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Clinical Cancer Research

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Imaging, Diagnosis, Prognosis

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Plasma Biomarkers as Predictors of Outcome in Patients with Advanced Hepatocellular Carcinoma

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Abstract

Purpose: Validated biomarkers of prognosis and response to drug have not been identified for patients with hepatocellular carcinoma (HCC). One of the objectives of the phase III, randomized, controlled Sorafenib HCC Assessment Randomized Protocol (SHARP) trial was to explore the ability of plasma biomarkers to predict prognosis and therapeutic efficacy.

Experimental Design: In SHARP, 602 patients with advanced HCC were randomized to receive either oral sorafenib 400 mg twice a day *per os* or matching placebo daily on a continuous basis. Ten plasma biomarkers implicated in the pathogenesis of HCC were measured in 491 patients at baseline and in 305 after 12 weeks of treatment. The candidate biomarkers were analyzed to identify correlates of prognosis or predictors of response to sorafenib.

Results: In both the entire patient population and the placebo cohort, baseline angiopoietin 2 (Ang2) and VEGF concentrations independently predicted survival. Clinical variables such as macroscopic vascular invasion, Eastern Cooperative Oncology Group (ECOG) performance status, and baseline α-fetoprotein and alkaline phosphatase concentrations also independently predicted survival in these groups. In the sorafenib cohort, trends toward enhanced survival benefit from sorafenib were observed in patients with high s-c-KIT or low hepatocyte growth factor concentration at baseline (P of interaction = 0.081 and 0.073, respectively).

Conclusions: The angiogenesis biomarkers Ang2 and VEGF were independent predictors of survival in patients with advanced HCC. In contrast, none of the biomarkers tested significantly predicted response to sorafenib. *Clin Cancer Res; 18(8); 2290–300. ©2012 AACR.*

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

The names of the investigators in the Sorafenib Hepatocellular Carcinoma Assessment Randomized Protocol (SHARP) Investigators Study Group are listed in the Appendix, in the Supplementary Section.

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Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide and is associated with the second lowest 5-year survival rate of all tumor types (1). Moreover, HCC incidence and mortality rates appear to be increasing in the United States and other countries (2). Management of the disease, nonetheless, has improved over the last decade, largely as a result of advances in chemoembolization techniques and the advent of molecularly targeted therapy. The multikinase inhibitor sorafenib was shown in 2 randomized, double-blind, placebo-controlled trials to confer a significant survival benefit in patients with advanced HCC (3, 4), thereby establishing sorafenib as the standard systemic therapy for this indication (5, 6).

Biomarkers predicting patient prognosis or response to therapy may advance the potential of personalized medicine in cancer treatment (7). Previous studies have evaluated the correlation between baseline α-fetoprotein (AFP) concentration and patient outcomes (8–10). Additional studies have correlated various markers with survival in patients with HCC (11). They include the expression of epithelial cell adhesion molecule (EpCAM), a hepatic stem cell marker in tumor tissue (12–14); expression of the miR-26 miRNA precursor (15); and a prognostic gene signature

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Translational Relevance

Validated biomarkers of patient prognosis and response to treatment have not yet been identified in patients with hepatocellular carcinoma (HCC). We assessed whether baseline concentrations of 10 biomarkers and changes in their concentrations over 12 weeks could predict patient prognosis or response to treatment in the 602 patients enrolled in a registration phase III trial of sorafenib in patients with HCC. We found that the concentrations of two biomarkers, VEGF and Ang2, predicted patient survival, suggesting both may be included in prognostic staging systems for patients with HCC. We also found that concentrations of soluble c-KIT and hepatocyte growth factor tended to predict response to sorafenib. Further efforts are needed to identify biomarkers that can predict patient prognosis or response to treatment, thus allowing treatment to be individualized for patients with HCC.

in nontumor hepatic tissue (16). Because of the heterogeneity of HCC, however, the identification of biomarkers in this disease is somewhat complex. Although molecularly defined classes of HCC have not yet been linked to specific responses to treatment (17–19), signaling cascades involved in tumor proliferation and neoangiogenesis have been implicated in its pathogenesis. These cascades include several important kinases involved in tumor progression, several of which are pharmacologically relevant targets of sorafenib. The molecular targets of sorafenib include VEGFR-1, -2, and -3, platelet-derived growth factor receptor (PDGFR)-β, c-KIT, RET, FLT-3, and RAF (20, 21).

Previous investigations to identify prognostic biomarkers in patients with HCC have focused primarily on VEGF, angiopoietin 2 (Ang2), and hepatocyte growth factor (HGF; ref. 22–25), but these studies—conducted on tumor tissue (22–24) and hepatic vein (25) markers—have involved small numbers of patients. Biomarker evaluations in larger HCC patient populations, especially as part of randomized, placebo-controlled trials, may provide additional insight into the predictive and/or prognostic utility of specific markers.

One objective of the phase III Sorafenib HCC Assessment Randomized Protocol (SHARP) trial was to explore the ability of plasma biomarkers to predict patient prognosis and sorafenib efficacy. We therefore assayed plasma concentrations of 10 proteins that are either molecular targets of sorafenib [VEGF, soluble (s)-VEGFR-2 and -3, soluble c-KIT (s-c-KIT), and soluble Ras] or are known to interact with signaling pathways impacted by sorafenib and have been implicated in the pathogenesis of HCC on this basis [Ang2, basic fibroblast growth factor (bFGF), EGF, insulin-like growth factor (IGF)-2, and HGF] in patients who participated in the SHARP trial. To our knowledge, this is the largest study to date of these biomarkers in a randomized, placebo-controlled HCC trial population.

Patients and Methods

Patients and samples

The SHARP trial design has been described in detail (3). Eligible patients with advanced, measurable HCC, an Eastern Cooperative Oncology Group (ECOG) performance status (PS) $\leq$2 (26), and Child–Pugh class A ($n = 602$) status were randomized to sorafenib 400 mg twice a day ($n = 299$) or matching placebo ($n = 303$). The primary endpoints were overall survival (OS) and time to symptomatic progression (TTSP); secondary endpoints included time to progression (TTP) by independent radiological review, disease control rate (DCR), and safety.

