reduce the daily dose by 200 mg (400 mg if started at 1000 mg) for Hb <10 g/dL; and discontinue if Hb is <8.5 g/dL.[81]

The standard dose for Peg-IFN-α-2a in the treatment of patients with compensated cirrhosis is 90 μg/kg/week. The criteria for dose reduction and discontinuation during treatment are as follows: reduce the dose to 45 μg/mL in the case of a neutrophil count <1000/μL, and to 22.5 μg/mL in the case of a neutrophil count <750/μL; and cease both Peg-IFN-α-2a and ribavirin in the case of a neutrophil count <500/μL, platelet count <50 000/μL or Hb <8.5 g/dL.[156] The starting doses for ribavirin are as for co-administration with Peg-IFN-α-2b. The criteria for ribavirin dose reduction or discontinuation when a decline in Hb occurs during treatment are: reduce the daily dose by 400 mg (600 mg if started at 1000 mg) for Hb <11 g/dL during treatment weeks 1–4, or Hb <10 g/dL during treatment weeks 5–48. For patients with heart conditions or a history of the same, in addition to the above criteria, if a decline in Hb ≥2 g/dL in comparison to the pretreatment level persists for 4 weeks, reduce the daily dose by 400 mg (600 mg if started at 1000 mg). If Hb remains <12 g/dL 4 weeks after the dose reduction, cease ribavirin.[80]

IFN monotherapy. Apart from patients with genotype 1 and a high viral load, IFN monotherapy should be selected for patients unable to tolerate Peg-IFN + ribavirin combination therapy due to adverse reactions such as anemia or depression. At present, IFN-β and human lymphoblastoid IFN (HLBI), an IFN-α formulation, are approved for national medical coverage for the treatment of patients with compensated cirrhosis with HCV genotype 1 and a low viral load, and genotype 2. They are not approved for patients with genotype 1 and a high viral load (≥100 kIU/mL for IFN-β, ≥500 kIU/mL for HLBI). Japanese clinical trials of IFN-β in the treatment of patients with compensated cirrhosis with genotype 1 and a low viral load, and genotype 2, yielded SVR rates in the patients administered 126 doses of 44% (8/18) in the genotype 1 low viral load group (<1 Meq/mL), 19% (3/16) in the genotype 2 high viral load group (≥1 Meq/mL) and 46% (6/13) in the genotype 2 low viral load group.[157] In a Japanese multicenter collaborative trial of HLBI in the treatment of HCV compensated cirrhosis, SVR rates in the group administered HLBI 6 MU consecutive daily for 2 weeks, then 3 MU three times weekly for 46 weeks, were 50% (1/2) in the genotype 1 low viral load group (<100 kIU/mL), 25% (3/12) in the genotype 2 high viral load group (≥100 kIU/mL) and 67% (4/6) in the genotype 2 low viral load group.[158] In both studies, efficacy increased with increased treatment duration. Furthermore, greater efficacy was seen with genotype 2 than genotype 1, and with a low viral load than with a high viral load. The rate of discontinuation due to adverse reactions was similar to that with chronic hepatitis C, and although the incidence of influenza-like syndrome and abnormal laboratory test results was high, no cirrhosis-specific adverse events were reported. In an overseas trial of Peg-IFN monotherapy in the treatment of patients with cirrhosis, SVR rates and biochemical efficacy were both superior to standard IFN therapy. A randomized prospective study comparing standard non-pegylated-IFN-α and Peg-IFN-α-2a reported SVR rates in patients administered non-pegylated-IFN-α-2a 3 MU three times/week, Peg-IFN-α-2a 90 μg/week and 180 μg/week to be 8% (7/88), 15% (14/96) and 30% (26/87), respectively. No difference was seen between groups in terms of tolerability.[159]

HLBI therapy aiming for viral clearance comprises HLBI 6 MU weekly for 2 consecutive weeks, then 3–6 MU three times weekly. The criteria for dose reduction and discontinuation during HLBI treatment are as follows: reduce the dose or increase the interval between doses in the case of a platelet count ≥30 000/μL and <50 000/μL, and discontinue in the case of a white blood cell counts <1500/μL, platelet count <30 000/μL or ALT level ≥500 U/L.[160]

IFN-β therapy is usually commenced at 6 MU, and is administered 3–6 MU consecutive daily until treatment week 6, then 3 MU three times a week. The criteria for dose reduction and discontinuation during IFN-β treatment are as follows: reduce the dose or increase the interval between doses in the case of a white blood cell counts <1500/μL, neutrophil count <750/μL or platelet count <50 000/μL, and discontinue in the case of a white blood cell counts <1000/μL, neutrophil count <500/μL or platelet count <25 000/μL.[133] For both HLBI and IFN-β, if HCV RNA becomes undetectable before treatment week 12, as for chronic hepatitis C, the treatment period should be extended to 48–72 weeks.

