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Original Article

Changes in Plasma Vascular Endothelial Growth Factor at 8 Weeks After Sorafenib Administration as Predictors of Survival for Advanced Hepatocellular Carcinoma

Kaoru Tsuchiya, MD, PhD[1]; Yasuhiro Asahina, MD, PhD[2,3]; Shuya Matsuda, MD[1]; Masaru Muraoka, MD[1]; Toru Nakata, MD[1]; Yuichiro Suzuki, MD[1]; Nobuharu Tamaki, MD[1]; Yutaka Yasui, MD[1]; Shoko Suzuki, MD[1]; Takanori Hosokawa, MD[1]; Takashi Nishimura, MD, PhD[1]; Ken Ueda, MD[1]; Teiji Kuzuya, MD, PhD[1]; Hiroyuki Nakanishi, MD, PhD[1]; Jun Itakura, MD, PhD[1]; Yuka Takahashi, MD, PhD[1]; Masayuki Kurosaki, MD, PhD[1]; Nobuyuki Enomoto, MD, PhD[4]; and Namiki Izumi, MD, PhD[1]

BACKGROUND: A new predictive biomarker for determining prognosis in patients with hepatocellular carcinoma (HCC) who receive sorafenib is required, because achieving a reduction in tumor size with sorafenib is rare, even in patients who have a favorable prognosis. Vascular endothelial growth factor (VEGF) receptor is a sorafenib target. In the current study, the authors examined changes in plasma VEGF concentrations during sorafenib treatment and determined the clinical significance of VEGF as a prognostic indicator in patients with HCC. **METHODS:** Plasma VEGF concentrations were serially measured in 63 patients with advanced HCC before and during sorafenib treatment. A plasma VEGF concentration that decreased >5% from the pretreatment level at 8 weeks was defined as a "VEGF decrease." An objective tumor response was determined using modified Response Evaluation Criteria in Solid Tumors 1 month after the initiation of therapy and every 3 months thereafter. **RESULTS:** Patients who had a VEGF decrease at week 8 (n = 14) had a longer median survival than those who did not have a VEGF decrease (n = 49; 30.9 months vs 14.4 months; $P = .038$). All patients who had a VEGF decrease survived for >6 months, and the patients who had both a VEGF decrease and an α-fetoprotein response (n = 6) survived during the observation period (median, 19.7 months; range, 6.5-31.0 months). In univariate analyses, a VEGF decrease, radiologic findings classified as progressive disease, and major vascular invasion were associated significantly with 1-year survival; and, in multivariate analysis, a VEGF decrease was identified as an independent factor associated significantly with survival. **CONCLUSIONS:** A plasma VEGF concentration decrease at 8 weeks after starting sorafenib treatment may predict favorable overall survival in patients with advanced HCC. ***Cancer* 2014;120:229-37.**

KEYWORDS: antiangiogenic therapy, biomarker, hepatocellular carcinoma, prognosis, α-fetoprotein.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver (70%-85%) and a major cause of mortality. It is the fifth and seventh most frequent cancer and the second and sixth most frequent cause of cancer death in men and women, respectively.[1] At early stages or at Barcelona Clinic Liver Cancer stage A, a 5-year survival rate of 60% to 70% can be achieved in well selected patients with HCC who undergo surgical therapies (liver resection or transplantation) or locoregional procedures (ie, radiofrequency ablation).[2] However, treatment of advanced HCC that is not amenable to surgical or locoregional therapies remains a challenge in clinical practice.

Sorafenib is an oral, small-molecule tyrosine kinase inhibitor that blocks the synthesis of several intracellular proteins considered to be important for tumor progression, including the platelet-derived growth factor receptor beta, raf kinase, and the vascular endothelial growth factor (VEGF) receptor. VEGF is a homodimetric glycoprotein with a molecular weight of 45 kDa. The VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and a structurally related molecule: placental growth factor. Three high-affinity VEGF tyrosine kinase receptors (VEGFRs) have been identified:

Corresponding author: Namiki Izumi, MD, PhD, Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino-shi, Tokyo 180-8610, Japan; Fax: (011) 81-422-32-9551; nizumi@musashino.jrc.or.jp

[1]Department of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo, Japan; [2]Department of Gastroenterology and Hepatology, Tokyo Medical and Dental University, Tokyo, Japan; [3]Department of Liver Disease Control, Tokyo Medical and Dental University, Tokyo, Japan; [4]First Department of Internal Medicine, University of Yamanashi, Yamanashi, Japan

The first 2 authors contributed equally to this article.

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VEGFR-1, VEGFR-2, and VEGFR-3. VEGFR-2 is the principal receptor that promotes the proangiogenic action of VEGF-A and has been the principal target of antiangiogenic therapies, although additional studies have underlined the importance of signaling through VEGFR-1. In 2 phase 3, placebo-controlled, randomized trials, sorafenib treatment significantly improved the time to tumor progression (TTP) and overall survival (OS) of patients with advanced HCC.[3,4] In those trials, however, no statistically significant pretreatment factors that predicted responses after patients started receiving sorafenib were identified.[5] Therefore, in clinical practice, it is extremely important to identify a predictive post-treatment biomarker that is associated with the treatment efficacy of sorafenib and the prognosis of patients after they start receiving sorafenib.

In general, the efficacy of treating solid tumors with systemic chemotherapy agents is assessed by radiologic findings. In 2010, Lencioni and Llovet published a modification of the Response Evaluation Criteria in Solid Tumors (RECIST).[6] However, the modified RECIST can be used only for typical HCC. Advanced HCCs often have atypical vascular patterns; therefore, evaluating tumor response to sorafenib is difficult with radiologic findings alone. Alternatively, α-fetoprotein (AFP) is the most popular tumor marker for HCC, and it has been reported that early AFP responses are a useful surrogate marker for predicting treatment response and prognosis in patients with advanced HCC who receive cytotoxic and antiangiogenic agents.[7-9] However, approximately 30% of patients with advanced HCC in the Sorafenib HCC Assessment Randomized Protocol (SHARP) trial had normal AFP concentrations.[10] Therefore, the identification of a new biomarker that can complementarily predict the efficacy of sorafenib and the prognosis of patients is necessary.

In a mouse model, an increase in hepatic VEGF levels was observed at 24 hours, 72 hours, and 120 hours after the administration of sorafenib,[11] suggesting that a change in VEGF levels may also occur during sorafenib therapy in humans. Therefore, we evaluated plasma VEGF changes during sorafenib treatment in patients with advanced HCC to determine whether VEGF has potential as a new biomarker for the prediction of treatment efficacy and prognosis after sorafenib administration.

MATERIALS AND METHODS

Patient Selection

Between December 2009 and August 2012, 95 consecutive patients with advanced, inoperable HCC received treatment with sorafenib at Musashino Red Cross Hospital. The diagnosis of HCC was based on guidelines established by the Liver Cancer Study Group of Japan[12] and the American Association for the Study of Liver Diseases[13] or by pathologic examination. According to these guidelines, a diagnosis of HCC is confirmed by histology or by characteristic radiologic findings, such as typical arterial enhancement of the tumor followed by a washout pattern in the images in the portal venous phase or the equilibrium phase on dynamic spiral computed tomography (CT) imaging or contrast-enhanced magnetic resonance imaging. Inclusion criteria were predefined as follows: 1) patients were alive 8 weeks after beginning treatment; and 2) patients had plasma VEGF and serum AFP concentrations evaluated at baseline, at 4 weeks, and at 8 weeks. Of 95 patients, 23 were unavailable for a week-8 VEGF measurement for the following reasons: 7 patients stopped sorafenib therapy because of erythema multiforme (grade 2-3) and started other therapies (radiation therapy or cytotoxic chemotherapy) within 1 month after starting sorafenib, 4 patients moved to another location before week 8, 5 patients refused to undergo a plasma VEGF measurement at week 8, and 7 patients were not available for obtaining VEGF concentration results. These 23 patients and 9 other patients who died within 8 weeks were excluded from the study. Hence, in total, 63 patients fulfilled the inclusion criteria. At enrollment, all patients had metastatic or locally advanced HCC that was not amenable to surgery or locoregional therapies, including transcatheter arterial chemoembolization (TACE) and local ablation. Written informed consent was obtained from all patients, and the ethics committee at Musashino Red Cross Hospital approved the study in accordance with the Declaration of Helsinki.

Sorafenib Treatment

The initial daily dose of sorafenib was 800 mg in 28 patients, 400 mg in 28 patients, and 200 mg in 7 patients. A reduced initial dose was allowed for patients who had the following factors: advanced age (≥80 years), gastrointestinal varices with a risk of bleeding, low body weight (<50 kg), and a poor performance status (≥2). In total, 60 patients underwent multiphase-multidetector CT imaging before starting sorafenib, 1 month after starting sorafenib, and every 3 months thereafter. Radiologic responses to therapy were evaluated according to modified RECIST. In all patients, serial measurements of plasma VEGF and serum AFP concentrations were performed before and after the receipt sorafenib and every month thereafter, with an allowance of ± 1 week. The endpoint of the current study was OS. In the follow-up visit after sorafenib administration, the medication was discontinued if progressive disease

(PD) was identified despite treatment, if intolerable adverse events occurred, or if inappropriate liver function was observed. Other palliative treatments or best supportive care were provided subsequently. An AFP response was defined as a decrease ≥20% in the serum AFP concentration during 8 weeks of treatment.

