)	40 (11–162)
Alpha fetoprotein (AFP) (ng/mL)	246.6 (2.8–184,100.0)

Table 2 Drug-related adverse events

	Any	Grade 3/4
Overall incidence	53 (98)	36 (68)
Hematological		
Hemoglobin	1 (2)	0
Leukocytes	4 (8)	0
Platelets	14 (26)	3 (6)
Dermatologic events		
Hand–foot skin reaction	39 (72)	14 (26)
Rash	27 (50)	7 (13)
Alopecia	9 (17)	
Gastrointestinal events		
Anorexia	12 (22)	4 (7)
Diarrhea	17 (32)	0
Vomiting	3 (6)	1 (2)
Fatigue	22 (41)	0
Voice changes	2 (4)	0
Hypertension	14 (26)	0
Abdominal pain not otherwise specified	5 (9)	0
Bleeding	4 (8)	2 (4)
Laboratory		
AST	30 (55)	13 (24)
ALT	28 (52)	8 (15)
Bilirubin	15 (28)	6 (11)
Amylase	15 (28)	3 (6)
Liver failure	10 (19)	

Liver failure is defined as encephalopathy, massive ascites, or jaundice

Table 3 Adverse events causing dose reduction

	Number of patients (%)
Patients requiring dose reduction	45 (83)
Hand–foot skin reaction	21 (38)
AST/ALT	8 (15)
Rash	7 (13)
Liver failure	4 (7)
Anorexia	2 (4)
Bleeding	2 (4)
Vomiting	1 (2)
Time to dose reduction	
<2 weeks	24 (44)
≥2 weeks to <4 weeks	12 (22)
≥4 weeks	9 (17)

treatment discontinuation were liver failure ($n = 4$, 7%), HFSR ($n = 4$, 6%), fatigue ($n = 3$, 6%), and abdominal pain not otherwise specified ($n = 3$, 6%). The median time to treatment failure (TTF; defined as the period from first treatment to discontinuation of sorafenib treatment, progression, or death) was 2 months (Fig. 1).

Table 4 Adverse events leading to treatment discontinuation

	Number of patients (%)
Any adverse events	17 (31)
Liver failure	4 (7)
Hand–foot skin reaction	3 (6)
Fatigue	3 (6)
Abdominal pain not otherwise specified	3 (6)
Anorexia	2 (4)
Rash	2 (4)

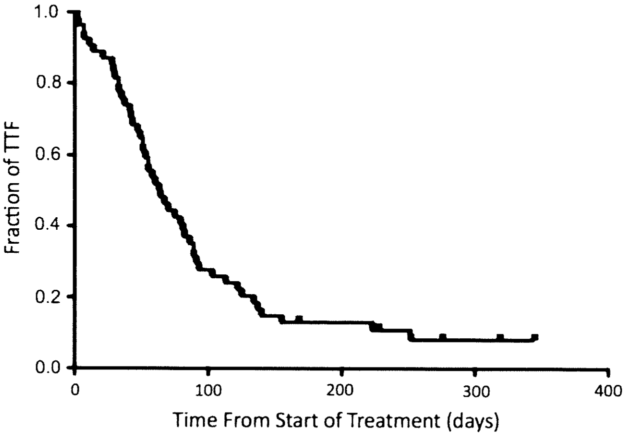

Fig. 1 Kaplan–Meier analysis of time to treatment failure (TTF). The median TTF was 2 months

Efficacy

According to RECIST version 1.1, one patient (2%) had a partial response, 25 patients had stable disease (57%), and the disease control rate (DCR; defined as no disease progression for ≥4 weeks) was 34% (Table 5).

At the time of analysis, with a median follow-up of 5.7 months (range 0.5–13.3), 49 patients had discontinued treatment (92%) and 28 patients were dead (52%). The overall median survival was 6.9 months (Fig. 2)

Discussion

The SHARP and Asia-Pacific studies, large, multicentre, phase III, randomized, double-blind, placebo-controlled trials of sorafenib, revealed a survival benefit and the tolerability of sorafenib in advanced HCC patients. However, considering the varying etiologies and treatment strategies for HCC in different regions [4], it is unclear whether these results apply to Japanese HCC patients. In Japan, high-risk groups for HCC, such as cirrhosis or hepatitis patients, undergo ultrasonography every 3–4 months and CT or MRI every 6–12 months for the early detection of HCC. Because we find HCC when it is earlier, Japanese HCC

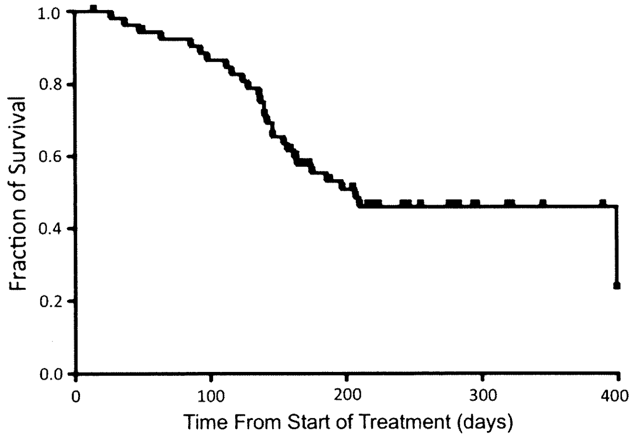

Fig. 2 Kaplan-Meier analysis of overall survival (OS). The median OS was 6.9 months