Low-dose IFN maintenance therapy. If HCV RNA does not become undetectable before treatment week 12 with Peg-IFN + ribavirin combination therapy or IFN monotherapy, a changeover to low-dose IFN maintenance therapy should be made with the aim of improving ALT levels and inhibiting hepatocellular carcinogenesis. Low-dose IFN or Peg-IFN maintenance therapy is useful in patients with liver cirrhosis in preventing progression

of liver disease and the development of HCC.[19,47,51] It is not effective in all patients, however, and discontinuation of treatment should be considered if improvement is not seen in ALT levels (≤40 IU/L) or AFP levels (≤10 ng/mL) within 6 months.

IFN therapy for decompensated cirrhosis

Patients with decompensated cirrhosis are at high risk of death due to liver failure, and liver transplant is the most effective treatment in suitable cases. However, post-transplant recurrence of hepatitis C causes allograft failure in approximately 30% of recipients within 5 years, so in overseas countries, pretransplant IFN therapy is administered with the aim of HCV eradication or suppression.[161,162] Several studies have demonstrated the efficacy of Peg-IFN (± ribavirin) therapy in patients with HCV genotype 2.[163–165] Patients with decompensated cirrhosis are at high risk of thrombocytopenia, anemia, infections and liver decompensation, however, and treatment discontinuation due to severe cytopenias is common. Serious bacterial infections associated with IFN therapy have been reported to be more common in patients with patients with Child–Pugh C than in Child–Pugh A/B disease.[166]

Treatment of patients with thrombocytopenia

In patients with marked thrombocytopenia associated with hypersplenism, it is difficult to introduce Peg-IFN or ribavirin combination therapy. Measures such as splenectomy or partial splenic embolization (PSE) are employed to increase the platelet count before commencing IFN therapy.[167–169] In Japan, mainly in patients with Child–Pugh A disease, Peg-IFN (± ribavirin) therapy is commenced following splenectomy or PSE. An increase in the platelet count is seen in almost all patients following either procedure, and high SVR rates are seen in patients with HCV genotype 2. However, postoperative complications including overwhelming post-splenectomy infection, portal vein thrombosis and hepatic dysfunction have been reported following both splenectomy and PSE.[168–170] The thrombopoietin receptor agonist, eltrombopag, has been developed overseas as an oral agent that increases platelet counts,[171] but it is not yet available for clinical use in Japan.

Recommendations:

1 *In patients with compensated cirrhosis (Child–Pugh class A) associated with HCV, aggressive IFN therapy should be commenced with the aims of preventing hepatocellular carcinogenesis and liver failure. This patient group requires careful observation during treatment due to the high incidence of adverse reactions such as cytopenias.*
2 *Patients with compensated cirrhosis associated with HCV should be given Peg-IFN + ribavirin combination therapy, irrespective of genotype or viral load. The standard dose is 1.0 μg/kg/week for Peg-IFN-α-2b and 90 μg/week for Peg-IFN-α-2a. The usual treatment period is 48 weeks, although consideration should be given to response-guided therapy and the discontinuation criteria for chronic hepatitis C.*
3 *Patients with compensated cirrhosis associated with HCV genotype 1 and a lower viral load, or genotype 2, not suited to combination therapy with ribavirin, should be administered HLBI or IFN-β monotherapy. HLBI therapy commences with HLBI 6 MU consecutive daily for 2 weeks, then 3–6 MU three times weekly. IFN-β therapy is usually commenced with 6 MU daily for a week, followed by 3 MU daily for 5 weeks, then 3 MU three times a week from treatment week 7. For both HLBI and IFN-β, if HCV RNA becomes undetectable before treatment week 12, the treatment period should be extended to 48–72 weeks.*
4 *If HCV RNA does not become undetectable before treatment week 12 with Peg-IFN + ribavirin combination therapy or IFN monotherapy in patients with compensated cirrhosis associated with HCV, long-term HLBI therapy at a dose of 3 MU three times weekly should be commenced with the aim of inhibiting hepatocellular carcinogenesis. Treatment should be discontinued if improvement is not seen in ALT levels (≤40 IU/L) or AFP levels (≤10 ng/mL) within 6 months.*
5 *The efficacy of IFN therapy is low in patients with decompensated cirrhosis associated with HCV (Child–Pugh class B and C). In particular, patients with Child–Pugh class C do not tolerate IFN therapy well, and serious adverse reactions such as cytopenias and infections have been reported, so IFN therapy is not recommended in this patient group.*
6 *If IFN therapy is being considered in a patient with compensated HCV cirrhosis associated with a platelet count <50 000/μL, one option is to perform splenectomy or PSE before commencing IFN therapy.*