Plasma VEGF Measurements

Serial serum samples were collected prospectively from each patient. Venous blood samples were drawn into a serum separator tube and centrifuged at ×1800*g* for 10 minutes, and plasma samples were stored at −80°C until measurement. Plasma VEGF concentrations were measured quantitatively using an enzyme-linked immunosorbent assay kit (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions. We defined a decrease in the plasma VEGF level >5% from the pretreatment level at 8 weeks as a "VEGF decrease."

Statistical Analysis

Categorical variables were compared using the chi-square test, and continuous variables were compared using the Mann-Whitney test. All tests of significance were 2-tailed, and P values < .05 were considered statistically significant. OS curves were calculated using the Kaplan-Meier method, and differences between groups were assessed using the log-rank test. OS was determined as the interval between the date of treatment initiation and either death or the last visit. A Cox proportional-hazards model was used to determine the factors associated with OS. In univariate analyses, clinical and biologic parameters (sex, age, etiology, albumin, bilirubin concentrations, Child-Pugh class, plasma VEGF concentrations, and serum AFP concentrations) and tumor factors (vascular invasion and distant metastasis) were included. A logistic regression model was used to identify the factors associated with 1-year survival after the receipt of sorafenib. All statistical analyses were performed using StatView (version 5.0) software (Abacus Concepts, Berkeley, Calif).

RESULTS

Patient Characteristics

In total, 63 patients were enrolled in this study, and their characteristics are listed in Table 1. The diagnosis of HCC was confirmed by histology in 11 patients and by typical radiologic findings based on established guidelines in the remaining 52 patients. In all, 51 patients had previously received other therapeutic modalities, including 22 patients who previously received radiofrequency ablation, 22 who previously underwent TACE, 1 who previously received transcatheter arterial chemoinfusion, and 6 who previously underwent hepatic resection. Twelve patients had received sorafenib as initial therapy for HCC. Among the 63 enrolled patients, 33 were seropositive for hepatitis C virus antibody, 8 were seropositive for hepatitis B surface antigen, and 22 were seronegative for both hepatitis C virus antibody and hepatitis B surface antigen. Eighteen patients had evidence of extrahepatic metastasis, and 18 had major vascular invasion. No patient was lost to follow-up in this study.

TABLE 1. Characteristics of Study Patients With Advanced Hepatocellular Carcinoma (n = 63)

Characteristic	Median [Range]
Age, y	70 [40-85]
Sex: No. of men (%)	53 (84.1)
Baseline AFP, ng/mL	114 [2.0-98440]
Baseline plasma VEGF, pg/mL	288 [60-1580]
Treatment duration, mo	4.1 [0.1-28.3]
Overall survival, mo	9.3 [2.0-30.9]

Abbreviations: AFP, α-fetoprotein; VEGF: vascular endothelial growth factor.

Pretreatment Plasma VEGF Concentration and Prognosis and Extent of Hepatocellular Carcinoma

Pretreatment plasma VEGF concentrations in the 9 patients who died within 8 weeks were significantly higher than in the patients who survived beyond 8 weeks (813 ± 630 pg/mL vs 384 ± 18 pg/mL; P = .0024). Consistent with a previous study (the SHARP trial; Llovet et al[3]), our data suggested that the pretreatment plasma VEGF concentration is a useful prognostic factor for sorafenib therapy. However, there was no significant difference in OS between patients who had pretreatment plasma VEGF concentrations ≤450 pg/mL (n = 46) and those who had concentrations >450 pg/mL (n = 17; P = .731). The pretreatment plasma VEGF concentration could not predict prognosis for the patients who survived beyond 8 weeks.

We compared the size and extent of HCC between patients who had low plasma VEGF concentrations (≤450 pg/mL) and high plasma VEGF concentrations (>450 pg/mL). No difference was observed in the size or extent of HCC at baseline between patients with lower versus higher pretreatment plasma VEGF concentrations.

Association Between Changes in Plasma VEGF Concentrations and Overall Survival

The median OS assessed by the Kaplan-Meier method was 16.3 months for all 63 patients enrolled in the study

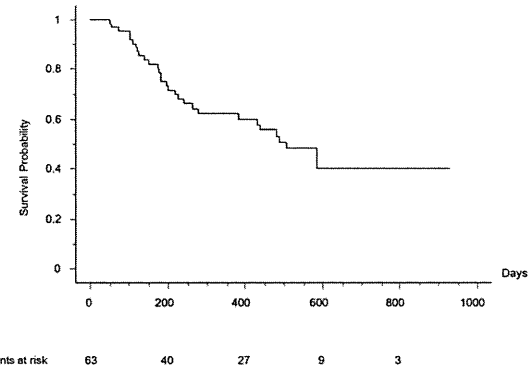

Figure 1. This Kaplan-Meier plot illustrates overall survival for all patients in the study.

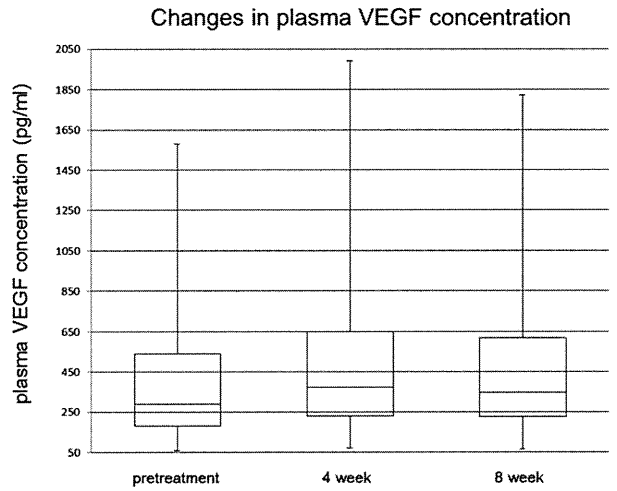

Figure 2. Changes in plasma vascular endothelial growth factor (VEGF) concentrations are illustrated.

(Fig. 1). Plasma VEGF concentrations at baseline, at 4 weeks, and at 8 weeks after the initiation of sorafenib treatment were 288 pg/mL (range, 60-1580 pg/mL), 372 pg/mL (range, 69-1990 pg/mL), and 347 pg/mL (range, 64-1840 pg/mL), respectively (Fig. 2). Plasma VEGF concentrations increased within 4 weeks after the administration of sorafenib in 47 of 63 patients (74.6%). The median survival of patients who had a decrease in their plasma VEGF concentration at week 4 (n = 16) and an increase in their plasma VEGF concentration at week 4 (n = 47) were 19.5 months and 16.8 months, respectively; and there was no significant difference in OS between changes in plasma VEGF at 4 weeks ($P = .645$). However, patients who had a VEGF decrease at week 8 (n = 14) had a longer median survival than those who did not have a VEGF decrease (n = 49; 30.9 months vs 14.4

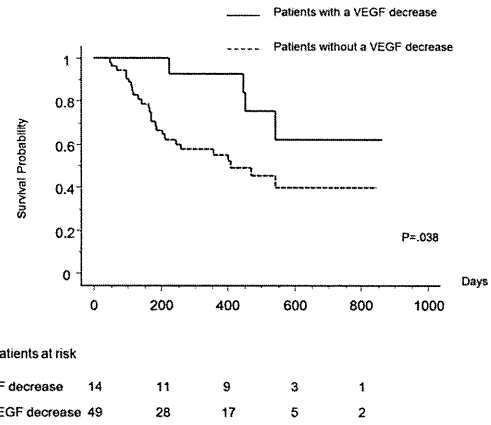

Figure 3. This Kaplan-Meier plot illustrates overall survival according to changes in vascular endothelial growth factor (VEGF) concentration.

months; $P = .038$) (Fig. 3), suggesting that a decrease in VEGF concentration 8 weeks after starting sorafenib treatment is closely associated with a favorable prognosis. The median percentage of decrease in the plasma VEGF concentration was 18.3% (range, 7%-41.7%). There were no differences in any pretreatment patient characteristics, including HCC stage and Child-Pugh score, between patients who did and did not have a VEGF decrease (Table 2).

Relation Between Radiologic Findings or Serum α-Fetoprotein Concentration and Overall Survival

The best radiologic responses to therapy assessed by modified RECIST were classified as a complete response (CR) (n = 4), a partial response (PR) (n = 16), stable disease (SD) (n = 34), and PD (n = 9). Fourteen patients had a VEGF decrease, and their best radiologic responses were a CR (n = 2), a PR (n = 2), SD (n = 9), and PD (n = 1). There was no significant difference in OS between the patients who had an objective response (CR + PR) and those with SD. The survival of patients who had PD was significantly worse than that of the patients without PD (median OS, 5.8 months and 19.4 months, respectively; $P = .0006$). There was no significant difference in OS between patients who had an AFP response and those who did not have an AFP response within the group that did not have PD (ie, those who attained a CR, a PR, or SD [the non-PD group]) (Fig. 4). There also was no significant difference ($P = .111$) between patients who did and did not have an AFP response among those in the non-PD group who had had an elevated AFP at baseline.