Table 5 Response rates using the response evaluation criteria in solid tumors

Response ($n = 44$)	Number of patients (%)
Complete response	0
Partial response	1 (2)
Stable disease	25 (57)
Progressive disease	18 (41)
DCR	15 (34)

DCR is the disease control rate, defined as the proportion of patients who had a best response rating of a complete response, partial response, or stable disease that was maintained for ≥4 weeks from the first manifestation of the rating

patients are often able to undergo surgery, local ablation, and TACE. Despite the efficacy of these procedures, patients frequently develop recurrence or disease progression after these treatments. In contrast, in much of the rest of Asia, the majority of patients are present with advanced disease, with large tumors, multiple tumors, and portal tumor thrombosis. These patients are less likely to receive curative treatment [18]. Furthermore, the liver function of HBV-related HCC patients tends to be better than that of HCV-related HCC patients. Shiratori et al. [2] reported that 38.6, 39.3, and 22.1% of cases presented as Child-Pugh A, B, and C when the severity of cirrhosis was classified in Japanese HCV-related HCC patients. By contrast, among the HBV-related HCC patients, 65.2, 26.1, and 8.7% cases presented as Child-Pugh A, B, and C. Additionally, liver function might worsen with the repetition of local therapies because sorafenib was only given to Child-Pugh A patients. Fewer HCV-related HCC patients (52%) were included in the present analysis compared with the general HCC prevalence in Japan (71–75%) [2, 6].

In the SHARP study, common drug-related adverse events were diarrhea (39%), fatigue (22%), HFSR (21%), rash (16%), alopecia (14%), anorexia (14%), and nausea (11%) [14]. Dose reduction due to adverse events was needed in 26% of subjects. The most common adverse events leading to dose reduction were diarrhea (8%), HFSR (5%), and rash (3%) [14]. Treatment was discontinued because of adverse events in 38%. The most frequent adverse events leading to sorafenib discontinuation were gastrointestinal events (6%), fatigue (5%), and liver dysfunction (5%) [14]. In comparison, in the Asia-Pacific study, the common drug-related adverse events were HFSR (45.0%), diarrhea (25.5%), alopecia (24.8%), fatigue (20.1%), rash (18.8%), hypertension (18.8%), and anorexia (12.8%) [15]. Dose reduction due to adverse events was needed in 30.9%, and treatment was discontinued due to adverse events in 19.5% [15]. The most common drug-related adverse events resulting in dose reduction were HFSR (11.4%) and diarrhea (7.4%) [15]. Compared with these studies, we observed a higher incidence of adverse events, especially HFSR, rash, hypertension, and liver failure.

The incidence of HFSR and rash in the Asia-Pacific study was higher than in the SHARP study [14, 15]. In a phase I study of a small population of Japanese patients with HCC, five of the six patients experienced HFSR and four experienced rash; these patients were Child-Pugh A receiving 400 mg twice daily [16]. In a phase II study of Japanese patients with advanced renal cell carcinoma [19], HFSR occurred in 55% and rash occurred in 37.4%. Asian patients, particularly Japanese, frequently develop HFSR. Although it is possible that the physiological difference is partly associated with race, prevention and management of HFSR are required in Japanese patients.

Regarding hypertension, Wu et al. [20] reported a 23.4% (95% CI 16.0–32.9%) overall incidence from a systemic review and meta-analysis of nine studies of renal cell cancer or other solid tumor. Hypertension was experienced by 14 patients (26%) in our study; no case was grade 3/4. Varying rates of hypertension have been reported, with a 5% incidence in the SHARP study and an 18.8% incidence in the Asia-Pacific study. In our study, the incidence of hypertension was comparable with that reported by Wu et al., although it was slightly higher compared with that reported in the SHARP and Asia-Pacific studies.

Liver failure occurred in ten patients (19%), while it was uncommon in the SHARP and Asia-Pacific studies. Nevertheless, Ozenne et al. [21] reported that seven (21%) French patients with Child-Pugh A experienced liver failure. The SHARP and Asia-Pacific studies showed the efficacy of sorafenib in carefully selected patients with advanced HCC. Liver failure may occur with the use of sorafenib in an unselected cirrhotic population. In our study, the median time to experience liver failure was 33 days (range 7–115); liver failure can happen in the early days of treatment. Furthermore, a common adverse event leading to treatment discontinuation was liver failure (7%).

In our study, 43 patients required dose reduction due to adverse events (83%). This was more frequent than in either the SHARP or Asia-Pacific studies. The most common adverse event leading to dose reduction was HFSR (43%) [12, 13]. Our patients suffered more HFSR than those in the SHARP and Asia-Pacific studies [12, 13]. The cause may be differences, such as age or race. Nevertheless, treatment discontinuation due to HFSR was required in only 6% of the patients; in the majority of the patients, it could be controlled by dose reduction. This concurred with the finding that two of seven patients with Child-Pugh A experienced HFSR when they took 400 mg daily in the Japanese phase I study [16].

