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TOPIC HIGHLIGHT

2015 Advances in Hepatocellular Carcinoma

Predictive biomarkers of sorafenib efficacy in advanced hepatocellular carcinoma: Are we getting there?

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Abstract

Sorafenib is the current standard treatment for advanced hepatocellular carcinoma (HCC), but its efficacy is modest with low response rates and short response duration. Predictive biomarkers for sorafenib efficacy are necessary. However, efforts to determine biomarkers for sorafenib have led only to potential candidates rather than clinically useful predictors. Studies based on patient cohorts identified the potential of blood levels of angiopoietin-2, hepatocyte growth factor, insulin-like growth factor-1, and transforming growth factor-β1 for predicting sorafenib efficacy. Alpha-fetoprotein response, dynamic contrast-enhanced magnetic resonance imaging, and treatment-related side effects may serve as early surrogate markers. Novel approaches based on super-responders or experimental mouse models may provide new directions in biomarker research. These studies identified tumor amplification of *FGF3/FGF4* or *VEGFA* and tumor expression of phospho-Mapk14 and phospho-Atf2 as possible predictive markers that await validation. A group effort that considers various prognostic factors and proper collection of tumor tissues before treatment is imperative for the success of future biomarker research in advanced HCC.

Key words: Hepatocellular carcinoma; Predictive marker; Prognosis; Sorafenib

Core tip: A predictive biomarker of sorafenib efficacy in advanced hepatocellular carcinoma is a clinical unmet need. Previous studies identified the potential of blood levels of angiopoietin-2, hepatocyte growth factor, insulin-like growth factor-1, and transforming growth factor-β1 for predicting sorafenib efficacy. Alpha-fetoprotein response, dynamic contrast-enhanced magnetic resonance imaging, and treatment-related side effects may serve as early surrogate markers. Novel approaches based on super-responders or experimental mouse models may provide new research directions. A group effort that considers various prognostic factors and proper collection of tumor tissues before treatment is imperative for the success of future biomarker research in advanced hepatocellular carcinoma.

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INTRODUCTION

Advanced hepatocellular carcinoma (HCC) is defined as metastatic or locally advanced HCC not amenable to locoregional therapy, including surgery, local ablation, or transcatheter arterial chemoembolization (TACE). Advanced HCC is known for its extremely poor prognosis. Patients who received supportive care only for advanced HCC exhibited a median overall survival (OS) of only 4.2 and 7.9 mo in East Asian and Western countries, respectively[1,2].

In most countries, sorafenib is the only approved therapy for advanced HCC because the drug has been demonstrated to provide survival benefits in 2 randomized, placebo-controlled, double-blind phase Ⅲ clinical trials[1,2]. Sorafenib is a multikinase inhibitor of several growth factor signaling pathways; it inhibits the vascular endothelial growth factor (VEGF) pathway by inhibiting VEGF receptors, and the mitogen-activated protein kinase (MAPK) pathway by inhibiting Raf[3].

Although sorafenib can prolong OS, its efficacy is modest. In the 2 phase Ⅲ clinical trials comparing sorafenib with a placebo as first-line therapy for advanced HCC, the objective response rate (ORR) of sorafenib was only 2% to 3%; the disease control rate (DCR) was approximately 34% to 43%, and survival prolongation was fewer than 3 mo[1,2]. Numerous investigators have attempted to develop new therapies for improving treatment outcomes of advanced HCC. However, phase Ⅲ studies comparing sunitinib, brivanib, and linifanib to sorafenib all failed, so did a phase Ⅲ study comparing sorafenib with erlotinib with sorafenib alone[4-8]. As of 2015, sorafenib remains the only approved therapy for advanced HCC although it was approved for this use in the United States in 2007.

Because sorafenib is the only approved drug for advanced HCC but exhibits a relatively modest activity, a biomarker to predict sorafenib efficacy can assist in identifying the minority of patients who are likely to benefit from the treatment. Numerous studies have attempted to determine predictive markers for sorafenib efficacy by analyzing the associations between potential markers and patient outcomes. However, most such studies were based on single-arm studies that lack a comparable arm of patients treated with a placebo, making differentiating predictive markers from prognostic markers difficult[9]. Furthermore, using objective tumor response to measure treatment efficacy, a strategy that has been adopted for other cancer types, may not be appropriate for sorafenib in HCC treatment because of the extremely low ORR of the drug[1,2,10]. OS is also not an ideal endpoint for identifying predictive biomarkers of sorafenib in HCC treatment because it cannot be used to differentiate predictive markers from prognostic markers. DCR, time to progression (TTP), and progression-free survival (PFS) have thus been adopted as alternative endpoints in these studies. However, DCR, TTP, and PFS are endpoints that consider not only treatment efficacy but also inherent tumor behavior.