Two 6-mL blood samples were collected at baseline (screening visit) and after 12 weeks of treatment, by venipuncture or through a Porta-a-Cath implantable venous access system, into a Vacutainer containing potassium EDTA. The blood samples were gently inverted and centrifuged within 10 to 15 minutes at 4°C for 10 minutes; if a refrigerated centrifuge was not available, the tubes were chilled on ice for 5 to 15 minutes and centrifuged in a standard centrifuge for 10 minutes. Plasma samples were frozen upright at ≤ -70°C within 20 minutes of centrifugation and kept frozen until ready for shipment to the sponsor.

Biomarker assays

Plasma biomarker concentrations were measured by commercially available ELISA kits for Ang2, EGF, bFGF, VEGF, sVEGFR-2, sVEGFR-3, HGF, and s-c-KIT (R&D Systems; catalog numbers DANG20, DEG00, HSFB75, DVE00, DY349, DHG00, and DSCR00, respectively), IGF-2 (Diagnostic Systems Laboratories; catalog number DSL-10-2600), and all forms of circulating Ras (Oncogene Science Biomarker Group; catalog number 064900009), according to the manufacturers' instructions.

Outcomes

Clinical and outcome data used in these correlative analyses were obtained from the SHARP clinical trial database, with a May 12, 2006, cutoff date for TTP and a February 9, 2007, cutoff date for OS. To maximize statistical power and to provide the largest number of noncensored data points, we used an OS cutoff date for biomarker analysis that was approximately 4 months later than the October 17, 2006, cutoff date reported previously (3).

Statistical analysis

Statistical analyses were conducted with SAS and R software. For each biomarker, samples were dichotomized into 2 groups, as described later. Cox regression models and Kaplan–Meier analyses were used to assess the relationships of OS and TTP with baseline biomarker concentrations (for prognostic value) and changes in biomarker concentrations. Multivariate Cox proportional hazard models were used to evaluate the prognostic value for survival of these biomarkers, as well as of treatment group (sorafenib or placebo) and clinical variables previously identified as

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prognostic (3). The clinical variables included in the model are listed in relevant tables. Clinical variables in binned biomarker groups were compared using the *F* tests. The relationship between baseline biomarker levels and sorafenib treatment effect was evaluated using a Cox proportional hazards model with an interaction term. One-way ANOVA was used to compare changes in biomarker concentration from baseline to week 12 in the sorafenib and placebo groups.

All binary cutoff values were defined before data analysis. In the absence of clinical information on a cutoff value differentiating low and high baseline concentration of a plasma biomarker, we used the median concentrations: 11.3 ng/mL for s-c-KIT, 1,042.9 pg/mL for Ras, 8,653 pg/mL for sVEGFR-2, 39,587 pg/mL for sVEGFR-3, 6,061.1 pg/mL for Ang2, 7.5 pg/mL for bFGF, 30.4 pg/mL for EGF, and 797.7 ng/mL for IGF-2. If, however, clinical data substantiated use of a nonmedian cutoff value to differentiate low from high baseline biomarker concentration, then that nonmedian value was used. For example, serum HGF concentration above the 78th percentile (corresponding to 1.0 ng/mL) was associated with poor survival in 55 patients with inoperable HCC (27); we therefore used the 75th percentile (3,279.1 pg/mL) as the cutoff value distinguishing low and high plasma HGF concentration. (We did not use the absolute value of 1.0 ng/mL as a cutoff value due to possible differences in serum and plasma concentrations). Although a phase III trial of sorafenib in patients with advanced renal cell carcinoma showed a trend toward greater sorafenib benefit in patients with VEGF level above the median, this relationship became significant when the 75th percentile was used as the cutoff value (28). We therefore used the 75th percentile (101.9 pg/mL) to differentiate a low from a high baseline VEGF concentration. To analyze the correlation between change in biomarker concentration and outcome, the median percent changes among all patients was used as the cutoff value to differentiate low from high changes.

Because the SHARP trial biomarker analyses were exploratory and hypothesis generating, no *P* value corrections for multiple testing were conducted.

Results

Populations of patients evaluated for biomarkers

A total of 602 patients were randomized in the SHARP trial, 299 to sorafenib and 303 to placebo (3). Trial centers submitted baseline plasma samples from 499 patients; 12-week samples were analyzed only if a baseline sample was available from the same patient. Ultimately, usable plasma samples were received from 491 patients (81.6%) at baseline and from 305 (50.7%) at 12 weeks. All usable plasma samples were assayed for all 10 proteins, as plasma volume allowed; because plasma volumes varied, the number of patients with data available for each biomarker ranged from 485 to 491 at baseline and from 274 to 305 at 12 weeks.

Baseline demographic and disease characteristics of patients in the biomarker subpopulations were similar to those in the overall SHARP population, as were the clinical benefits of sorafenib. In the SHARP biomarker population, OS in the sorafenib and placebo groups was 10.8 and 8.5 months, respectively [HR, 0.72; 95% confidence interval (CI), 0.58–0.90], and TTP was 5.3 and 3.0 months (HR, 0.60; 95% CI, 0.46–0.79), respectively. In comparison, OS of the sorafenib and placebo groups in the overall SHARP population was 10.7 and 7.9 months (HR, 0.69; 95% CI, 0.55–0.87), respectively, and TTP was 5.5 and 2.8 months (HR, 0.58; 95% CI, 0.45–0.74), respectively.