TABLE 2. Characteristics of Patients Categorized According to Variation in Vascular Endothelial Growth Factor Levels at 8 Weeks of Sorafenib Treatment

Characteristic	No. of Patients (%)		
	With VEGF Decrease, n = 14	Without VEGF Decrease, n = 49	*P*
Age, y	72	69	.325
Sex: Men	11 (78.6)	42 (85.7)	.679
Body weight, kg	58.3	62.3	.175
Cause of disease			.210
Hepatitis B	0 (0)	8 (16.3)	
Hepatitis C	9 (64.3)	24 (49)	
Other	5 (35.7)	17 (34.7)	
Prior treatment			.797
Yes	11 (78.6)	40 (81.6)	
No	3 (21.4)	9 (18.4)	
Baseline bilirubin, mg/dL	0.8	1.0	.375
Baseline albumin, g/dL	3.4	3.6	.190
Child-Pugh score			.178
5	7 (50)	30 (61.2)	
6	7 (50)	16 (32.7)	
7	0 (0)	3 (6.1)	
Maximum tumor size, cm			.892
≤5	8 (57.1)	22 (44.9)	
>5	6 (42.9)	27 (55.1)	
No. of tumors			.883
≤3	10 (71.4)	34 (69.4)	
>3	4 (28.6)	15 (30.6)	
Extrahepatic disease			.502
Yes	3 (21.4)	15 (30.6)	
No	11 (78.6)	34 (69.4)	
Site of metastatic disease			
Lung	1	7	
Bone	1	4	
Lymph node	1	3	
Lung and bone	0	1	
Major vascular invasion			.739
Yes	3 (21.4)	15 (30.6)	
No	11 (78.5)	34 (69.4)	

Abbreviations: VEGF: vascular endothelial growth factor.

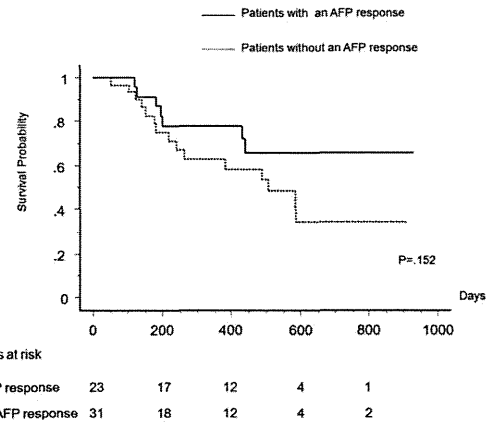

Figure 4. This Kaplan-Meier plot illustrates overall survival according to α-fetoprotein (AFP) response in patients without progressive disease (PD), classified as non-PD (ie, those who had a complete response, a partial response, or stable disease) according to modified Response Evaluation Criteria in Solid Tumors.

It is noteworthy that all patients who had a VEGF decrease and an AFP response survived during the observation period (median, 19.7 months; range, 6.5-31.0 months). In patients without a VEGF response (n = 49), there was no significant difference in OS between those who did and did not have an AFP response ($P = .147$). Of 49 patients who did not have a VEGF decrease at 8 weeks, 19 patients were able to survive beyond 1 year after starting sorafenib. Nine patients without a VEGF decrease at 8 weeks survived for >18 months.

Prognostic Factors After Sorafenib Administration

In univariate analysis, among all patients, a VEGF decease and an AFP response were associated significantly with OS after starting sorafenib. Major vascular invasion and PD, as evidenced by radiologic findings after sorafenib administration, also were significant prognostic factors. To predict which patients would have a highly favorable prognosis, the prognostic factors associated with 1-year survival after starting sorafenib were assessed in univariate and multivariate analyses. In the univariate analysis, a VEGF decrease, PD, and major vascular invasion were associated significantly with survival (Table 3). In the multivariate analysis, which was performed using those factors as covariates, a VEGF decrease was identified as an independent factor associated significantly with survival (Table 3). There was a significant difference in OS among the 3 groups (patients with a VEGF decrease and non-PD, patients without a VEGF decrease but non-PD, and patients without a VEGF decrease and PD; $P = .0013$) (Fig. 5). Only 1 patient who had a VEGF decrease was classified with PD. All 4 patients who had a VEGF decrease and an objective response (CR or PR) were able to survive during the observation period.

Adverse Events During Sorafenib Treatment

The overall incidence of treatment-related adverse events was 100%. The rate of discontinuation of sorafenib as a result of adverse events was 22.2%. Adverse events that led to the discontinuation of sorafenib treatment were liver dysfunction (63.6%), hand-foot skin reaction (18.2%), interstitial pneumonia (9.1%), and rash (9.1%). Dose reductions because of adverse events occurred in 62 patients. The most frequent adverse event leading to dose reductions was liver dysfunction (33.9%). In addition,

TABLE 3. Prognostic Factors Associated With 1-Year Survival After Sorafenib Administration

Risk Factor	OR (95% CI)[a]	*P*
Univariate analysis		
Age, by every 10 y	1.47 (0.75-2.87)	.266
Sex		
Women	1.00	
Men	0.26 (0.50-1.39)	.116
HBV infection		
Negative	1.00	
Positive	0.33 (0.06-2.02)	.231
HCV infection		
Negative	1.00	
Positive	1.23 (0.41-3.74)	.714
Albumin, by every 1 g/dL,	1.34 (0.45-3.99)	.604
Total bilirubin, by every 1 mg/dL	0.79 (0.28-2.25)	.656
Pre-AFP, by every 10 ng/mL	1.00 (1.00-1.00)	.161
Tumor size, cm		
<5	1.00	
≥5	0.42 (0.14-1.32)	.147
No. of tumors		
≤3	1.00	
≥4	0.26 (0.06-1.08)	.064
Major vascular invasion		
Yes	1.00	
No	4.00 (1.12-14.4)	.034
Extrahepatic metastasis		
Yes	1	
No	1.82 (0.56-5.90)	.320
5% VEGF decrease at wk 8		
No	1.00	
Yes	11.1 (1.29-94.6)	.028
PD		
No	1.00	
Yes	0.16 (0.29-0.86)	.033
Objective response: CR + PR		
No	1.00	
Yes	1.63 (0.49-5.42)	.426
AFP response		
No	1.00	
Yes	2.76 (0.80-9.52)	.107
Multivariate analysis[b]		
5% VEGF decrease at wk 8		
No	1.00	
Yes	10.0 (1.02-91.3)	.041
PD		
No	1.00	
Yes	0.20 (0.29-1.39)	.104
Major vascular invasion		
Yes	1.00	
No	3.03 (0.71-12.9)	.134

Abbreviations: AFP, α-fetoprotein; CI, confidence interval; CR, complete response; HBV, hepatitis B virus; HCV, hepatitis C virus; PD, progressive disease; PR, partial response; VEGF, vascular endothelial growth factor.
[a] The ORs for 1-year survival were calculated using logistic regression analysis.
[b] In the multivariate logistic analysis, a 5% VEGF decrease, PD, and portal invasion were included as covariates.

the incidence of adverse events was not related to plasma VEGF concentrations.

DISCUSSION

In the current study, we demonstrated that plasma VEGF concentrations change dynamically during sorafenib therapy, and changes in VEGF concentration are closely associated with OS in patients who receive treatment with sorafenib. VEGF is the major mediator of angiogenesis in HCC, and several studies have correlated VEGF concentrations with the prognosis of patients who have advanced HCC.[5,14-21]

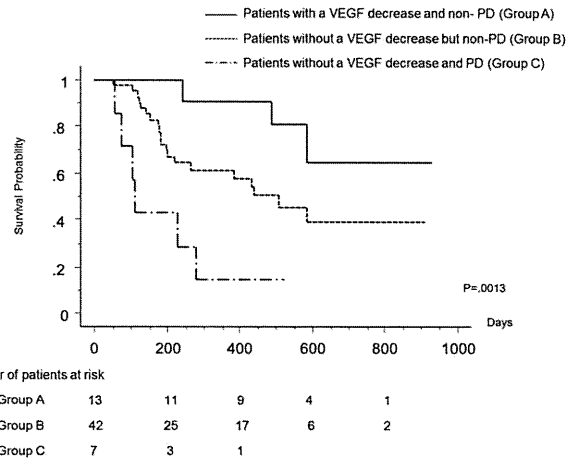

Figure 5. This Kaplan-Meier plot illustrates overall survival according to the combination of vascular endothelial growth factor (VEGF) changes and radiologic findings classified by modified Response Evaluation Criteria in Solid Tumors. Non-PD indicates patients who did not have progressive disease (PD) (ie, those who had a complete response, a partial response, or stable disease).