3.9 Management of patients with normal ALT levels

In a study of Peg-IFN + ribavirin combination therapy and hepatocellular carcinogenesis in 809 patients with chronic hepatitis C and normal pretreatment ALT levels (male/female, 269/540; average age, 57 ± 11

years; genotype 1/2, 550/247; mean observation period, 36.2 ± 16.5 months), in the group with platelet counts ≥150 000/μL ($n = 586$) no significant difference was seen in the incidence of HCC according to therapeutic effect, with 1.5% of non-responders developing HCC within 3 years. In the group with platelet counts <150 000/μL ($n = 323$), however, the cumulative incidence of HCC was high at 10.1% in non-responders, with no cases of HCC among the responders or relapsers. These results demonstrated that Peg-IFN + ribavirin therapy significantly inhibits hepatocellular carcinogenesis ($P < 0.001$).[172] The efficacy of Peg-IFN + ribavirin combination therapy is similar in patients with normal and elevated ALT levels.[173,174]

Accordingly, antiviral therapy should be considered even in patients with ALT levels ≤30 IU/mL if their platelet count is <150 000/μL. On the other hand, antiviral therapy does not need to be commenced immediately in patients with an ALT level ≤30 IU/mL and a platelet count ≥150 000/μL, and follow-up while waiting for the next generation DAAs is a reasonable option. ALT levels may rise during the follow-up period, however, and treatment is indicated if the patient has a strong desire to commence antiviral therapy. At present, the available evidence regarding patients with normal ALT levels is mainly related to Peg-IFN + ribavirin combination therapy, although high therapeutic efficacy can also be anticipated with telaprevir + Peg-IFN + ribavirin combination therapy in this patient group.

Recommendation:

Antiviral therapy for patients with normal ALT levels (ALT, ≤30 IU/mL) can be administered in the same way as for patients with elevated ALT levels. Aggressive therapy is particularly desirable in patients with platelet counts <150 000/μL.

4. PROTECTIVE THERAPY

THE AIM OF protective therapy is not HCV clearance, but rather to reduce inflammation and inhibit the progression of fibrotic change in the hepatic tissue. The indications for protective therapy in patients with chronic hepatitis C are: patients with abnormal ALT and AST levels unable to undergo IFN or other antiviral therapy; patients who failed to achieve viral clearance with antiviral therapy; and patients who do not wish to undergo antiviral therapy. UDCA and SNMC are the protective therapies that have been scientifically shown to be useful.

UDCA

Ursodeoxycholic acid is a bile acid formulation, approved for use in doses of 600–900 mg daily by national medical insurance. The main mechanism of action of UDCA in hepatitis is a hepatocytoprotective effect. Other postulated mechanisms of action include protection of the hepatocyte cell membrane by substitution of UDCA for other cytotoxic bile acids, antioxidative stress affects, immunoregulatory effects and anti-apoptotic effects.[175]

Improvement of liver function is seen from UDCA doses of 150 mg/day.[176,177] In a Japanese nationwide multicenter double-blind trial, significantly greater improvement was seen in AST, ALT and γ-glutamyl transpeptidase levels in the groups administered 600 and 900 mg/day than in those given 150 mg/day.[176] Accordingly, the UDCA dose for the treatment of chronic hepatitis C is generally 600 or 900 mg/day. Adverse reactions are mainly gastrointestinal symptoms such as epigastric discomfort, diarrhea and constipation, but these are generally mild. A retrospective study of inhibition of hepatocellular carcinogenesis by UDCA reported that it significantly reduced the incidence of HCC.[178]