Prognostic value of plasma biomarkers for all patients

We first analyzed the prognostic value of plasma biomarkers in all randomized patients in the SHARP trial. Both baseline Ang2 and baseline VEGF concentrations correlated with survival (Fig. 1A and B). The median survival of patients with low and high baseline Ang2 concentrations was 14.1 and 6.3 months, respectively, whereas the median survival of patients with low and high baseline VEGF concentrations was 10.6 and 6.2 months, respectively. A multivariate analysis that included all 10 baseline plasma biomarkers, treatment group, and the predictors of survival previously identified in patients with advanced HCC (3) showed that, among the entire SHARP population, both baseline Ang2 and VEGF retained independent prognostic value, along with treatment group, ECOG PS, macrovascular invasion, and baseline plasma levels of AFP and alkaline phosphatase (Table 1).

Biomarkers prognostic in the placebo group

Univariate analyses of the potential prognostic value of the 10 candidate biomarkers in the placebo group alone showed that low baseline concentrations of Ang2, VEGF, and HGF, and high baseline concentrations of IGF-2, correlated with better OS (Fig. 1C–F). Low baseline concentrations of Ang2 also correlated with longer TTP (HR, 1.52; $P = 0.016$; data not shown), with Ang2 being the only biomarker prognostic for both OS and TTP. Baseline levels of bFGF and the other biomarkers assayed did not correlate with prognosis among patients in the placebo group (data not shown). Baseline concentrations of HGF, VEGF, s-c-KIT, Ang2, and IGF-2 correlated with other clinical/demographic variables associated with poor outcome in advanced HCC, including an ECOG PS of 1 or 2; macroscopic vascular invasion and/or extrahepatic spread; and concentrations of AFP, albumin, alkaline phosphatase, and bilirubin (Table 2). In addition, multivariate analysis—which included all 10 biomarkers plus clinical factors previously found to be prognostic in patients with advanced HCC (3)—showed that baseline Ang2 and VEGF concentrations were independently prognostic for OS ($P = 0.002$ each).

Biomarkers prognostic in the sorafenib group

Because sorafenib is the current standard of care worldwide for patients with advanced HCC (3–5, 28–30), we analyzed the correlation of clinical factors and biomarkers with outcome in the sorafenib group of the SHARP study.

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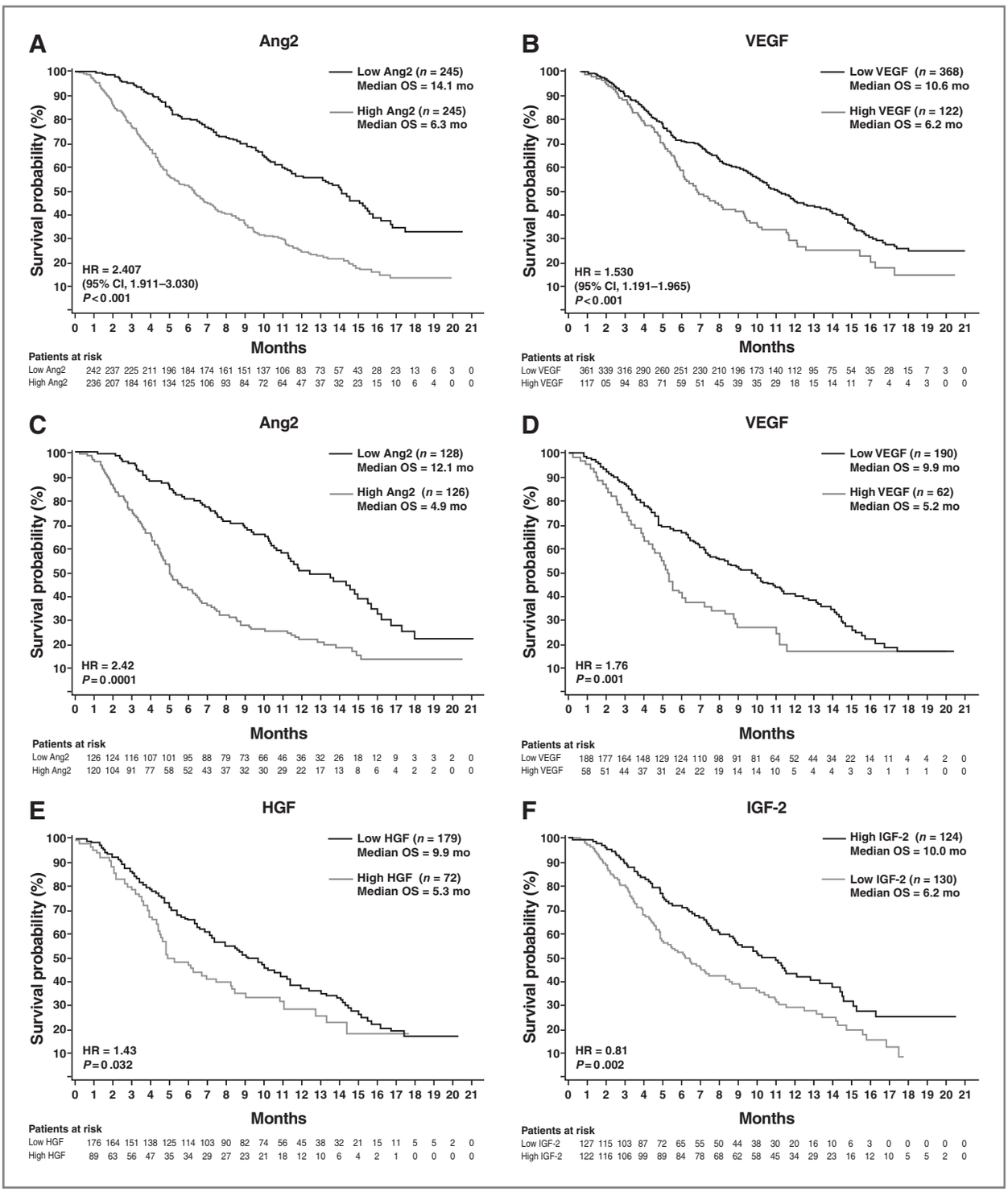

Figure 1. Univariate analyses of baseline plasma biomarkers as prognostic factors in advanced HCC. Ang2 (A) and VEGF (B) versus OS in the full cohort of patients. C, Ang2, (D) VEGF, (E) HGF, and (F) IGF-2 versus OS in the placebo cohort.