Recently, a new staging system was proposed that includes the plasma VEGF concentration along with the Cancer of the Liver Italian Program (CLIP) score; this new system—known as the V-CLIP score—classifies patients with advanced HCC more appropriately into a homogeneous prognostic group.[22] Therefore, the concentration of circulating VEGF is included as a candidate prognostic marker for HCC, especially in patients with advanced disease. The objective of our study was to elucidate the important question of whether an on-treatment change in VEGF is a potentially useful new biomarker for predicting prognosis in patients who survive beyond 8 weeks, because such an on-treatment predictor among patients who have relatively longer survival has not yet been elucidated. In this study, plasma VEGF concentrations increased from pretreatment levels within 4 weeks of starting sorafenib in 47 of 63 patients (74.6%). This was followed by a decrease in plasma VEGF levels at 8 weeks in 68.1% of patients. A possible mechanism of this transient increase in VEGF after starting sorafenib may be related to a reactive increase against the inhibition of VEGF activity or hypoxia induced by sorafenib. This

hypothesis is supported by the demonstration that plasma VEGF concentrations increased shortly after treatment with TACE.[24-26] It is believed that these increases in plasma VEGF concentration are related to the induction of tissue hypoxia.[27] However, the peak time point of VEGF elevation during sorafenib administration was different from that previously reported in TACE, in which a transient elevation of VEGF was observed within 7 days after TACE.[24-26] This observed difference may be related to the continuous induction of hypoxia by sorafenib administration.

It is noteworthy that, in our study, decreases in plasma VEGF observed within 8 weeks of sorafenib administration were associated with better OS. One possible reason for this association may be that the decrease in VEGF concentrations reflects a decrease in the number of tumor cells secreting VEGF. An association between changes in VEGF concentrations and disease progression was observed in a previous study of an anti-VEGF antibody, bevacizumab, in patients with advanced HCC.[23] In that study, plasma VEGF-A concentrations decreased from baseline in all patients after 8 weeks of bevacizumab therapy and increased to near baseline levels in 5 of 6 patients at the time of disease progression. Unfortunately, plasma VEGF-A levels after 8 weeks of bevacizumab in that study were available for only 8 of 46 patients who were enrolled the study, and plasma VEGF-A levels after 4 weeks were not evaluated. In our study, all patients were evaluated before and every 4 weeks after starting sorafenib. Moreover, we demonstrated the usefulness of plasma VEGF concentrations at 8 weeks and not at 4 weeks. Zhu et al[28] reported that plasma levels of VEGF and placental growth factor increased after cediranib, a pan-VEGFR tyrosine kinase inhibitor monotherapy for advanced HCC. In that study, progression-free survival was correlated inversely with baseline levels of VEGF, soluble VEGFR2 (sVEGFR2), and basic fibroblast growth factor and with on-treatment levels of basic fibroblast growth factor and insulin-like growth factor-1; and progression-free survival was directly associated with on-treatment levels of interferon-γ. Because changes of VEGF concentrations during therapy were not identified as a prognostic factor in the study by Zhu et al, biomarkers that predict prognosis may be different among different types of tyrosine kinase inhibitors. Jayson et al[29] reported that plasma VEGF-A in patients who received bevacizumab was potentially predictive and prognostic in metastatic breast, gastric, and pancreatic cancers; however, it was only prognostic (and not predictive) in metastatic colorectal cancer, nonsmall cell lung cancer, and renal cell carcinoma. In our study, we measured plasma VEGF concentrations and not plasma VEGF-A concentrations. Sorafenib is a multikinase inhibitor, whereas bevacizumab is a humanized monoclonal antibody that recognizes and blocks VEGF-A expression. Further studies to evaluate the clinical usefulness of determining VEGF and VEGF-A concentrations during sorafenib therapy are necessary in various cancers. Although the precise mechanism underlying the association between serial changes in VEGF and disease progression is unclear, the findings of the current study are extremely valuable for clinical practice in predicting the prognosis of patients who receive treatment with sorafenib.

Llovet et al[5] studied plasma biomarkers as predictors of outcome in patients with advanced HCC. They measured plasma biomarkers in 491 patients at baseline and in 305 patients after 12 weeks in a phase 3, randomized, controlled trial (the SHARP trial). Those authors concluded that angiopoietin-2 and VEGF were independent predictors of survival in patients with advanced HCC and that none of the tested biomarkers significantly predicted response to sorafenib. In our study, by measuring plasma VEGF monthly, we demonstrated that the changes 8 weeks after starting sorafenib were important for predicting OS.

It has been reported that modified RECIST guidelines are useful for predicting efficacy and prognosis after patients with advanced HCC receive treatment with sorafenib.[30] However, modified RECIST can only be used for typical hypervascular HCC, and not for atypical HCC, including poorly differentiated HCC and diffuse-type HCC. Moreover, the percentage of patients in our study who had PD was only 11.1% (9 of 63 patients), and the objective response rate (CR + PR vs SD) could not predict OS, suggesting that using only modified RECIST guidelines was insufficient for predicting OS in most patients who received sorafenib (non-PD patients). Therefore, it is important to identify a predictive biomarker for those patients who can expect long survival during sorafenib therapy, although their radiologic findings may not be categorized as objective responses.

From this point of view, decreases in VEGF observed in non-PD patients at week 8 may identify patients who have a favorable prognosis. According to our results, the median survival of patients who had a VEGF decrease was extremely good at 31.0 months, and we demonstrated that a VEGF decrease, but not modified RECIST or AFP, was the only significant post-therapeutic factor associated with favorable survival after sorafenib administration (Table 3). In our study, all

patients who had both a VEGF decrease and an AFP response survived during the observation period (median, 19.7 months). Taken together, the combination of a plasma VEGF decrease, an AFP response, and modified RECIST is useful for predicting an extremely favorable prognosis.

This study had a few limitations. The first was our subanalysis of consecutive patients. However, the median survival for the 23 excluded patients who were available for estimation was equivalent to that of the included patients (16.8 months); therefore, it is unlikely that selection bias affected our results. The second limitation is that we measured only plasma VEGF concentrations. In previous studies, many factors, including VEGF-A, short VEGF-A isoform, sVEGFR1, sVEGFR2, sVEGFR3, angiopoietin-2, and insulin-like growth factor-2, were evaluated as biomarkers. However, to our knowledge, this is the first clinical study to demonstrate the early dynamic changes in plasma VEGF concentrations in patients who received sorafenib. Finally, the number of patients in this study was relatively small to make recommendations to physicians. Our results indicated that patients who have decreased VEGF concentrations at 8 weeks have a favorable prognosis, regardless of their radiologic findings. However, further studies with a larger number of patients will be necessary to propose new recommendations.

In conclusion, changes in plasma VEGF concentrations during sorafenib treatment are dynamic in patients with advanced HCC, and an observed decrease in the plasma VEGF concentration 8 weeks after starting sorafenib is associated significantly with favorable OS. Today, because many clinical trials of new molecular-targeted agents for HCC are being conducted, it is necessary for hepatologists and oncologists to determine the time when alternative agents should be started as a second or third line of treatment. Our results have potentially important clinical implications for physicians and may influence their decisions regarding a treatment strategy for advanced HCC in individual patients.

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CONFLICT OF INTEREST DISCLOSURES

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Inhibition of hepatocellular carcinoma by PegIFNα-2a in patients with chronic hepatitis C: a nationwide multicenter cooperative study

Namiki Izumi · Yasuhiro Asahina · Masayuki Kurosaki · Gotaro Yamada · Tsutomu Kawai · Eiji Kajiwara · Yukishige Okamura · Takayuki Takeuchi · Osamu Yokosuka · Kazuya Kariyama · Joji Toyoda · Mie Inao · Eiji Tanaka · Hisataka Moriwaki · Hiroshi Adachi · Shinji Katsushima · Masatoshi Kudo · Kouichi Takaguchi · Yoichi Hiasa · Kazuaki Chayama · Hiroshi Yatsuhashi · Makoto Oketani · Hiromitsu Kumada

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Abstract
Background We investigated whether the administration of maintenance doses of interferon prevented hepatocellular carcinoma (HCC) in patients with chronic hepatitis C.
Methods Study 1: A multicenter, retrospective, cooperative study was carried out to determine whether long-term administration of low-dose peginterferon alpha-2a (PegIFNα-2a) prevented HCC development in patients with chronic hepatitis C. In total, 594 chronic hepatitis C patients without a history of HCC were enrolled and treated with 90 μg PegIFNα-2a administered weekly or bi-weekly for at least 1 year. Study 2: HCC developed in 16 of 99 additional patients without PegIFNα-2a treatment during 3.8 years of observation. A propensity-matched control study was then carried out to compare the incidence of

N. Izumi (✉) · Y. Asahina · M. Kurosaki
Department of Gastroenterology and Hepatology, Musashino Red-Cross Hospital, Musashino, Japan
e-mail: nizumi@musashino.jrc.or.jp