In our series, 44% of the patients required dose reduction within 2 weeks and the median daily dose was 450 mg (range 182–800), demonstrating that it is difficult for Japanese patients to continue sorafenib treatment at 400 mg twice daily. Treatment was discontinued because of adverse events in 31% of our patients, which was similar to the rate in the SHARP study, but higher than in the Asia-Pacific study. Adverse events could be managed by dose reduction in the majority of patients. Therefore, careful follow-up is recommended.

The median overall survival was 10.7 months in the SHARP trial and 6.5 months in the Asia-Pacific trial. The differences in survival time might have been caused by differences in patient background. Patients in the Asia-Pacific study displayed more extrahepatic spread, more hepatic tumors, a worse ECOG-PS, and increased concentrations of AFP compared with patients in the SHARP study [14, 15]. The median survival time was 9.2 months in a phase II study [17] and 15.6 months in a Japanese phase I study [16], although Child-Pugh B patients were included in both of these studies. More recently, two retrospective studies from Europe showed that the median survival times for Child-Pugh A patients were 8.9 [21] and 8.3 months [22]. The median overall survival in our series was 6.9 months, although the survival benefits cannot be directly compared, as this was a retrospective study. Our study included many patients with higher serum AFP levels, suggesting the inclusion of highly advanced cases in the present study.

In summary, the present study demonstrated that sorafenib was generally tolerated in Japanese HCC patients because the probability of treatment discontinuation due to adverse events was acceptable, although most patients needed dose reduction. The overall safety profile of sorafenib was similar to that seen in previous studies in patients with HCC, except for the higher rates of HFSR, rash, and liver failure.

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Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C

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Background & Aims: Assessment of the risk of hepatocellular carcinoma (HCC) development is essential for formulating personalized surveillance or antiviral treatment plan for chronic hepatitis C. We aimed to build a simple model for the identification of patients at high risk of developing HCC.

Methods: Chronic hepatitis C patients followed for at least 5 years (n = 1003) were analyzed by data mining to build a predictive model for HCC development. The model was externally validated using a cohort of 1072 patients (472 with sustained virological response (SVR) and 600 with nonSVR to PEG-interferon plus ribavirin therapy).

Results: On the basis of factors such as age, platelet, albumin, and aspartate aminotransferase, the HCC risk prediction model identified subgroups with high-, intermediate-, and low-risk of HCC with a 5-year HCC development rate of 20.9%, 6.3–7.3%, and 0–1.5%, respectively. The reproducibility of the model was confirmed through external validation ($r^2 = 0.981$). The 10-year HCC development rate was also significantly higher in the high- and intermediate-risk group than in the low-risk group (24.5% *vs.* 4.8%; $p < 0.0001$). In the high-and intermediate-risk group, the incidence of HCC development was significantly reduced in patients with SVR compared to those with nonSVR (5-year rate, 9.5% *vs.* 4.5%; $p = 0.040$).

Conclusions: The HCC risk prediction model uses simple and readily available factors and identifies patients at a high risk of HCC development. The model allows physicians to identify patients requiring HCC surveillance and those who benefit from IFN therapy to prevent HCC.

Keywords: Decision tree; Prediction; Pegylated interferon; Ribavirin; Risk.
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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide [1] and its incidence is increasing in many countries [2]. Chronic viral hepatitis is responsible for 80% of all HCC cases [2]. The need to conduct HCC surveillance should be determined according to the risk of HCC development because this surveillance is cost-effective only in populations with an annualized cancer development rate of ⩾1.5% [3]. The annualized rate of developing HCC from type C liver cirrhosis is 2–8% [4–6], indicating that this population with type C liver cirrhosis needs surveillance. However, the annualized rate of HCC development is <1.5% in patients with chronic hepatitis C but without cirrhosis and the benefit of surveillance for all patients with chronic hepatitis has not yet been established [3]. HCC surveillance may be needed for patients with advanced fibrosis because the risk of HCC development increases in parallel with the progression of liver fibrosis [7,8]. Liver biopsy is the most accurate means of diagnosing fibrosis, but a single liver biopsy cannot indicate long-term prognosis because liver fibrosis progresses over time. Serial liver biopsies are not feasible because of the procedure's invasiveness. Moreover, factors other than fibrosis, such as advanced age, obesity, sex, lower albumin, and low platelet counts, also contribute to the development of HCC from chronic hepatitis C [8–11]. Therefore, these factors must be considered while assessing the risk of HCC development.

A meta-analysis of controlled trials [12] has shown that interferon (IFN) therapy reduced the rate of HCC development in patients with type C liver cirrhosis. However, there was a marked heterogeneity in the magnitude of the prevention effect of IFN on HCC development among the studies, probably due to the large differences in the baseline rate of HCC development among the different trials [12]. Whether the incidence of HCC development could be reduced in all patients with chronic hepatitis C, especially in those without liver cirrhosis, remains to be elucidated.

Data mining analysis, unlike conventional statistical analysis, is performed in an exploratory manner without considering a predefined hypothesis. Decision tree analysis, the major component of data mining analysis, is used to extract relevant factors from among various factors. These relevant factors are then combined in an orderly sequence to identify rules for predicting the incidence of the target outcome [13]. Data mining analysis has been used to define prognostic factors in various diseases [14–20]. In the field of hepatic diseases, data mining analysis has proven to be a useful tool for predicting early response [21], sustained virological response (SVR) [22–25], relapse [26], and adverse events [27] in patients with chronic hepatitis C treated with pegylated interferon (PEG-IFN) plus ribavirin (RBV). The findings of data mining analysis are expressed as flowcharts and are therefore easily understood [28] and readily available for clinical use, even by physicians without a detailed understanding of statistics.