Multivariate analysis showed that s-c-KIT, HGF, and Ang2 concentrations were independent prognostic factors for OS in patients treated with sorafenib. Although VEGF concentration was prognostic for patients in the all-patient and placebo cohorts, it was not prognostic for patients treated with sorafenib.

To determine whether plasma biomarkers could predict response to sorafenib, we analyzed the interaction between

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Table 1. Multivariate analyses of the sorafenib, placebo, and all-patient cohorts to identify factors independently prognostic for OS in patients with HCC

	Placebo cohort		Sorafenib cohort		All-patient cohort	
Baseline factor	***P* value**	**HR (95% CI)**	***P* value**	**HR (95% CI)**	***P* value**	**HR (95% CI)**
Treatment (sorafenib vs. placebo)	NA	—	NA	—	0.041	0.78 (0.61–0.99)
ECOG PS (0 vs. >0)	NS	—	NS	—	0.016	1.36 (1.06–1.75)
AFP (≤ vs. >200 ng/mL)	0.007	1.58 (1.13–2.21)	0.015	1.57 (1.09–2.26)	0.001	1.49 (1.17–1.89)
MVI (present vs. absent)	0.0003	1.88 (1.34–2.66)	0.005	1.74 (1.19–2.55)	<0.0001	1.81 (1.43–2.32)
EHS (present vs. absent)	NS	—	0.016	1.60 (1.09–2.34)	NS	—
Alkaline phosphatase (≤ vs. >median)	0.013	1.56 (1.10–2.22)	0.002	1.82 (1.26–2.63)	0.0003	1.59 (1.24–2.04)
s-c-KIT (≤ vs. >11.3 ng/mL)	NS	—	0.004	0.56 (0.38–0.83)	NS	—
HGF (≤ vs. >3,279.1 pg/mL)	NS	—	0.017	1.69 (1.10–2.60)	NS	—
VEGF (≤ vs. >101.9 pg/mL)	0.002	1.97 (1.28–3.04)	NS	—	0.015	1.48 (1.08–2.03)
Ang2 (≤ vs. >6,043.5 pg/mL)	0.002	1.84 (1.25–2.70)	0.034	1.59 (1.04–2.43)	0.001	1.58 (1.20–2.07)

NOTE: Analyses included the following baseline variables: all biomarker values, ECOG PS, AFP, macroscopic vascular invasion (MVI), extrahepatic spread (EHS), albumin score, alkaline phosphatase, bilirubin score, prothrombin time, presence of ascites, and, for the all-patient cohort analysis, treatment group. Factors with $P < 0.05$ in one or more of the analyses are shown.
Abbreviations: NA, not applicable; NS, not significant ($P > 0.05$).

each baseline biomarker and sorafenib treatment effect. We found that patients with a high baseline s-c-KIT concentration tended to show greater improvements in OS (P of interaction = 0.081; Fig. 2A and B) and TTP (P of interaction = 0.052; Fig. 3A and B) in response to sorafenib than in those with a low s-c-KIT level. Conversely, patients with low baseline HGF tended to derive greater benefit from sorafenib in both OS (P of interaction = 0.073; Fig. 2C and D) and TTP (P of interaction = 0.396; data not shown) than those with high HGF concentration. In addition, patients with high baseline bFGF tended to show greater sorafenib-associated improvement in TTP (P of interaction = 0.078; Fig. 3C and D) than those with low bFGF level; however, a similar association was not observed for bFGF and OS benefit, where both high and low bFGF groups benefited equally from sorafenib treatment (P of interaction = 0.46; data not shown). Although baseline Ang2 concentrations correlated with OS in multivariate analysis of the sorafenib cohort alone, the biomarker treatment interaction analysis did not correlate with sorafenib-associated survival benefit (P of interaction = 0.80; data not shown).

Treatment-induced changes in plasma biomarker concentrations and correlation with outcome

We found that the change from baseline to week 12 in mean plasma concentration of 8 biomarkers differed significantly between the sorafenib and placebo groups (Fig. 4). For example, mean plasma s-c-KIT concentration decreased significantly in the sorafenib group but was essentially unchanged in the placebo group ($P < 0.0001$). Mean plasma HGF decreased in the sorafenib group but increased in the placebo group ($P < 0.0001$). In the sorafenib group, mean plasma concentration of VEGF increased significantly ($P = 0.010$), and mean concentrations of sVEGFR-2 ($P < 0.0001$) and sVEGFR-3 ($P < 0.0001$) decreased significantly, compared with levels in the placebo group. Interestingly, the mean concentration of Ang2—a biomarker we found to be independently prognostic for survival—increased in the placebo group but did not change significantly in the sorafenib group ($P < 0.0001$). Mean levels of bFGF and the other biomarkers tested did not change differently between the sorafenib and placebo groups.

Cox regression models and Kaplan–Meier analyses were conducted to examine the relationships between changes in biomarker concentrations and outcome. Ang2 increases of at least 5.1% (the median change in Ang2) were associated with shorter OS and TTP (data not shown) in both the sorafenib (OS, $P < 0.0001$; TTP, $P = 0.0002$) and placebo (OS, $P < 0.0001$; TTP, $P < 0.0001$) cohorts, reflecting our finding that Ang2 is a biomarker of poor prognosis in patients with HCC. A decrease in HGF level of >2.7% (the median change in HGF) was associated with longer TTP ($P = 0.042$) but not longer OS ($P = 0.0521$) among sorafenib-treated patients and with both longer TTP ($P < 0.0001$) and OS ($P < 0.000001$) among patients who received placebo. A decrease in mean IGF-2 level of >11.2% (the median change in IGF-2) was associated with shorter OS ($P = 0.005$) and a trend toward shorter TTP ($P = 0.075$) among sorafenib-treated patients as well as shorter OS ($P < 0.0001$) and shorter TTP ($P = 0.009$) among patients who received placebo. No associations between change in biomarker concentration and outcome (either OS or TTP) were identified for bFGF or the other biomarker candidates tested ($P > 0.05$ for all).