SNMC

The main constituent of SNMC is glycyrrhizin, a compound extracted from the liquorice root. The mechanisms of action of SNMC in the treatment of hepatic dysfunction are derived from anti-inflammatory effects related to the steroid-like properties of glycyrrhizin, and hepatocyte cell membrane protective effects. These actions are considered to lead to improved ALT levels. In a Japanese double-blind trial of SNMC 40 mL daily for 1 month, significant improvement in AST and ALT levels was seen in the SNMC group in comparison with the placebo group.[179,180] Doses are 40–100 mL daily or alternate daily, although Japanese dosage comparison trials found significantly greater improvement in ALT levels with 100 mL than with 40 mL.[181,182] In another study, long-term administration of SNMC significantly inhibited progression to liver cirrhosis in comparison with the control group.[183] Adverse reactions to SNMC include hypokalemia and hypertension.

Studies of inhibition of hepatocellular carcinogenesis by SNMC found that the incidence of HCC was significantly lower in the treatment group than in the control group.[183,184] SNMC therapy has also been found to significantly reduce the incidence of HCC in non-responders to IFN therapy.[185,186]

UDCA + SNMC combination therapy

An RCT comparing SNMC monotherapy and UDCA + SNMC combination therapy found significantly greater improvement in ALT levels in the combination therapy group.[187] This combination is useful in reducing inflammation.

Recommendation:

Oral UDCA and i.v. SNMC, or both in combination, are recommended as protective therapy in patients with chronic hepatitis C.

5. THERAPEUTIC PHLEBOTOMY

IRON METABOLISM PLAYS an important role in patients with chronic hepatitis C. Iron is an essential metal, and a constituent of important proteins, including Hb. When iron is present in excess, however, cytotoxic hydroxyl radicals are produced, causing oxidative stress. Therapeutic phlebotomy was devised as a supportive therapy for patients with chronic hepatitis C because oxidative stress associated with iron overload is a factor in progression of liver disease. Restriction of dietary iron is also important in the management of patients undergoing iron reduction therapy. As for protective therapy, therapeutic phlebotomy is indicated in patients with chronic hepatitis C with abnormal ALT and AST levels unable to undergo IFN or other antiviral therapy, patients who failed to achieve viral clearance with antiviral therapy and patients who do not wish to undergo antiviral therapy.

In 1994, a Japanese study reported that therapeutic phlebotomy lowered ALT levels in patients with chronic hepatitis C.[188] A Japanese multicenter RCT also confirmed improvement in ALT levels with therapeutic phlebotomy.[189] Other studies have reported a 50% decrease in ALT levels in 80% of patients, and normalization of ALT levels in 40–70% of patients.[190,191] Histological studies have reported inhibition of progression,[192] and even improvement,[193] of histological changes. Long-term therapeutic phlebotomy has been reported to significantly inhibit hepatocellular carcinogenesis.[190]

In general, therapeutic phlebotomy involves removal of 200–400 mL blood at 1–2-week intervals with the aim of reducing the serum ferritin level to ≤20 ng/mL. If the Hb level drops below 9–10 g/dL, phlebotomies are discontinued to allow recovery of hematopoietic function. After the target has been reached, therapeutic phlebotomies are performed as appropriate with reference to ferritin and Hb levels. Adverse reactions are rare, involving bradycardia and hypotension associated with the vagal reflex.

An additive effect is seen when therapeutic phlebotomy is performed in conjunction with UDCA or SNMC therapy. Greater reduction in ALT levels was seen with UDCA in combination with therapeutic phlebotomy than with UDCA monotherapy.[194] In patients on SNMC therapy, further reduction in ALT levels was seen with the addition of small volume phlebotomies.[195] The combination of therapeutic phlebotomy with another therapy with a different mode of action provides additional improvement in ALT levels.

Recommendations:

Therapeutic phlebotomy is a useful therapeutic modality in patients with chronic hepatitis C. Its use in combination with a protective therapy, oral UDCA or i.v. SNMC should also be considered.