G. Yamada
Department of Internal Medicine, Kawasaki Hospital of Kawasaki Medical University, Okayama, Japan

T. Kawai
Department of Gastroenterology, Kanbara General Hospital, Fuji, Japan

E. Kajiwara
Department of Gastroenterology, Shinnittetsu Yahata Memorial Hospital, Kitakyushu, Japan

Y. Okamura
Department of Gastroenterology, Sano Kousei Hospital, Kitakyushu, Japan

T. Takeuchi
Department of Gastroenterology, Notogawa Hospital, Higashioumi, Japan

O. Yokosuka
Department of Gastroenterology and Hepatology, Chiba University, Chiba, Japan

K. Kariyama
Department of Hepatology, Okayama Citizens' Hospital, Okayama, Japan

J. Toyoda
Department of Gastroenterology and Hepatology, Sapporo Kousei Hospital, Sapporo, Japan

M. Inao
Department of Gastroenterology and Hepatology, Saitama Medical University, Moroyama, Japan

E. Tanaka
Second Department of Internal Medicine, Shinshu University, Matsumoto, Japan

H. Moriwaki
Department of Gastroenterology and Hepatology, Gifu University, Gifu, Japan

H. Adachi
Department of Hepatology, Tonami General Hospital, Tonami, Japan

S. Katsushima
Department of Gastroenterology, Kyoto Medical Center, Kyoto, Japan

M. Kudo
Department of Gastroenterology and Hepatology, Kinki University, Higashiosaka, Japan

K. Takaguchi
Department of Gastroenterology, Kagawa Central Hospital, Takamatsu, Japan

HCC between the 59 patients who received low-dose PegIFNα-2a (PegIFNα-2a group) and 59 patients who did not receive PegIFNα-2a treatment (control group), matched for sex, age, platelet count, and total bilirubin levels.

Results Study 1: HCC developed in 49 patients. The risk of HCC was lower in patients with undetectable hepatitis C virus RNA, ≤40 IU/L alanine aminotransferase (ALT), or ≤10 ng/L alpha-fetoprotein (AFP) 24 weeks after the start of therapy. Study 2: The incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group.

Conclusions Low-dose and long-term maintenance administration of PegIFNα-2a decreased the incidence of HCC in patients with normalized ALT and AFP levels at 24 weeks compared with patients without normal ALT and AFP levels.

Keywords Chronic hepatitis C · Hepatocellular carcinoma · Peginterferon

Introduction

Hepatocellular carcinoma (HCC), the sixth most common cancer worldwide, often develops because of long-term hepatitis B or C virus infection [1, 2]. In particular, chronic hepatitis C and hepatic cirrhosis increase the risk of HCC; the annual incidence of tumor development in such patients may be as high as 2–4 % [3–5]. The incidence of HCC decreases in patients who achieve a sustained virological response (SVR) to interferon (IFN) treatment, although the incidence remains high in non-SVR patients [6–9]. A detailed analysis of HCC development revealed that chronic hepatitis C patients aged 65 years or more, especially those with advanced fibrosis of the liver, were at an increased risk of developing HCC [10]. For patients 65 years or older with advanced liver fibrosis, the dose of ribavirin is often reduced or the agent is discontinued, resulting in lower SVR rates in those with discontinuation of ribavirin. Establishing an effective treatment strategy for preventing the development of HCC is important for these high-risk patients.

Factors related to the development of HCC have been analyzed in patients who did not achieve an SVR even after IFN treatment; advanced fibrosis of the liver and high levels of serum alanine aminotransferase (ALT), and alpha-fetoprotein (AFP) are risk factors for HCC development [11, 12]. A randomized controlled trial was conducted in Western countries to determine whether combined peginterferon and ribavirin treatment with weekly administration of 90 μg peginterferon alpha-2a (PegIFNα-2a) could prevent HCC in non-responders. A 3.5-year follow up showed that administration of a maintenance dose of PegIFNα-2a did not reduce tumor incidence in these patients [13]. However, after 8.5 years of observation, the incidence of HCC was decreased among those in the PegIFNα-2a group with cirrhosis [14]. Meanwhile, Bruix et al. [15] reported that maintenance therapy with PegIFNα-2b did not prevent HCC in chronic hepatitis C patients with cirrhosis. In Japan, long-term low-dose administration of natural IFN has been reported to decrease the incidence of HCC [16]. In light of these conflicting results, investigations should be carried out in a large number of patients with chronic hepatitis C to resolve the question of whether IFN treatment prevents the development of HCC.

We carried out a multicenter retrospective cooperative study of patients with chronic hepatitis C to determine whether those treated with 90 μg PegIFNα-2a without ribavirin had a reduced incidence of HCC compared with those not treated with IFN.

Patients and methods

Study 1: analysis of risk factors for HCC in patients treated with long-term low-dose-PegIFNα-2a

In total, at 21 hepatitis centers throughout Japan, 743 patients with hepatitis C who had received 90 μg of PegIFNα-2a therapy weekly or bi-weekly for 1 year or more without having received the full dose (180 μg) since December 2003 were examined retrospectively for the development of HCC. The end of enrollment in this study was the end of December 2008 and the end of follow up was the end of December 2010. Patients with a history of HCC before the start of therapy and those with a therapy period of less than 48 weeks were excluded, leaving 594 patients who had undergone long-term administration of PegIFNα-2a for analysis. At the 21 centers involved in this

Y. Hiasa
Department of Gastroenterology and Hepatology, Ehime University, Matsuyama, Japan

K. Chayama
Department of Gastroenterology and Hepatology, Hiroshima University, Hiroshima, Japan

H. Yatsuhashi
Department of Gastroenterology and Hepatology, Nagasaki Medical Center, Nagasaki, Japan

M. Oketani
Department of Gastroenterology and Hepatology, Kagoshima University, Kagoshima, Japan

H. Kumada
Department of Hepatology, Toranomon Hospital, Tokyo, Japan

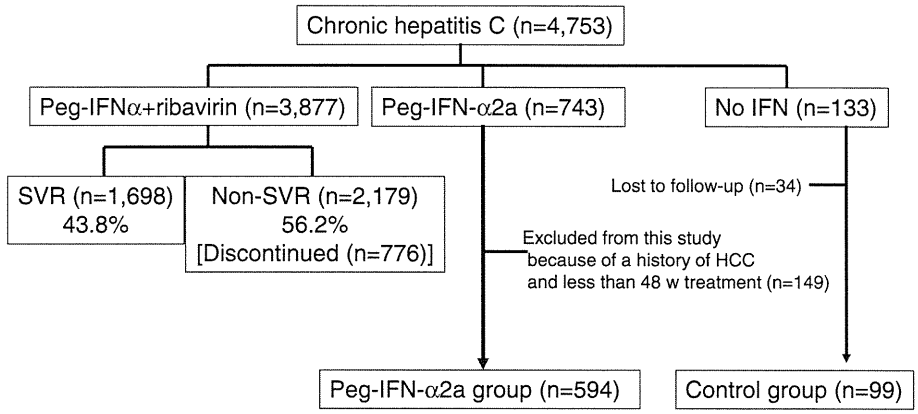

Fig. 1 Flow diagram of the patients' enrollment in the study. *Peg-IFNα* pegylated interferon α, *SVR* sustained viral response, *HCC* hepatocellular carcinoma, *w* week

study, 4,753 patients with chronic hepatitis C had been treated; Peg-IFN and ribavirin combination treatment had been administered to 3,877 patients, 743 patients had received Peg-IFN alone, and 133 patients had not agreed to receive IFN (a flow diagram of the enrollment of patients in this study is shown in Fig. 1). In the patients with Peg-IFN and ribavirin combination treatment, the SVR rate was 43.8 %; SVR was not achieved in 2,179 patients, and in 776 of these patients, the combination therapy was discontinued owing to adverse events or the patient's choice. Patients who failed to achieve an SVR were not included in this study, because the incidence of HCC is known to be reduced even in non-responders to IFN [17].

The backgrounds of the 594 patients studied are shown in Table 1. Findings from the liver biopsies of the patients were classified according to international standards [18]. Long-term PegIFNα-2a treatment is approved by the Japanese Medical Insurance system. Written informed consent was obtained from all patients prior to participation in this study. The study design was approved by the regional ethics committees of the 21 centers involved in this study, including the Musashino Red Cross Hospital, in accordance with the Helsinki Declaration. The 743 patients treated with PegIFNα-2a alone were not indicated for Peg-IFNα and ribavirin combination therapy because of anemia or heart disease. The 133 patients who did not agree to receive IFN served as the control group (see Fig. 1). A large proportion of the 594 study patients had advanced fibrosis of the liver and active inflammation. A dose of 90 μg PegIFNα-2a was administered to 512 and 82 patients weekly and biweekly, respectively, according to the patients' wishes. There were no significant differences between the weekly and biweekly groups in the patients' background data (data not shown).