In the present study, data mining analysis was used to identify risk factors for HCC development in a cohort of patients with chronic hepatitis C who had been followed for at least 5 years. An HCC risk prediction model was constructed on the basis of simple and generally available tests because the goal was to make the model easy to use in the clinic. The suitability, reproducibility, and generalizability of the results were validated using the data of an external cohort that was independent of the model derivation cohort.

Materials and methods

Patients

The model derivation cohort consisted of 1003 chronic hepatitis C patients without cirrhosis who had a non-sustained virological response (nonSVR) to previous IFN administered at the Musashino Red Cross Hospital and were followed for at least 5 years. Patients who had SVR or those who were followed for less than 5 years were not included. An analytical database on age, body mass index, albumin, aspartate aminotransferase (AST) levels, alanine aminotransferase (ALT) levels, γ-glutamyltransferase (GGT) levels, total bilirubin levels, total cholesterol levels, hemoglobin levels, and platelet count at the start of the observation was created. Histological data such as fibrosis stage, activity grade, or degree of steatosis was not included in the database because the goal of the present study was to make the model on the basis of simple and generally available tests. The patients who developed HCC more than 5 years after the start of the observation were considered not to have developed HCC by the 5-year point because the model was intended to predict HCC development within 5 years. The 1072 chronic hepatitis C patients included in the external validation cohort were treated with PEG-IFN and RBV at the University of Yamanashi, Tokyo Medical and Dental University, Osaka University, Osaka City University, Nagoya City University, or Toranomon Hospital and followed for at least 5 years. Among them, 600 had nonSVR and 472 had SVR. Data from nonSVR patients in this external cohort were used for external validation of the HCC prediction model. To assess the preventive effect of PEG-IFN plus RBV therapy on HCC development, the cumulative HCC development rate was compared between SVR and nonSVR patients in the external validation cohort after stratification by the risk of HCC development as determined by data mining analysis. Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committees of all concerned hospitals.

HCC surveillance and diagnosis

HCC surveillance was conducted by performing abdominal ultrasonography every 4–6 months. Contrast-enhanced computer tomography, magnetic resonance imaging, or angiography were performed when abdominal ultrasonography suggested a new lesion suspicious for HCC. Classical HCC was diagnosed for tumors showing vascular enhancement with washout on at least two types of diagnostic imaging. Tumor biopsy was used to diagnose tumors with non-classical imaging findings.

Statistical analysis

The IBM-SPSS Modeler 13 (IBM SPSS Inc., Chicago, IL, USA) was used for decision tree analysis. The statistical methods used have been described previously [21,22,24–27]. In brief, the software searched the analytical database for the factor that most effectively predicted HCC development and for its cutoff value. The patients were divided into two groups according to that predictor. Each divided group was repeatedly assessed and divided according to this 2-choice branching method. Branching was stopped when the number of patients decreased to ⩽20 to avoid over fitting. Finally, an HCC risk prediction model was created through this analysis. The model classified patients into subgroups with different HCC development rates in a flowchart form. For model validation, nonSVR patients from an external cohort were individually fitted into the model and classified into the subgroups and the HCC development rates of those subgroups were then calculated. The suitability and reproducibility of the model were validated by comparing the subgroup HCC development rates of the model derivation group to those of the validation group.

On univariate analysis, Student's *t*-test was used for continuous variables and Fisher's exact test was used for categorical data. Logistic regression was used for multivariate analysis. A log-rank test for Kaplan–Meier analysis was used to statistically test HCC development rates over time. *p*-Values of <0.05 were considered significant. SPSS Statistics 18 (IBM SPSS Inc.) was used for these analyses.

Results

Univariate and multivariate analysis of factors associated with HCC development

The baseline characteristics of patients are shown in Table 1. The 5-year HCC development rate in the model derivation group was 6.2%, which did not differ significantly from the rate of 6.0% in the nonSVR group of the external cohort, but the rate of 2.0% in the SVR group of the external cohort was significantly lower than that in the model derivation group ($p = 0.0003$) and the nonSVR group of the external cohort ($p = 0.0012$). On univariate analysis, the factors found to be associated with HCC development in the model derivation cohort were age, AST levels, albumin levels, total cholesterol levels, and platelet count. On multivariate analysis, age (odds ratio 1.086), albumin levels (odds ratio 0.248), and platelet count (odds ratio 0.842) were significant predictors of HCC development (Table 2).

HCC risk prediction model by data mining analysis

The results of decision tree analysis are presented in Fig. 1. Age was selected as the first predictor. The 5-year HCC development rate was 3.4% in younger patients (<60 years) and 8.6% in older patients (⩾60 years). The second predictor for younger patients (<60 years) was platelet count. The HCC development rate was 6.9% in patients with a lower platelet count ($<150 \times 10^9$/L) and 0.8% in patients with a higher count ($\geqslant 150 \times 10^9$/L). The second predictor for older patients (⩾60 years) was also platelet count. The HCC development rate was 13.1% in patients with a lower platelet count ($<150 \times 10^9$/L) and 1.8% in patients with a higher count ($\geqslant 150 \times 10^9$/L). The third predictor was albumin levels,

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Table 1. Baseline characteristics of patients for model deviation and external validation.