With these potential limitations in mind, in this paper we summarize the current understanding of predictive markers for sorafenib in advanced HCC treatment. Tables 1 and 2 present the key findings of the studies discussed in this review. Studies examining only prognostic markers are excluded. We also discussed novel approaches for finding predictive markers for sorafenib. Although the results of these studies have not been validated, advances in methods may assist in future biomarker studies.

BLOOD FACTORS

Vascular endothelial growth factor-A

A key target of sorafenib is the VEGF receptor-2; consequently, the ligand VEGF-A has been studied as a potential predictor for sorafenib efficacy. In research based on SHARP study, a phase Ⅲ trial conducted in Western countries comparing sorafenib with a placebo as first-line therapy for advanced HCC, Llovet *et al*[11] found that baseline plasma VEGF-A concentration exhibited no predictive values although low plasma VEGF-A was associated with improved prognosis. This finding was comparable to that of a biomarker study based on a single-arm clinical trial that showed the association between low plasma VEGF-A level with improved OS but not a higher DCR or improved PFS[12].

A small study with 30 patients demonstrated that low serum VEGF-A levels were associated with a higher DCR but not improved PFS or OS[13]. The study is the only research to indicate the potential for VEGF-A as a predictive marker, but its small scale and failure to

Table 1 Studies on predictive and prognostic markers for advanced hepatocellular carcinoma - serum or plasma markers

	Authors	Treatment	Findings supportive of		
			Predictive markers	Prognostic markers	Others
VEGF-A	Llovet *et al*[11]	Sorafenib *vs* placebo	No predictive values	Low VEGF-A → longer OS	-
	Shao *et al*[12]	Sorafenib plus UFT	No predictive values	Low VEGF-A → longer OS	-
	Miyahara *et al*[13]	Sorafenib	High VEGF → PD	No prognostic values	Not associated with PFS
IL-6 & 8	Shao *et al*[12]	Sorafenib plus UFT	-	High IL-6 and IL-8 → shorter OS	High IL-6 and IL-8 → shorter PFS
Ang-2	Llovet *et al*[11]	Sorafenib *vs* placebo	No predictive values	High Ang-2 → shorter OS	-
	Miyahara *et al*[13]	Sorafenib	High Ang-2 → PD	No associations	High Ang-2 → shorter PFS
IGF-1	Shao *et al*[20]	Sorafenib plus UFT or bevacizumab plus capecitabine	High IGF-1 → better DCR	High IGF-1 → longer OS	High IGF-1 → longer PFS
TGF-β1	Lin *et al*[23]	Sorafenib with or without UFT	-	High TGF-β1 → shorter OS	High TGF-β1 → shorter PFS
HGF	Llovet *et al*[11]	Sorafenib *vs* placebo	Low HGF → better efficacy[1]	-	-
	Miyahara *et al*[13]	Sorafenib	High HGF → PD	No associations	High HGF → shorter PFS
G-CSF	Miyahara *et al*[13]	Sorafenib	High G-CSF → PD	No associations	High G-CSF → shorter PFS
AFP response	Shao *et al*[31]	Sorafenib or thalidomide with tegafur/uracil Bevacizumab with capecitabine	AFP response → longer DCR	AFP response → longer OS	AFP response → longer PFS
	Personeni *et al*[32], Yau *et al*[33], and Kuzuya *et al*[34]	Sorafenib	AFP response → longer DCR	AFP response → longer OS	AFP response → longer PFS
	Nakazawa *et al*[35]	Sorafenib	AFP elevation → PD	AFP elevation → shorter OS	AFP elevation → shorter PFS
Circulating endothelial cells or progenitors	Shao *et al*[46]	Sorafenib plus UFT	Increase in post-treatment total CEC or viable CEC → PD[1]	High CEP → shorter OS	High CEP → shorter PFS
Neutrophil-to-lymphocyte ratio	Zheng *et al*[52]	Sorafenib	High ratio → shorter TTP	High ratio → shorter OS	-
ERK activity changes	Caraglia *et al*[55]	Sorafenib + octreotide	Post-treatment increase → PD	-	-
Soluble c-Kit	Llovet *et al*[11]	Sorafenib *vs* placebo	High c-Kit → better efficacy[1]	-	-
Leptin	Miyahara *et al*[13]	Sorafenib	High leptin → PD	No associations	High leptin → shorter PFS
LDH	Faloppi *et al*[56]	Sorafenib	-	LDH decrease → longer OS	LDH decrease → longer PFS