The median duration of follow up in the PegIFNα-2a group was 1,273 days (range 228–2,768 days) and HCC was observed in 49 of the 594 patients (Table 1). Pre-treatment and on-treatment factors associated with the development of HCC were analyzed by Student's *t*-test, the Mann–Whitney *U*-test, and the χ^2 test (Table 2). Independent factors for the development of HCC were assessed by multivariate analysis using logistic regression. The

Table 1 Background data of patients treated with PegIFNα-2a ($n = 594$)

	$n = 594$
Age (years)	61.7 ± 11.7
Sex (male/female)	258/336
BMI	23.2 ± 3.3
Genotype (1/2)	443/151
Diagnosis (ASC/CH/LC)	4/460/130
History of excess alcohol consumption (≥60 g/day; yes/no)	118/376
Fibrosis (F0, 1, 2/F3, 4)	443/151
Inflammatory activity (A0, 1/A2, 3)	469/125
Diabetes mellitus (no/yes)	499/95
LDL cholesterol (mg/dL)	94.2 ± 31.1
Fasting blood sugar (mg/dL)	106.3 ± 28.5
White blood cell count (/mm^3)	4,360 ± 1,470
Red blood cell count (×10^6/μL)	423.8 ± 56.4
Hemoglobin (g/dL)	13.3 ± 1.8
Platelet count (×10^3/μL)	137 ± 56
Albumin (g/dL)	4.0 ± 0.5
Total bilirubin (mg/dL)	0.8 ± 0.6
AST (IU/L)	65.8 ± 47.8
ALT (IU/L)	72.1 ± 68.0
Gamma-GTP (IU/L)	55.2 ± 51.3
Esophageal varices (no/yes)	344/31
Alpha fetoprotein (ng/L)	6.9 (4.2–13.8)
Once weekly or biweekly PegIFNα-2a	512:82
Baseline HCV RNA (KIU/mL)	1,024 (73–2,130)
Development of HCC (no/yes)	545/49

PegIFN pegylated interferon, *BMI* body mass index, *ASC* asymtomatic carrier, *CH* chronic hepatitis, *LC* liver cirrhosis, *LDL* low-density lipoprotein, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GTP* guanosine triphosphate, *HCV* hepatitis C virus, *HCC* hepatocellular carcinoma

Values are means ± SD, with ranges in parentheses

incidence of HCC was analyzed according to the ALT, AFP, and hepatitis C virus (HCV) RNA levels 24 weeks after the start of PegIFNα-2a administration by using the Kaplan–Meier method. The risk of HCC was analyzed, using the Kaplan–Meier method, only in the non-responders with detectable HCV RNA during PegIFNα-2a administration by dividing them according to the ALT and AFP levels 24 weeks after the start of therapy. The incidence of HCC was compared between the patients with ALT levels of <41 IU/L and those with levels of ≥41 IU/L, and between patients with serum AFP levels of <10 ng/L and those with levels of ≥10 ng/mL at 24 weeks after starting treatment, because at most of the centers participating in the this study, the upper normal range of serum ALT is set at 40 IU/L, and the most significant difference in the incidence of HCC was observed between the PegIFNα-2a and control group with the cut-off serum ALT set at 41 IU/L and cutoff serum AFP set at 10 ng/mL, 24 weeks after starting treatment. The HCV RNA level was measured using the Amplicor Monitor method with a lower detection limit of 50 IU/L (Roche Diagnostics, Tokyo, Japan). A history of excess alcohol consumption was determined as >60 g alcohol per day in order to exclude alcoholic liver disease.

An asymptomatic carrier was defined as a patient with a serum ALT level within the normal range and minimal inflammation or fibrosis in the biopsied tissues of the liver. Chronic hepatitis was defined as mild-to-severe fibrosis of the liver according to liver biopsy [18]. The diagnosis of liver cirrhosis was based on the results of histological examination of the biopsied liver tissues.

Study 2: incidence of HCC in the PegIFNα-2a therapy and non-administration (control) groups in comparison with propensity-matched controls

Ninety-nine of the 133 chronic hepatitis C patients who had not received IFN were examined as controls; patients in this group received liver-protective agents such as glycyrrhizin or were untreated, and the group was observed for more than 1 year. None of the individuals in the control groups had received IFN alone or PegIFNα and ribavirin combination treatment. They were treated for a median of 1,395 days (range 75–6,556 days). Fifty-nine of these patients underwent liver biopsy before the treatment and were considered the control group for the propensity-matched study. For the propensity-matched study, 59 patients were selected from the PegIFNα-2a group according to their age, sex, platelet count, and total bilirubin levels, which had been identified as independent pretreatment risk factors for the development of HCC in Study 1. The rates of HCC were analyzed using the Kaplan–Meier method, and the risk of HCC was analyzed particularly in patients with advanced fibrosis of the liver (F3 and F4).

Table 2 Comparison of HCC and non-HCC patients with long-term PegIFNα-2a administration ($n = 594$)

	Patients with or without development of HCC		p value
	With HCC ($n = 49$)	Without HCC ($n = 545$)	
Pretreatment parameters			
Age (years)	63.8 ± 1.7	61.3 ± 0.5	<0.05
Sex (male/female)	32/17	226/319	<0.01
BMI	24.0 ± 0.5	23.1 ± 0.2	n.s.
Genotype (1/2)	47/6	397/148	n.s.
History of excess alcohol consumption (≥60 g/day; yes/no)	11/38	107/338	n.s.
Fibrosis (F0, 1, 2/F3, 4)	25/24	418/127	<0.001
Inflammatory activity (A0, 1/A2, 3)	7/42	462/83	<0.001
Diabetes mellitus (no/yes)	38/11	461/84	n.s.
LDL cholesterol (mg/dL)	88.2 ± 9.0	94.7 ± 2.6	n.s.
White blood cell count (/mm^3)	4,355 ± 210	4,360 ± 64	n.s.
Red blood cell count (×10^6/μL)	420.8 ± 8.1	424.1 ± 2.6	n.s.
Hemoglobin (g/dL)	13.6 ± 0.3	13.3 ± 0.1	n.s.
Platelet count (×10^3/μL)	106 ± 8	140 ± 2	<0.001
Albumin (g/dL)	3.8 ± 0.1	4.0 ± 0.1	<0.001
Total bilirubin (mg/dL)	1.2 ± 0.1	0.8 ± 0.1	<0.001
AST (IU/L)	78.1 ± 6.8	64.6 ± 2.1	n.s.
ALT (IU/L)	72.8 ± 9.7	72.0 ± 2.9	n.s.
Gamma-GTP (IU/L)	68.7 ± 7.5	53.9 ± 2.3	n.s.
Alpha fetoprotein (ng/L)	17.1 (4.4–36.8)	16.7 (4.1–23.1)	n.s.
Esophageal varices	29.0 % (9/31)	6.4 % (22/344)	<0.01
On-treatment parameters			
ALT (IU/L)	59.4 ± 5.7	44.6 ± 1.8	<0.05
Alpha fetoprotein (ng/L)	9.8 (4.6–17.4)	5.5 (3.7–11.1)	<0.01
HCV RNA level (KIU/mL)	236 (<0.5–2,210)	21 (<0.5–1,780)	<0.05

n.s. not significant

Statistical analysis

Categorical data were compared using the χ^2 test or Fisher's exact test. The distributions of continuous variables were analyzed using Student's t-test and the Mann–Whitney U-test for two groups. Multivariate analysis was

conducted using logistic regression. The cumulative incidence curve was determined using the Kaplan–Meier method and differences between groups were assessed by the log-rank test. For all methods, the level of significance was set at $p < 0.05$. Multivariate analysis of the risk of HCC was carried out using the Cox proportional hazard model. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 11.0 (SPSS, Chicago, IL, USA). In Study 1, age, sex, platelet count, and total bilirubin levels were identified as independent factors for the development of HCC; therefore, these factors were selected for the propensity-matched control study (Study 2) in which 59 patients from the PegIFNα-2a group were included.

Results

Study 1

We analyzed the factors involved in the development of HCC in patients who received 90 μg PegIFNα-2a weekly or biweekly for more than a year. The incidence of HCC did not differ significantly between the groups treated with PegIFNα-2a weekly and biweekly (34 of 512 vs. 15 of 82, respectively). As shown in Table 2, univariate analysis revealed statistically significant differences in the pretreatment parameters including age, sex, fibrosis of the liver, platelet count, albumin level, and total bilirubin, between patients who developed HCC and those who did not. Endoscopy was carried out in 375 patients, and esophageal varices were noted in 31 of them. The incidence of HCC was higher in patients with esophageal varices than in those without varices [29.0 % (9 of 31) vs. 6.4 % (22 of 344)]. Assessment of on-treatment factors by univariate analysis revealed statistically significant differences in serum ALT, AFP, and HCV RNA levels 24 weeks after the start of PegIFNα-2a maintenance treatment (Table 2).

Multivariate analysis including pretreatment parameters revealed that age, sex, fibrosis of the liver, platelet count, and total bilirubin were independent risk factors for HCC development (Table 3). Multivariate analysis including on-treatment parameters identified ALT levels of ≥41 IU/L and AFP levels of ≥10 ng/L 24 weeks after the start of the PegIFNα-2a therapy as independent risk factors for HCC development (Table 3).