	Model derivation (n = 1003)	External cohort, non-SVR (n = 600)	External cohort, SVR (n = 472)
Sex: Male/Female*	463 (46%)/540 (54%)	306 (51%)/294 (49%)	299 (63%)/173 (37%)
Age (yr)	57.3 (11.1)	55.9 (9.6)	51.4 (10.6)
Body mass index (kg/m²)	23.5 (3.2)	23.4 (3.3)	23.3 (3.1)
Albumin (g/dl)	4.1 (0.3)	4.0 (0.4)	4.0 (0.3)
AST (IU/L)	64.2 (36.5)	67.3 (43.8)	62.5 (48.3)
ALT (IU/L)	80.6 (55.1)	81.2 (62.3)	88.6 (82.1)
GGT (IU/L)	59.3 (50.5)	67.6 (65.1)	55.7 (71.2)
Total cholesterol (mg/dl)	172.1 (31.5)	168.2 (31.0)	174.3 (33.7)
Platelet (10^9/L)	154.0 (53.0)	153.7 (53.2)	176.6 (49.7)
Hemoglobin (g/dl)	13.3 (1.5)	14.2 (1.5)	14.4 (1.4)
HCC development within 5 years: n (%)*	62 (6.2%)	36 (6.0%)	10 (2.0%)

Data expressed as mean (standard deviation) unless otherwise indicated.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.
*Data expressed as number of patients (percentage).

whose cutoff value was 3.75 g/dl in patients with a higher platelet count (⩾150 × 10^9/L). The HCC development rate was 6.3% when albumin levels were lower (<3.75 g/dl) and 1.5% when levels were higher (⩾3.75 g/dl). The cutoff value for albumin levels was 4.0 g/dl in patients with a lower platelet count (<150 × 10^9/L). The HCC development rate was 20.9% when albumin levels were lower (<4.0 g/dl) and 6.4% when levels were higher (⩾4.0 g/dl). The fourth and final predictor was AST levels. The HCC development rate was 7.3% when AST levels were at least 40 IU/L and 0% when the levels were <40 IU/L. On the basis of this analysis, seven subgroups with a 5-year HCC development rate of 0–20.9% were identified. The area under the receiver operating characteristic curve according to the HCC risk prediction model was 0.817.

External validation of the HCC risk prediction model with an independent external cohort

Six hundred nonSVR patients from an external cohort were fitted into the HCC risk prediction model and classified into the seven subgroups. The 5-year HCC development rate of these subgroups was 0–17.9%. The HCC development rate in the individual subgroups of the model derivation group was closely correlated to that in the corresponding subgroups of the external validation group (Fig. 2; correlation coefficient r^2 = 0.981). The HCC development rate in the subgroup of patients with the highest risk of HCC development (high-risk group) according to the model older age (⩾60 years) with a lower platelet count (<150 × 10^9/L) and lower albumin levels (<4.0 g/dl) was 20.9% in the model derivation

Table 2. Multivariable analysis of factors associated with subsequent development of HCC within 5 years.

	Odds ratio	95% CI	*p* value
Age	1.086	1.029-1.146	0.003
Albumin	0.248	0.100-0.613	0.003
Platelet	0.842	0.769-0.921	<0.0001

CI, confidence interval.

group and 17.9% in the external validation group. The intermediate-risk group or the patients with an HCC development rate of at least 5% consisted of the following three subgroups: (1) older age (⩾60 years), lower platelet count (<150 × 10^9/L), higher albumin levels (⩾4.0 g/dl), and higher AST levels (⩾40 IU/L); (2) older age (⩾60 years), higher platelet count (⩾150 × 10^9/L), and lower albumin levels (<3.75 g/dl); and (3) younger age (<60 years) and lower platelet count (<150 × 10^9/L). In these intermediate-risk groups, the 5-year HCC development rate was 6.3–7.3% in the model derivation group and 5.3–7.9% in the external validation group. The low-risk group consisted of the following three subgroups: (1) younger age (<60 years) and higher platelet count (⩾150 × 10^9/L); (2) older age (⩾60 years), lower platelet count (<150 × 10^9/L), higher albumin levels (⩾4.0 g/dl), and lower AST levels (<40 IU/L); and (3) older age (⩾60 years), higher platelet count (⩾150 × 10^9/L), and higher albumin levels (⩾3.75 g/dl). In these low-risk groups, the 5-year HCC development rate was 0–1.5% in the model derivation group and 0–2.9% in the external validation group.

Predictability of the HCC risk prediction model on HCC development rate beyond 5 years

Cumulative HCC development rates in the high-, intermediate-, and low-risk groups were compared over time using the Kaplan–Meier method. The 10-year rates were 28.9% in the high-risk group, 22.9% in the intermediate-risk group, and 4.8% in the low-risk group (Fig. 3A). The high and intermediate-risk group created by pooling data from the high- and intermediate-risk groups had a significantly higher cumulative HCC development rate than the low-risk group beyond 5 years (Fig. 3B; 5-year rate, 11.6% *vs.* 1.0%; 10-year rate, 24.5% *vs.* 4.8%; *p* <0.0001).