[1]Borderline significant. AFP: Alpha-fetoprotein; CEC: Circulating endothelial cells; CEP: Circulating endothelial progenitors; DCP: Des-γ-carboxy prothrombin; DCR: Disease control rate; G-CSF: Granulocyte-colony stimulating factor; HGF: Hepatocyte growth factor; IGF: Insulin-like growth factor; IL: Interleukin; LDH: Lactate dehydrogenase; ORR: Objective response rates; OS: Overall survival; PD: Progressive disease; PFS: Progression-free survival; TTP: Time to progression; UFT: Tegafur/uracil; VEGF: Vascular endothelial growth factor.

demonstrate an association with TTP or PFS rendered the findings less convincing.

Interleukin-6 and interleukin-8

Interleukin (IL)-6 and IL-8 are important inflammatory response mediators that can promote angiogenesis[14,15]. Shao *et al*[12] analyzed the outcomes of patients who received sorafenib with metronomic chemotherapy as first-line therapy for advanced HCC and demonstrated that high pretreatment plasma IL-6 or IL-8 levels were associated with poor PFS and OS. Although the baseline pretreatment IL-6 or IL-8 levels were not associated with DCR, patients with progressive disease exhibited significantly more increases in IL-6 and IL-8 levels after treatment than did patients with disease control.

Shao *et al*[12] reported that IL-6 and IL-8 levels may predict sorafenib efficacy. However, the patient cohort in that study did not receive sorafenib alone. In addition, the study was small in scale and lacked validation. Further study is warranted.

Angiopoietin-2

Angiopoietin-2 (Ang-2) is a crucial angiogenic factor. By binding to its receptor Tie2, Ang-2 cooperates with the VEGF pathway to maintain normal physiological functions. In the presence of VEGF, Ang-2 destabilizes blood vessels and promotes vascular sprouting[16]. In cancers, Ang-2 is linked to not only angiogenesis but also invasive and metastatic phenotypes[16]. Although sorafenib exerts no significant activity against Tie2[3], the predictive value of Ang-2 has been explored in 2 studies.

As discussed, Llovet *et al*[11] conducted a large

Table 2 Studies on predictive and prognostic markers for advanced hepatocellular carcinoma -- tissue biomarkers, clinical parameters, and others

Markers	Authors	Treatment	Findings supportive of		
			Predictive markers	Prognostic markers	Others
Tissue biomarkers					
p-ERK expression	Abou-Alfa *et al*[57]	Sorafenib	High p-ERK → longer TTP	-	-
	Ozenne *et al*[58]	Sorafenib	No predictive values	-	-
	Chen *et al*[59]	Sorafenib	High p-ERK → longer TTP	-	-
p-c-Jun expression	Hagiwara *et al*[61]	Sorafenib	High p-c-Jun expression → poor response, shorter TTP	p-c-Jun expression → shorter OS	-
Clinical parameter					
DCE-MRI	Hsu *et al*[63]	Sorafenib plus UFT	High baseline K^{trans} or decreased K^{trans} after treatment→ higher DCR	Vascular response[1] → longer OS	Vascular response[1] → longer PFS
Positron emission tomography	Lee *et al*[68]	Sorafenib	-	Low SUV → longer OS	Low SUV → longer PFS
Hypertension	Estfan *et al*[71]	Sorafenib	Hypertension → longer TTP (?)[2]	Hypertension → longer OS	-
	Otsuka *et al*[72]	Sorafenib	No predictive values	No prognostic values	-
Skin toxicity	Otsuka *et al*[72]	Sorafenib	No predictive values	Skin toxicities → longer OS	-
	Lee *et al*[73]	Sorafenib	-	⩾ grade 2 skin toxicities → longer OS	⩾ grade 2 skin toxicities → longer PFS
	Vincenzi *et al*[74]	Sorafenib	Early[4] ⩾ grade 1 skin toxicities → longer DCR and TTP	Early ⩾ grade 1 skin toxicities → longer OS[3]	-
Hepatitis etiology	Shao *et al*[78]	Sorafenib *vs* other treatments	Synthesized hazard ratio for overall mortality: 0.65 in patients with HCV etiology and 0.87 in patients with non-HCV etiology		
Novel approaches					
FGF3/FGF4 amplification	Arao *et al*[79]	Sorafenib	*FGF3/FGF4* amplification → higher tumor response	-	-
VEGFA amplification	Horwitz *et al*[85]	Sorafenib	-	*VEGFA* amplification → longer OS	-
p-Mapk14, p-Atf2 expression	Rudalska *et al*[86]	Sorafenib	-	High p-Mapk14 or p-Atf2 expression → shorter OS	-