The incidence of HCC was significantly lower in patients with ALT levels of ≤40 IU/L than in those with ALT levels of ≥41 IU/L 24 weeks after the start of observation (Fig. 2). The incidence of HCC was also significantly lower in patients with AFP concentrations of <10 ng/mL at 24 weeks after the start of observation than in those with AFP concentrations of ≥10 ng/mL (Fig. 3). The dose of PegIFNα-2a was reduced to 45 μg in 16 patients because of neutropenia and thrombocytopenia. In addition, PegIFNα-2a was discontinued in 18 patients because of adverse events, including depression (7 patients), interstitial pneumonitis (3 patients), thrombocytopenia (3 patients), neutropenia (1 patient), itching (1 patient), and ascites (3 patients). No statistically significant differences were found between the patients with reduced dosage or treatment interruption and those without treatment modifications with respect to overall survival, HCC incidence, ascites formation, variceal bleeding, hepatic encephalopathy, and 2-point increases in the Child-Pugh score. No patients underwent liver transplantation.

Table 3 Independent risk factors for HCC development in patients treated with 90 μg PegIFNα-2a weekly or bi-weekly, evaluated by multivariate analysis (logistic regression analysis)

	Multivariate analysis		
	Odds ratio	95 % Confidence interval (CI)	*p*
Age (years) (every 5 years)	2.24	1.76–9.33	<0.005
Sex (male/female)	3.16	1.56–10.7	<0.005
Fibrosis (F3, 4/F0, 1, 2)	1.69	1.18–5.2	<0.01
Platelet count (<120 × 10^3/μL vs. ≥120 × 10^3/μL)	3.24	1.44–27.6	<0.01
Total bilirubin (mg/dL)	1.59	1.09–2.58	<0.05
ALT (at 24 weeks) (≥41 vs. <40 IU/L)	2.49	1.51–8.28	<0.05
AFP (at 24 weeks) (≥10 vs. <10 ng/L)	3.78	1.92–11.8	<0.01

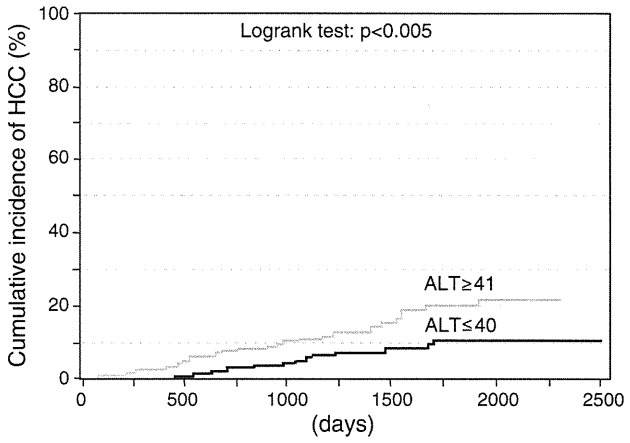

Fig. 2 Comparison of HCC rates in patients administered with PegIFNα-2a ($n = 594$) with respect to alanine aminotransferase (*ALT*) levels 24 weeks after the start of therapy. *Black line* patients with ALT ≥41 IU/L in the first 24 weeks, *gray line* patients with ALT ≤40 IU/L in the first 24 weeks

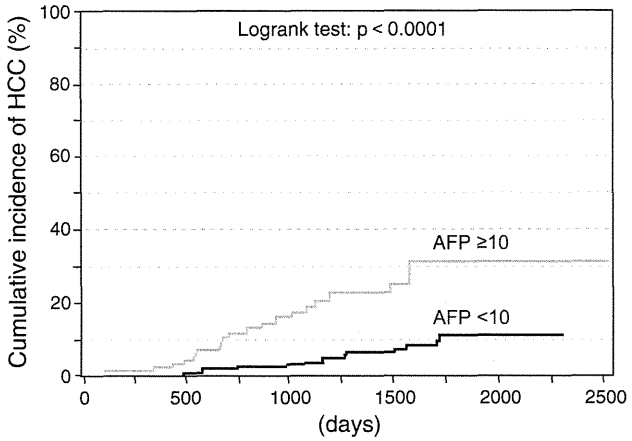

Fig. 3 Comparison of HCC rates in patients administered PegIFNα-2a ($n = 594$) with respect to alpha-fetoprotein (*AFP*) levels in the first 24 weeks after the start of therapy. *Black line* patients with AFP ≥10 ng/mL at 24 weeks, *gray line* patients with AFP <10 ng/mL at 24 weeks

Study 2

We compared the incidence of HCC between 59 patients in the control group and the same number of patients in the PegIFNα-2a group using the matched-pair test. The backgrounds of the patients are shown in Table 4. The PegIFNα-2a group had higher rates of advanced fibrosis (F3 and F4) and active inflammation (A2 and A3). No other differences were found between the two groups, except for the white blood cell count (Table 4).

Development of HCC was observed in 2 patients in the PegIFNα-2a group and 8 in the control group. The incidence of HCC was compared between the two groups, using the Kaplan–Meier method. The incidence of HCC in the PegIFNα-2a group was significantly lower than that in the control group (log-rank test, $p = 0.0187$; Fig. 4). Among the patients with advanced fibrosis of the liver (F3 and F4), those in the PegIFNα-2a group had a lower incidence of HCC than those in the control group. The independent risk factors for the development of HCC were analyzed using the stepwise Cox proportional hazard model. Only PegIFNα-2a administration and age were identified as independent risk factors for the development of HCC (Table 5).

Discussion

The number of HCC cases resulting from HCV infection continues to increase worldwide [19]. To date, IFN therapy is the most effective preventive measure against HCC in patients with chronic hepatitis C; furthermore, the incidence of HCC is reduced in patients who achieve an SVR to IFN [6–9] Therefore, achieving an SVR is the most effective approach for reducing the risk of developing HCC. In Japan, the incidence of HCC is elevated in older patients with hepatitis C. Corroborating this finding, the results of a Japanese study show a higher risk of HCC in patients aged 65 years and more [10]. Therefore, prevention of HCC in aged patients is an important challenge.

In the present multicenter, cooperative, retrospective study conducted in Japan, the incidence of HCC was reduced in patients who received 90 μg PegIFNα-2a weekly or biweekly and had AFP values of <10 ng/mL and ALT values of ≤40 IU/L 24 weeks after the start of the treatment. The results of the matched case–control study of the PegIFNα-2a group and the non-IFN control group show that the incidence of HCC was significantly lower in the PegIFNα-2a group than in the control group, especially in patients with advanced fibrosis of the liver (F3 and F4). However, there could have been a selection bias between

Table 4 Backgrounds of the patients in the propensity-matched control study (PegIFNα-2a group, $n = 59$; control group, $n = 59$)

	PegIFNα-2a group ($n = 59$)	Control group ($n = 59$)	p value
Age (years)	60.5 ± 13.0	63.3 ± 10.5	n.s.
Gender (male/female)	24/35	25/34	n.s.
BMI	22.9 ± 3.6	22.9 ± 3.4	n.s.
Genotype (1/2)	49/10	46/13	n.s.
History of excess alcohol consumption (60 g/day; yes/no)	10/49	4/55	n.s.
Fibrosis (F0, 1, 2/F3, 4)	37/22	43/16	<0.05
Development of HCC (F0–2/F3, 4)	1/1	1/7	n.s.
Inflammatory activity (A0,1/A2, 3)	19/40	30/29	<0.05
Diabetes mellitus (no/yes)	57/2	56/3	n.s.
LDL cholesterol (mg/dL)	95.3 ± 23.8	117.0 ± 4.2	n.s.
White blood cell count (/mm^3)	4,260 ± 1,239	5,193 ± 2,078	<0.05
Red blood cell count (×10^{-4}/μL)	430 ± 57.8	441 ± 44.9	n.s.
Hemoglobin (g/dL)	13.6 ± 1.5	13.6 ± 1.9	n.s.
Platelet count (×10^{-3}/μL)	14.5 ± 5.7	15.8 ± 5.7	n.s.
Albumin (g/dL)	4.1 ± 0.5	4.1 ± 0.4	n.s.
Total bilirubin (mg/dL)	0.7 ± 0.5	0.9 ± 0.7	n.s.
AST (IU/L)	58.3 ± 47.7	49.7 ± 26.6	n.s.
ALT (IU/L)	63.6 ± 68.7	58.0 ± 39.2	n.s.
Gamma-GTP (IU/L)	78.3 ± 81.3	55.3 ± 75.1	n.s.
Baseline alpha-fetoprotein (AFP) (ng/L)	7.2 (4.3–14.2)	7.7 (3.9–13.8)	n.s.
Baseline HCV RNA level (KIU/mL)	1,230 (24–3,870)	1,024 (38–3,110)	n.s.