Effect of response to PEG-IFN plus RBV therapy in the reduction of HCC development: analysis stratified by the HCC risk prediction model

The 600 nonSVR patients and 472 SVR patients in the external cohort were fitted into the HCC risk prediction model and

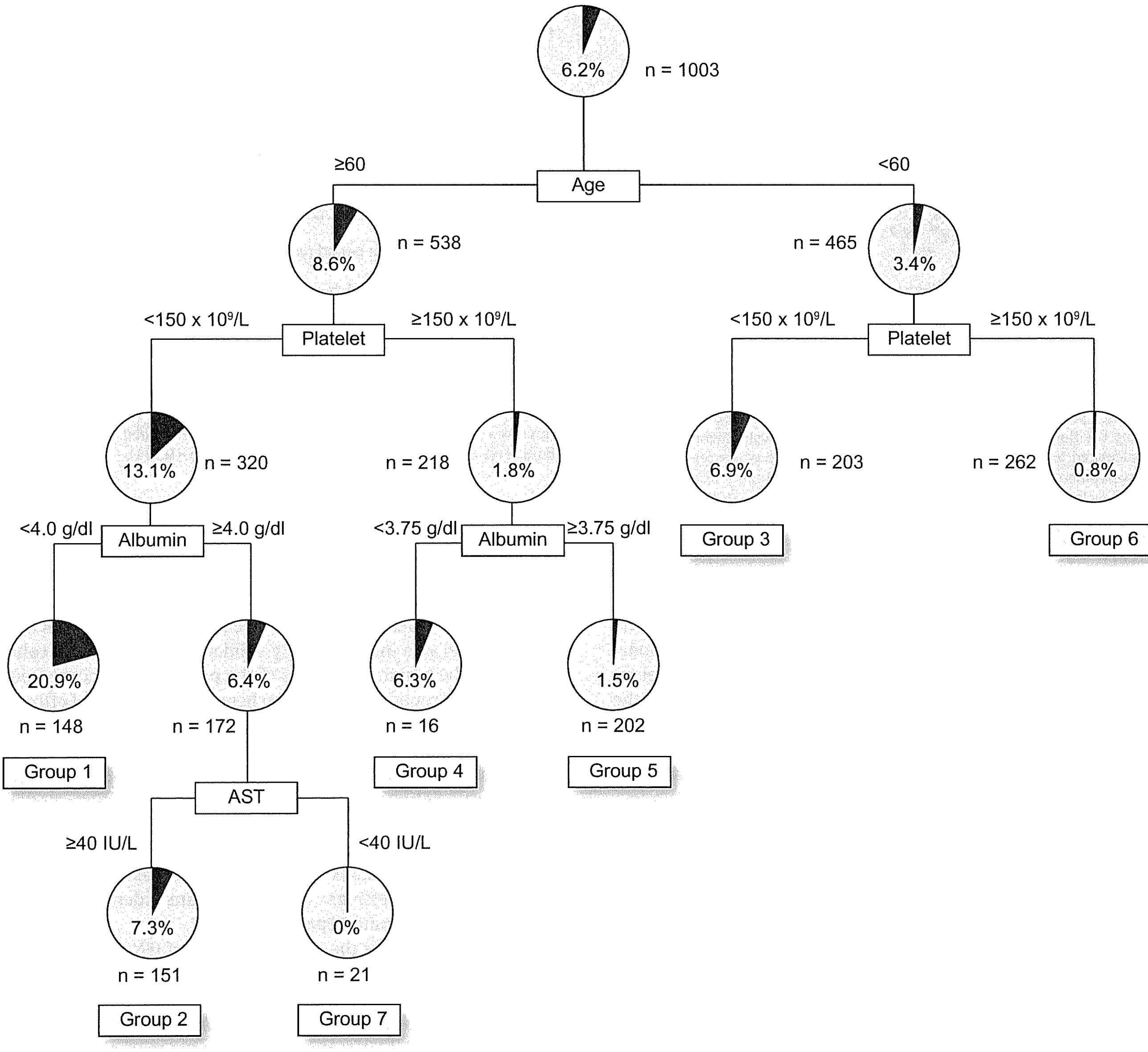

Fig. 1. The decision tree model of HCC development within 5 years. Boxes indicate the factors used to differentiate patients and the cutoff values for those different groups. Pie charts indicate the HCC development rate within 5 years for each group of patients after differentiation. Terminal groups of patients differentiated by analysis are numbered from 1 to 7.

classified into the high-and intermediate-risk group or the low-risk group, as defined above. The HCC development rate was significantly lower in SVR patients than in nonSVR patients in the high-and intermediate-risk group (5-year HCC rate, 9.5% *vs.* 4.5%; $p = 0.040$, log-rank test). In the low-risk group, the 5-year rate was 1.8% in nonSVR patients and 0.9% in SVR patients. Both rates were low and not significantly different ($p = 0.331$, log-rank test) (Fig. 4).