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Table 2. Univariate analyses of baseline biomarker concentrations and demographic/clinical variables

	HGF		VEGF		s-c-KIT		Ang2		IGF-2	
Demographic or clinical variable	**Mean level, pg/mL**	***P* value**	**Mean level, pg/mL**	***P* value**	**Mean level, ng/mL**	***P* value**	**Mean level, pg/mL**	***P* value**	**Mean level, pg/mL**	***P* value**
Sex										
Male	2,272.2	NS	94.2	NS	12.0	NS	7,672.5	NS	840.7	<0.0001
Female	2,754.2		93.2		12.4		8,175.0		1,374.7	
Age										
<65	2,768.5	NS	106.0	NS	12.5	NS	7,850.3	NS	972.7	0.0182
≥65	2,770.6		86.1		11.9		7,668.6		873.6	
ECOG PS										
0	2,778.6	NS	82.8	0.047	13.0	0.003	6,608.8	<0.0001	935.4	NS
1 or 2	2,760.3		106.0		11.1		8,917.6		889.9	
MVI and/or EHS										
Absent	2,635.4	NS	81.9	NS	12.6	NS	6,800.0	0.018	885.2	NS
Present	2,830.9		99.5		11.9		8,162.2		925.6	
AFP										
≤Median	2,549.5	0.011	97.4	NS	12.1	NS	6,887.8	0.0015	947.2	NS
>Median	2,989.0		90.7		12.1		8,590.7		879.2	
MVI										
No	2,570.4	0.002	94.4	NS	11.9	NS	6,889.6	<0.0001	960.8	0.001
Yes	3,096.4		93.4		12.4		9,133.1		835.1	
Albumin										
≤Median	3,145.5	<0.001	98.4	NS	12.8	0.032	9,273.2	<0.0001	754.6	<0.0001
>Median	2,398.8		89.7		11.4		6,189.2		1,073.0	
Alkaline phosphatase										
≤Median	2,472.1	<0.001	78.8	0.004	11.8	NS	6,305.7	<0.0001	929.6	NS
>Median	3,071.2		109.6		12.4		9,195.0		896.2	
Total bilirubin										
≤Median	2,412.8	<0.001	107.3	0.016	10.7	<0.0001	6,857.3	0.0003	1,056.9	<0.0001
>Median	3,122.2		81.1		13.5		8,598.9		771.7	

Abbreviations: EHS, extrahepatic spread; MVI, macroscopic vascular invasion; NS, not significant ($P > 0.05$).

Discussion

This study represents the largest effort to date to identify biomarkers of prognosis and response to sorafenib in patients with advanced HCC. This study was conducted in the setting of the phase III SHARP trial (3), which evaluated the efficacy and safety of sorafenib in patients with advanced HCC. We found that a number of plasma biomarkers—including Ang2, VEGF, HGF, and IGF-2—were predictors of prognosis in patients with advanced HCC, but none of the plasma markers tested significantly predicted response to sorafenib.

The most important finding of this study is the identification of Ang2 and VEGF as strong, independent predictors of survival in patients with HCC. Ang2 and VEGF are key signaling elements that drive angiogenesis, thereby enabling HCC growth and metastasis (31). To our knowledge, this study is the first to suggest that high plasma Ang2 concentrations at baseline are indicative of poor prognosis in patients with advanced HCC, suggesting that elevated levels of this angiogenic factor may be associated with more aggressive disease. Ang2 concentrations increased during treatment in the placebo group, suggesting poor outcome related to disease progression in this cohort. In contrast, Ang2 levels appear to be held constant during treatment with sorafenib, perhaps reflecting the more favorable outcome in this group. Furthermore, increases in Ang2 during treatment were associated with poorer outcomes in both groups, suggesting that measurements of Ang2 may have value in disease monitoring during treatment.

As elevated levels of VEGF at baseline indicate poor prognosis in patients with advanced HCC, a result consistent with previous findings (32–34), the increase in VEGF concentration observed after sorafenib treatment is at first glance counterintuitive, given the known survival advantages of sorafenib in patients with advanced HCC (3). However, treatment-induced increases in plasma concentration of VEGF (along with decreases in sVEGFR-2) have been consistently observed in other trials of sorafenib (35,