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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

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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. © 2013 The Authors. *Cancer* published by Wiley Periodicals, Inc. on behalf of American Cancer Society. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

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:

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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

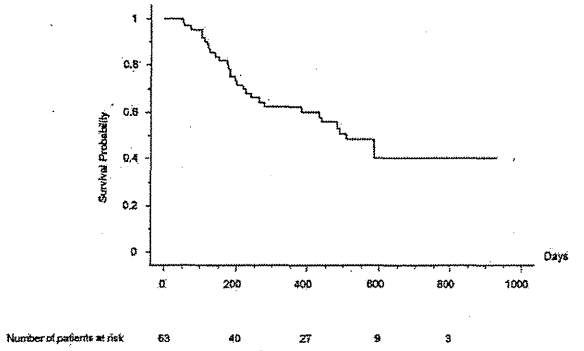

Figure 1. This Kaplan-Meier plot illustrates overall survival for all patients in the study.

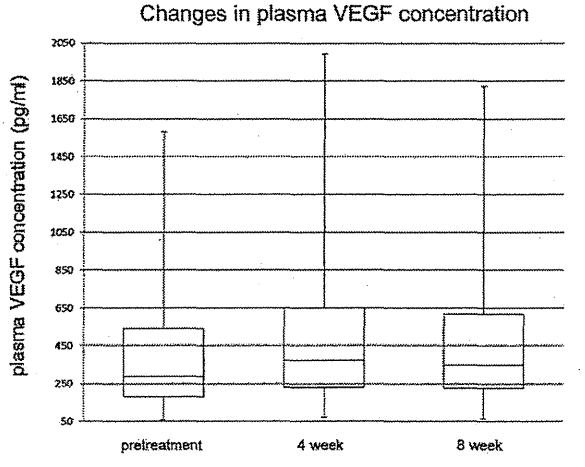

Figure 2. Changes in plasma vascular endothelial growth factor (VEGF) concentrations are illustrated.

(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

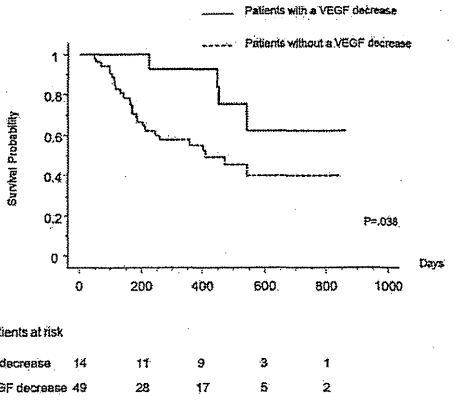

Figure 3. This Kaplan-Meier plot illustrates overall survival according to changes in vascular endothelial growth factor (VEGF) concentration.

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.

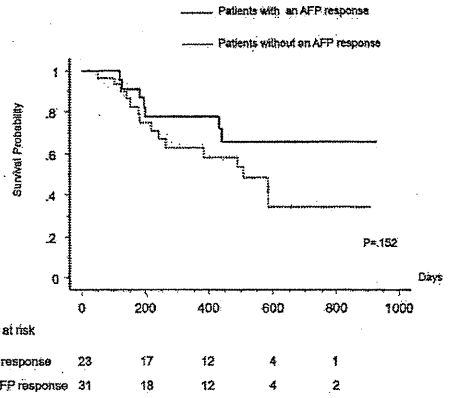

Figure 4. This Kaplan-Meier plot illustrates overall survival according to α-fetoprotein (AFP) response in patients without progressive disease (PD), classified as non-PD (ie, those who had a complete response, a partial response, or stable disease) according to modified Response Evaluation Criteria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

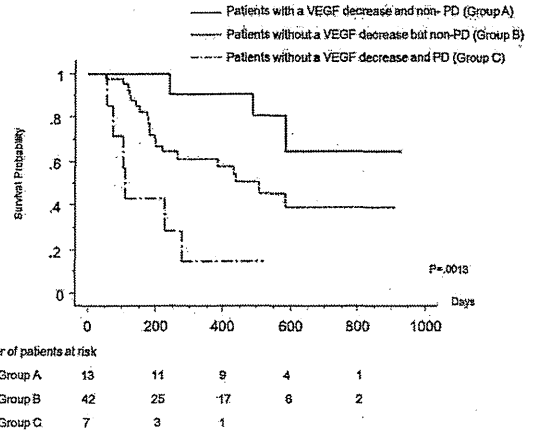

Figure 5. This Kaplan-Meier plot illustrates overall survival according to the combination of vascular endothelial growth factor (VEGF) changes and radiologic findings classified by modified Response Evaluation Criteria in Solid Tumors. Non-PD indicates patients who did not have progressive disease (PD)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

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]

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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