Discussion

An awareness of the risk of HCC development in the context of routine care for chronic hepatitis C is essential for formulating an HCC surveillance plan personalized for individual patients. The risk of developing HCC from chronic hepatitis is lower than that from cirrhosis [7]; therefore, across-the-board surveillance for chronic hepatitis C is not recommended [3]. A method to easily determine this risk, without performing serial liver biopsies, would be extremely significant clinically. In the present study, an HCC risk prediction model that included the factors such as age, platelet count, albumin levels, and AST levels was constructed. The model was found to have excellent reproducibility when validated with an external cohort. This model could identify subgroups of chronic hepatitis C patients at high risk of HCC development; the 5-year HCC development rate for the high- and intermediate-risk groups was 11.6%, yielding an annual incidence of 2.3%. This HCC risk prediction model requires only

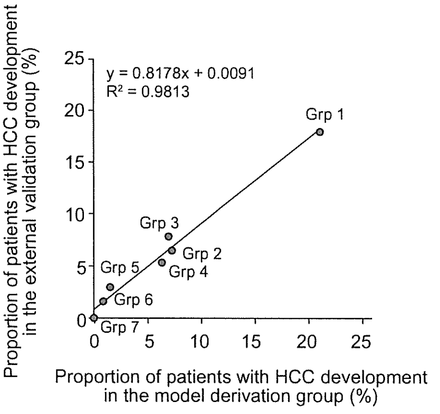

Fig. 2. External validation of the decision tree model with an independent cohort. Each patient in the external validation group was allocated to groups 1–7 following the flowchart of the decision tree. The HCC development rates were then calculated for each group and the graph plotted. The *x*-axis represents the HCC development rate in the model derivation group, and the *y*-axis represents the HCC development rate in the external validation group. The HCC development rates in each subgroup of patients are closely correlated between the model derivation group and the external validation group (correlation coefficient: $R^2 = 0.981$).

simple test values that are readily obtained in routine care and can therefore be easily used at the patient bedside. The model can be used to identify patients with a high risk of HCC development and therefore requiring surveillance, thereby allowing the formulation of surveillance plans personalized for individual patients.

Advanced fibrosis has been reported as independent risk factors for HCC development [7,8]. Platelet counts and albumin levels, which were factors selected for discrimination of the risk of HCC development, are closely related to the stage of fibrosis. Their correlation with the HCC risk has been repeatedly demonstrated [9–11,29–31]. The present study confirmed the impact of old age and advanced fibrosis, as reflected by low platelet counts and albumin levels. These results are consistent with our previous report [32]. What is unique to the present study was the study design to build a simple and reliable model for

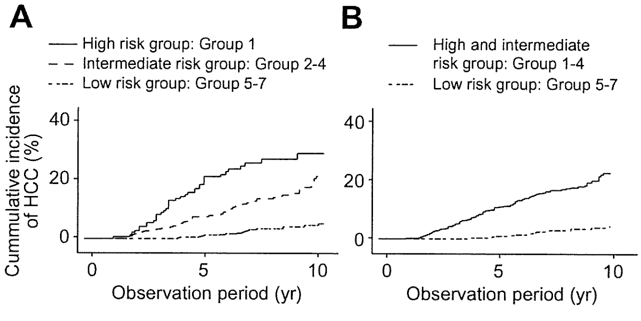

Fig. 3. Cumulative incidence of HCC development beyond 5 years in subgroups of patients defined by the decision tree model. Cumulative incidences of HCC in the groups classified by the decision tree model are compared. (A) The cumulative HCC development rate beyond 5 years is higher in the high- (group 1) and intermediate-risk (groups 2–4) groups compared to the low-risk group (groups 5–7). (B) The high and intermediate-risk group created by pooling data from the high- and intermediate-risk groups has a significantly higher cumulative HCC development rate than the low-risk group (5-year rate, 11.6% *vs.* 1.0%; 10-year rate, 24.5% *vs.* 4.8%; $p < 0.0001$).

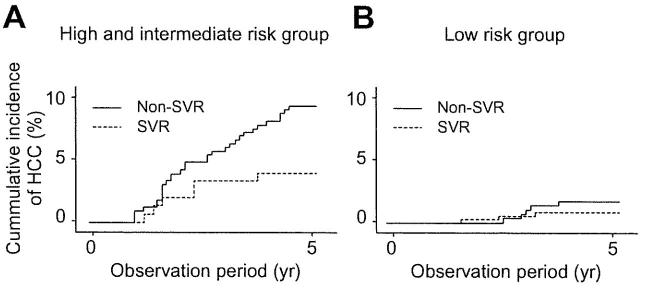

Fig. 4. Sustained virological response to PEG-IFN plus RBV therapy reduces the incidence of HCC development after stratification by the HCC risk. The 600 nonSVR patients and the 472 SVR patients in the external cohort were fitted into the HCC risk prediction model and classified into the high and intermediate-risk group or the low-risk group. The HCC development rate is significantly lower in SVR patients than in nonSVR patients in the high and intermediate-risk group (groups 1–4) (5-year HCC rate, 9.5% *vs.* 4.5%; $p = 0.040$). In the low-risk group (groups 5–7), the 5-year rate is 1.8% in nonSVR patients and 0.9% in SVR patients. Both rates are low and not significantly different ($p = 0.331$).

the prediction of HCC development that could be easily used in the clinic. For this purpose, a novel statistical method was used, histological factors were excluded in the analysis, the model derivation cohort was restricted to those who had nonSVR and had a long follow-up period duration (5 years), and the reproducibility of the model was independently validated by an external cohort. These are the major differences of the present study compared to our previous report. Many researchers have put a lot of efforts to formulate regression models for HCC prediction [9,10,33]. These prediction models are useful for identifying high-risk patients but are somewhat complicated to use at the bedside because they require calculations to be performed. Our prediction model is used simply by incorporating patients' data obtained through simple tests into the decision tree and following the flowchart. These prediction models based on factors easily accessible in routine clinical settings help physicians identify high-risk patients out of chronic hepatitis.