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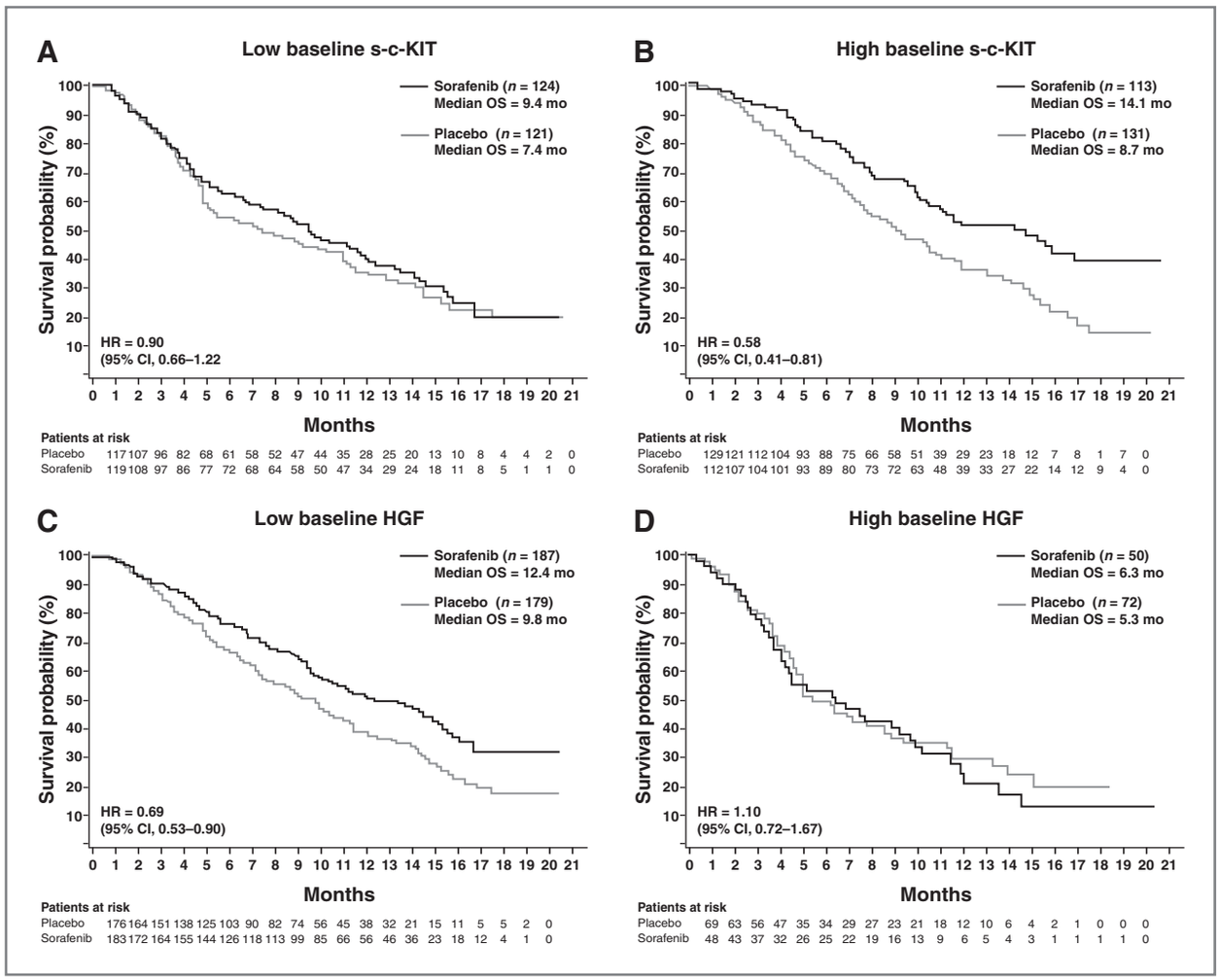

Figure 2. Analysis of baseline biomarkers as predictive factors for sorafenib benefit (OS). Low s-c-KIT (A) and high s-c-KIT (B), *P* value for biomarker treatment interaction = 0.081. C, low HGF and (D) high HGF, *P* value for biomarker treatment interaction = 0.073.

36) and with other agents inhibiting VEGFR-2 in HCC (37, 38) and other tumor types (39–41). Treatment with the anti-VEGF antibody bevacizumab has yielded mixed results, with increases in VEGF observed in some studies (40, 42) and decreases in VEGFR noted in others, including HCC (43, 44). Increases in VEGF, and associated decreases in VEGFR-2, have also been observed in nontumor-bearing mice after treatment with a VEGFR-2 inhibitor (45), suggesting that at least part of the increase in VEGF observed in humans is tumor independent. In the current study, the change in VEGF level observed during treatment did not correlate with outcome. Thus, these treatment-induced increases in VEGF are likely to be (at least in part) tumor independent and may not adversely affect the tumor due to efficient blockage of VEGFR signaling by sorafenib.

The role of the Ang-Tie2 pathway in oncogenesis has been reviewed recently (46). Increased expression of Ang2, particularly in conjunction with high VEGF-A concentration, correlated with poor outcomes in patients with breast cancer (47) and those with non–small cell lung cancer (NSCLC; ref. 48), as well as those with advanced HCC (49). Our biomarker analysis suggests that both molecules are independent predictors of survival in patients with HCC and provides a basis for novel opportunities for combination therapy in these patients.

Elevated HGF concentration was also identified as indicative of poor prognosis in the present study, although HGF did not retain significance in multivariate modeling. Commensurate with this finding, mean HGF levels decreased during treatment with sorafenib (and not in the placebo cohort), perhaps reflecting the more favorable outcome of the sorafenib group. Again, consistently, patients in either treatment group exhibiting HGF decreases greater than the median experienced better outcomes (longer OS and/or TTP). Taken together, these data suggest that HGF levels directly reflect HCC disease status, with low levels indicating favorable prognosis and decreasing levels suggesting disease improvement.