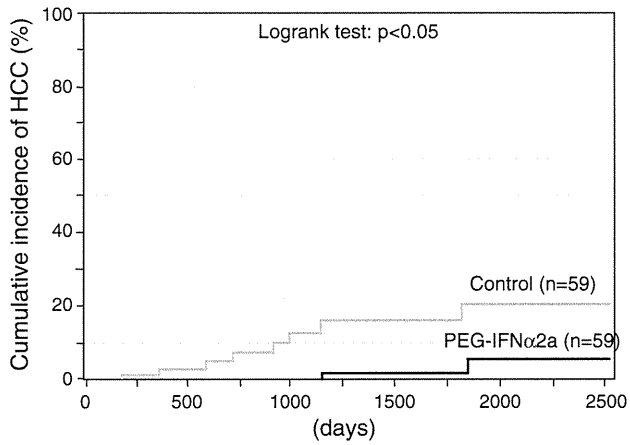

Fig. 4 Comparison of HCC rates between the long-term PegIFNα-2a administration group ($n = 59$) and non-administration group ($n = 59$) in the propensity-matched control study (Kaplan–Meier log-rank test, $p = 0.019$)

Table 5 Risk factors for HCC in the propensity-matched control study (Cox proportional hazard model)

Variables	Risk ratio	95 % CI	*p* value
PegIFN versus control	0.17	0.03–0.75	<0.05
Age (every 1 year)	1.12	1.02–1.25	<0.05
Fibrosis (F3, 4 vs. F0, 1, 2)	1.70	0.75–4.16	n.s.
Platelet count (every 10 × 10^3/μL)	0.89	0.73–1.09	n.s.
Albumin (every 1.0 g/dL)	0.80	0.10–6.68	n.s.
On-treatment AFP (<10 vs. ≥10 ng/L)	4.07	0.59–40.12	n.s.

the PegIFNα-2a group and the control group (patients who did not agree to receive IFN treatment), because this was a retrospective and non-randomized study. However, concordant with the findings of the HALT-C study [14], the present results show that PegIFNα-2a inhibits the development of HCC in patients with advanced fibrosis of the liver.

Recent studies show that polymorphisms in the host *IL28B* gene are important factors in the response to PegIFNα and ribavirin combination therapy [20, 21]. However, the mechanism of IL28B involvement in the response to PegIFNα and ribavirin has not been elucidated completely. A recent report has shown that IL28B is a significant factor in the development of HCC as well as in the response to IFN therapy [22]. Further studies are warranted to analyze the relationship between IL28B and inhibition of the development of HCC by PegIFNα in chronic hepatitis C.

Risk factors for the development of HCC have been discussed previously. Increased intrahepatic fat is involved in the development of HCC in chronic hepatitis C patients [23, 24]. In addition, diabetes-associated fat disorder [25, 26], hepatic iron overload [27], advanced fibrosis, older age, and fatty deposits in the liver are risk factors for HCC development [4]. Therefore, it is important to establish strategies to mitigate these risk factors to prevent the development of HCC and thus improve the outcomes of hepatitis C patients.

IFN therapy after HCC treatment is reported to inhibit the recurrence of tumors [28, 29], and a meta-analysis has revealed a trend toward inhibition of the recurrence of HCC [30, 31]. The prevention of HCC is an important issue that needs to be addressed to improve the survival of chronic hepatitis C patients. The findings of the present study and the HALT-C trial [14] indicate the effectiveness of long-term administration of maintenance IFN for preventing the development of HCC in chronic hepatitis C patients without an SVR. Improvement in ALT levels is also known to be an important predictor for the prevention of HCC [32]. A low AFP value during IFN administration is also recognized as a significant indicator of a lower risk of HCC [33, 34]. Recently, Osaki et al. [35] reported that a decrease of serum AFP during treatment with IFN was associated with a reduced incidence of HCC. Taking these findings and our own together, we conclude that maintenance administration of low-dose PegIFNα-2a weekly or biweekly to non-SVR patients with chronic hepatitis C decreases the incidence of HCC, especially in patients whose serum ALT and AFP levels are within the normal range 24 weeks after the start of treatment. The preventive effects of IFN against the development of HCC without elimination of the virus may be associated with its anticarcinogenic effects [16, 35]; however, the precise mechanism should be investigated.

The limitations of the present study are that it is retrospective and multicentric; therefore, potentially there may have been a selection bias. However, the reduction of the rate of development of HCC by maintenance administration of PegIFNα-2a in the patients in whom serum ALT and AFP levels were within the normal ranges 24 weeks after the start of treatment may be attributable to the anticarcinogenic effects of IFN without elimination of the virus.

Conclusion

The incidence of HCC was lower in non-SVR patients with chronic hepatitis C who were administered with maintenance low-dose PegIFNα-2a; especially in those whose serum ALT and AFP levels were within the normal ranges 24 weeks after the start of treatment.

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Conflict of interest Namiki Izumi received lecture fees from Chugai Co. and MSD Co. in 2011.

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Hepatitis C Virus NS4B Protein Targets STING and Abrogates RIG-I–Mediated Type I Interferon-Dependent Innate Immunity

Sayuri Nitta,[1*] Naoya Sakamoto,[1,2,6*] Mina Nakagawa,[1,2] Sei Kakinuma,[1,2] Kako Mishima,[1] Akiko Kusano-Kitazume,[1] Kei Kiyohashi,[1] Miyako Murakawa,[1] Yuki Nishimura-Sakurai,[1] Seishin Azuma,[1] Megumi Tasaka-Fujita,[1] Yasuhiro Asahina,[1,2] Mitsutoshi Yoneyama,[3] Takashi Fujita,[4,5] and Mamoru Watanabe[1]

Hepatitis C virus (HCV) infection blocks cellular interferon (IFN)-mediated antiviral signaling through cleavage of Cardif by HCV-NS3/4A serine protease. Like NS3/4A, NS4B protein strongly blocks IFN-β production signaling mediated by retinoic acid–inducible gene I (RIG-I); however, the underlying molecular mechanisms are not well understood. Recently, the stimulator of interferon genes (STING) was identified as an activator of RIG-I signaling. STING possesses a structural homology domain with flaviviral NS4B, which suggests a direct protein-protein interaction. In the present study, we investigated the molecular mechanisms by which NS4B targets RIG-I–induced and STING-mediated IFN-β production signaling. IFN-β promoter reporter assay showed that IFN-β promoter activation induced by RIG-I or Cardif was significantly suppressed by both NS4B and NS3/4A, whereas STING-induced IFN-β activation was suppressed by NS4B but not by NS3/4A, suggesting that NS4B had a distinct point of interaction. Immunostaining showed that STING colocalized with NS4B in the endoplasmic reticulum. Immunoprecipitation and bimolecular fluorescence complementation (BiFC) assays demonstrated that NS4B specifically bound STING. Intriguingly, NS4B expression blocked the protein interaction between STING and Cardif, which is required for robust IFN-β activation. NS4B truncation assays showed that its N terminus, containing the STING homology domain, was necessary for the suppression of IFN-β promoter activation. NS4B suppressed residual IFN-β activation by an NS3/4A-cleaved Cardif (Cardif1-508), suggesting that NS3/4A and NS4B may cooperate in the blockade of IFN-β production. *Conclusion*: NS4B suppresses RIG-I–mediated IFN-β production signaling through a direct protein interaction with STING. Disruption of that interaction may restore cellular antiviral responses and may constitute a novel therapeutic strategy for the eradication of HCV. (HEPATOLOGY 2013;57:46-58)

Type I interferon (IFN) plays a central role in eliminating hepatitis C virus (HCV) both under physiological conditions and when used as a therapeutic intervention.[1-3] In experimental acute-resolving HCV infection in chimpanzees, numerous IFN-related genes are expressed during clinical course of infection.[4] Viruses are recognized by cellular innate immune receptors, such as toll-like receptors, and a family of RIG-I–like receptors, such as retinoic-acid-inducible gene I (RIG-I) and melanoma-differentiation-associated gene 5 (MDA-5); host antiviral responses are then activated, resulting in the

From the [1]Departments of Gastroenterology and Hepatology; [2]Departments of Hepatitis Control, Tokyo Medical and Dental University, Tokyo, Japan; [3]Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, Chiba, Japan; [4]Laboratory of Molecular Genetics, Department of Genetics and Molecular Biology, Institute for Virus Research, Kyoto University, Kyoto, Japan; [5]Laboratory of Molecular Cell Biology, Graduate School of Biostudies, Kyoto University, Kyoto, Japan; and [6]Department of Gastroenterology and Hepatology, Hokkaido University, Hokkaido, Japan.

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BiFC, bimolecular fluorescence complementation; CARD, caspase recruitment domain; DAPI, 4',6-diamidino-2-phenylindole; dsRNA, double-stranded RNA; ER, endoplasmic reticulum; FACL4, fatty acid-CoA ligase, long chain 4; HCV, hepatitis C virus; IFN, interferon; IKKε, IκB kinase ε; IRF-3, interferon-regulatory factor 3; ISRE, interferon-stimulated response element; MAM, mitochondria-associated ER membrane; mKG, monomeric Kusabira-Green; PDI, protein disulphide-isomerase; pIRF-3, phosphorylated IRF3; poly(dA:dT), poly(deoxyadenylic-deoxythymidylic) acid; RIG-I, retinoic acid–inducible gene I; siRNA, small interfering RNA; SOCS, suppressor of cytokine signaling; STAT1, signal transducer and activator of transcription protein-1; STING, stimulator of interferon genes; TBK1, TANK binding kinase 1.

**These authors contributed equally to this work.*