Viral eradication is the short-term goal of IFN therapy, but the ultimate goal is the prevention of HCC occurrence. Previous reports have shown that SVR to IFN therapy suppresses HCC occurrence in patients with type C liver cirrhosis and chronic hepatitis [7,12,30,34,35]. However, there is a marked heterogeneity in the magnitude of the treatment effect on the risk of HCC among studies, probably due to differences in the baseline risk of HCC among different trials [12]. Thus, the question remains whether the preventive effect of IFN therapy on HCC development could apply to all patients with chronic hepatitis C, especially those without liver cirrhosis. The result of the present study indicated that among high- and intermediate-risk patients, as assessed with our HCC risk prediction model, the cumulative HCC development rate was significantly reduced in SVR patients compared with nonSVR patients. This finding suggests that patients with chronic hepatitis, in whom disease has not yet progressed to hepatic cirrhosis but who are at a high risk of HCC development, benefit from antiviral treatment. The preventive effect of IFN on HCC development was not evident in low-risk patients within 5 years of observation. A longer observation term may be required to analyze the possible effect of antiviral therapy in these patients. Application of the present model on treatment decision may have limitations in that effect to prevent HCC development may differ in newer therapeutic agents such as protease

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inhibitors [36,37], and that low-risk patients may also benefit from therapy after a longer term observation period such as 15–20 years.

Patients with chronic hepatitis often have no subjective symptoms accompanying their disease and therefore have a low consciousness of the disease. The broad array of adverse reactions and the high cost of IFN therapy are frequent hurdles in motivating patients to undergo therapy. However, patients may be convinced to undergo therapy or remain motivated for continued therapy if they are made aware of their risk of HCC development and the preventive effect of IFN on HCC development.

In conclusion, a reproducible HCC risk prediction model, which includes the factors such as age, platelet count, albumin levels, and AST levels, was constructed to predict the 5-year HCC development rate in patients with chronic hepatitis C. The model requires only a combination of readily available test values and can therefore be easily used at the bedside. The information provided by the model allows the physician to identify patients requiring IFN therapy for the prevention of HCC and formulate plans for imaging HCC surveillance.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Original Article

Cancer preventive effect of pegylated interferon α-2b plus ribavirin in a real-life clinical setting in Japan: PERFECT interim analysis

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Aim: This study was conducted to clarify the incidence of hepatocellular carcinoma (HCC) and the factors contributing to its occurrence by following chronic hepatitis C patients who received pegylated interferon (PEG-IFN) α-2b plus ribavirin (RBV) combination therapy.

Methods: Patients who received PEG-IFN α-2b and RBV combination therapy with no history of HCC or HCC within 3 months after the start of treatment were observed for the onset of HCC at 67 centers.

Results: Sustained virological response (SVR) was observed in 999 (53.5%) of 1865 patients eligible for analysis. During the observation period (median duration: 4 years and 3 months), HCC developed in 59 patients (3.1%). A significant difference was observed in the 5-year cumulative incidence of HCC between SVR and non-SVR patients (1.1% vs. 7.1%). Factors contributing to HCC selected in multivariate analysis were therapeutic efficacy, sex, age, alanine aminotransferase (ALT) level at 24 weeks after the end of treatment, and platelet count. Non-SVR patients with ALT improvement after the end of treatment had a significantly lower 5-year cumulative incidence of HCC than those without (3.4% vs. 11.0%). HCC developed in 10 patients who achieved SVR, and multivariate analysis indicated that ALT level at 24 weeks after the end of treatment was the only significant factor contributing to HCC.

Conclusion: Several known risk factors for HCC contributed to HCC in patients who received PEG-IFN α-2b and RBV combination therapy, and ALT abnormality after the end of treatment contributes to the onset of HCC in both non-SVR and SVR patients.

Key words: alanine aminotransferase, chronic hepatitis C virus, hepatocellullar carcinoma, pegylated inteferon, ribavirin

Abbreviations: AFP, alpha fetoprotein; ALT, alanine aminotransferase; BR, biochemical response; CHC, chronic hepatitis C; HCC, hepatocellular carcinoma; IFN, interferon; LVR, late virological response; NR, no response; NVR, nonvirological response; PEG-IFN, pegylated interferon; ; RBV, ribavirin; SVR, sustained virological response; TR, transient response.

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INTRODUCTION

THE INCREASE IN the incidence of hepatocellular carcinoma (HCC) in Japan peaked in 2004 and is now in a declining trend.[1] The HCC mortality rate, however, is still particularly high among developed countries,[2] and even now nearly 35 000 people die