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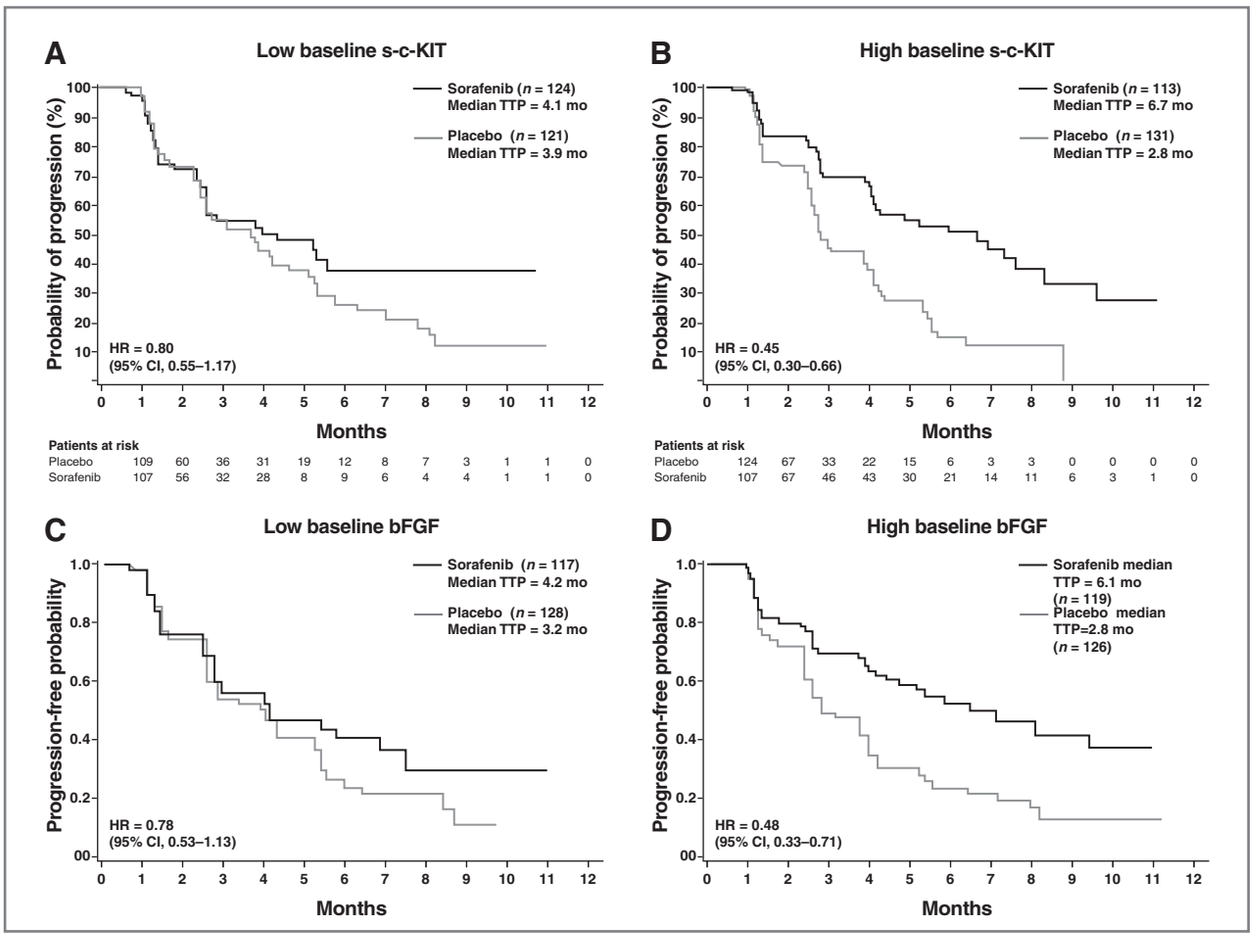

Figure 3. Analysis of baseline biomarkers as predictive factors for sorafenib benefit (TTP). Low s-c-KIT (A) and high s-c-KIT (B), *P* value for biomarker treatment interaction = 0.052. Low bFGF (C) and high bFGF (D), *P* value for biomarker treatment interaction = 0.078.

A few biomarkers predicting drug response have been confirmed in oncology, including HER2 expression and response to trastuzumab in breast and gastric cancers (50) and KRAS mutations and responses to cetuximab (51) and panitumumab (52) in colon cancer. The number is increasing steadily, including the recent approvals of vemurafenib for patients with BRAF V600E mutation-positive metastatic melanoma (53) and crizotinib for patients with NSCLC and anaplastic lymphoma kinase (ALK) rearrangement, as shown by fluorescence in situ hybridization tests (54). These predictive biomarkers have thus far been identified in tumor tissue rather than plasma samples. In the present study, we found that baseline plasma concentrations of s-c-KIT and HGF were independent predictors of survival in patients receiving sorafenib but showed only a nonsignificant trend as predictors of response to sorafenib treatment. The clinical significance of these results is uncertain as the role of s-c-KIT in the pathogenesis of HCC has not been consistently showed. Of further note are the results for HGF, a ligand that signals through the receptor tyrosine kinase c-MET. The HGF-MET cascade is relevant in hepatocarcinogenesis, and MET activation has been associated with poor outcome (55). In preclinical models, greater concentrations of sorafenib were required to inhibit the proliferation of HCC cells cultured with HGF than those without (56). Our clinical results may reflect this finding, in that patients with elevated HGF levels at baseline showed a trend toward deriving less benefit from sorafenib than those with low levels. Studies in NSCLC may explain this phenomenon, suggesting that HGF may be involved in conferring resistant to treatment with receptor tyrosine kinase inhibitors (57).

The clinical ramifications of the findings from this exploratory biomarker analysis of a large, randomized, placebo-controlled cohort of patients are 3-fold. First, we found that plasma Ang2 and VEGF concentrations, in addition to AFP concentration and other clinical parameters, are independent predictors of survival and should be considered prognostic biomarkers in patients with advanced HCC. Second, these prognostic biomarkers may prove valuable for the stratification of patients with advanced HCC before randomization in clinical trials. Finally, although trends of interest were identified in plasma s-c-KIT and HGF levels as predictive markers,

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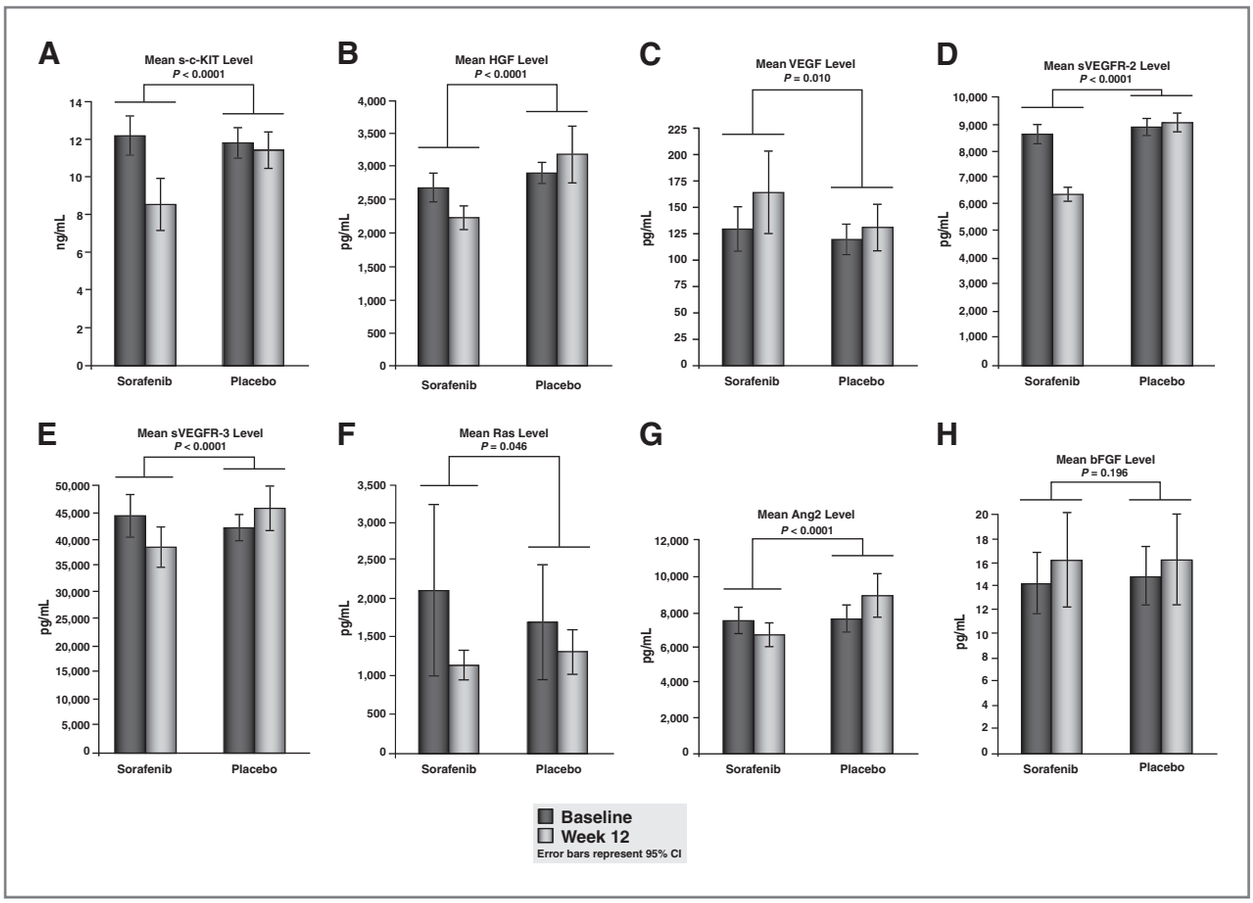

Figure 4. Analysis of the change in biomarker levels during treatment. Mean plasma concentrations of (A) s-c-KIT, (B) HGF, (C) VEGF, (D) sVEGFR-2, (E) sVEGFR-3, (F) Ras, (G) Ang2, and (H) bFGF at baseline (black bars) and at week 12 (gray bars). The *P* values compare the changes from baseline to week 12 in each biomarker concentration between the sorafenib and placebo groups by one-way ANOVA.

none of the plasma biomarkers tested reached statistical significance in predicting response to sorafenib. Before any of these biomarkers can be used clinically as surrogate markers of efficacy or response to sorafenib, further investigations are needed to confirm and validate their predictive and/or prognostic value.

Appendix

The following principal investigators (listed alphabetically by country) enrolled patients in the SHARP trial: **Argentina:** M.G. Pallota, J.J. Zarba; **Australia:** M. Boyer, S. Riordan, A. Strickland, N. Tebbutt, B. Thomson; **Belgium:** I. Borbath, J. De Greve, J.-L. Van Laethem, W. Van Steenbergen, H. Van Vlierberghe; **Brazil:** C. Barrios, A. Cosme de Oliveira; **Bulgaria:** I. Kotzev, D. Takov, K. Tchernev; **Canada:** K. Burak, M. Ma, P. Metrakos, C. Olweny, M. Sherman; **Chile:** C. Gamargo Garate, J. Martinez-Castillo; **France:** M. Beaugrand, J. Bennouna, J.-F. Blanc, J.-P. Bronowicki, F. Degos, S. Dominguez, J.-D. Grange, P. Hillon, J.-L. Raoul, J.-F. Seitz; **Germany:** H. Blum, P. Buggisch, W. Caspary, M. Dollinger, P.R. Galle, G. Gerken, B. Göke, M. Gregor, T. Greten, D. Häussinger, P. Hilgard, J. Scherübl, M. Scheulen, R. Schmid, U. Spengler, R. Wiest, S. Zeuzem; **Greece:** C. Arvanitakis, G. Germanidis, I. Katsos; **Israel:** A. Figer, S. Stemmer; **Italy:** D. Amadori, L. Bolondi, F. Cognetti, A. Craxi, F. Farinati, C. Gridelli, A. Martoni, V. Mazzaferro, C. Porta, S. Ricci, A. Sangiovanni, A. Santoro, F. Trevisani; **Mexico:** L.E. Cisnero Garza; **New Zealand:** E. Gane, A. O'Donnell; **Peru:** J. Leon, A. Lozano; **Poland:** J. Jassem, G. Rydzewska, A. Szawlowski, P. Tomczak; **Romania:** F. Badulescu, L. Miron; **Russia:** V. Kubyshkin; **Spain:** J. Bruix, A. Forner, J. Bustamante Schneider, M. Diago, J.L. Montero Alvarez, S. Pascual, L. RuÚz del Arbol, B. Sangro, R. Solá, J. Tabernero; **Switzerland:** B. Muellhaupt, A. Roth; **United Kingdom:** T.R. Jeffry Evans, S. Falk, T. Meyer, H. Reeves, P. Ross; **United States:** A. Befeler, T. Boyer, C. Britten, T. Byrne, G. Garcia-Tsao, P. Gold, A. Goldenberg, D. Heuman, P. Kennedy, A. Koch, J.M. Llovet, J. Marrero, M. Schilsky, J. Schwartz, M. Schwartz.

Disclosure of Potential Conflicts of Interest

J.M. Llovet and J. Bruix have participated in advisory activities for Bayer HealthCare Pharmaceuticals and Onyx Pharmaceuticals. No potential conflicts of interest were disclosed by the other authors.

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