[1]Defined as ⩾ 40% decreased in K^{trans} after treatment; [2]Statistical values of the comparison not reported; [3]Borderline statistical significance; [4]Within the first month of treatment. DCE-MRI: Dynamic contrast-enhanced magnetic resonance imaging; DCR: Disease control rate; OS: Overall survival; p-: Phospho-; PBMC: Peripheral blood mononuclear cells; PFS: Progression-free survival; TTP: Time to progression; UFT: Tegafur/uracil.

biomarker study based on SHARP study. The authors found that a high baseline plasma Ang-2 level was an independent factor for poorer OS but not for reduced sorafenib efficacy. Conversely, in a small retrospective study, a high serum Ang-2 level was associated with a lower DCR and poorer PFS[13]. The actual role of Ang-2 in predicting sorafenib efficacy warrants further investigations.

Insulin-like growth factor-1

The insulin-like growth factor (IGF) signaling pathway is key in regulating energy metabolism and growth[17]. The pathway is also critical in the carcinogenesis of many cancers. Neoplastic tissues frequently express the ligands of the pathway, IGF-1 and IGF-2[17]. However, most circulating IGF-1 and IGF-2 are synthesized by the liver[18]. Thus, IGF-1 levels appear to be associated with liver reserves[19].

Shao *et al*[20] explored the predictive role of serum IGF-1 levels by examining patients who were enrolled in 2 phase Ⅱ clinical trials. The patients received either sorafenib with metronomic chemotherapy or bevacizumab with capecitabine as first-line therapy for advanced HCC. In the study, high pretreatment serum IGF-1 levels were associated with a higher DCR and improved PFS and OS.

In a study of patients with all-stage HCC, Kaseb *et al*[19,21] demonstrated that plasma IGF-1 levels could serve as a satisfactory prognostic marker. Because the study by Shao *et al*[20] was based on single-arm clinical trials, there is a chance that their findings were attributed to the prognostic role of IGF-1, rather than the ability of IGF-1 in predicting sorafenib efficacy. Nevertheless, the vast difference in DCR between patients with high and low levels of IGF-1 (71% *vs* 39%) in the study by Shao *et al*[20] still suggests that circulating IGF-1 levels may be useful in predicting sorafenib efficacy in HCC treatment. Additional validation studies are warranted.

Transforming growth factor-β1

Activation of the transforming growth factor (TGF)-β signaling pathway has been shown to promote tumor progression, particularly at late and advanced tumor stages. Blood TGF-β1 levels were higher in patients with HCC than in patients with chronic hepatitis or cirrhosis[22]. Lin and Shao *et al*[23] reported that patients who received sorafenib containing regimens for advanced HCC exhibited poorer PFS and OS when they had higher pretreatment serum TGF-β1 levels. The DCRs were similar between patients with higher and lower serum TGF-β1 levels. In addition, serum

TGF-β1 levels significantly increased before disease progression.

Although these findings suggest that TGF-β is associated sorafenib resistance, the current data are insufficient to justify using TGF-β levels as a predictive marker for sorafenib efficacy in HCC treatment.

Hepatocyte growth factor

Hepatocyte growth factor (HGF) is the ligand for c-Met, the overexpression of which has been reported in HCC[24]. The HGF and c-Met pathway was demonstrated to be involved in sorafenib resistance[25]. In a biomarker study based on SHARP study, low HGF was found to be associated with improved sorafenib efficacy, but the findings reached only borderline statistical significance[11]. In a small retrospective study, patients with higher HGF levels were less likely to have disease control and exhibited shorter PFS[13]. These 2 studies have demonstrated the potential of HGF as a predictive marker for sorafenib efficacy. Validation studies are warranted.

Alpha-fetoprotein

Alpha-fetoprotein (AFP) is a glycoprotein expressed by HCC and secreted into the blood of approximately 70% of HCC patients[26]. For patients who undergo curative hepatectomies for localized HCC, pretreatment AFP levels are associated with prognosis[27]. A few studies have also reported that patients with advanced HCC and higher pretreatment AFP levels exhibited poorer prognosis[11,28-30]. However, little association between pretreatment AFP levels and DCR, TTP, or PFS has been determined[28,29]. The predictive value of pretreatment AFP levels for sorafenib efficacy is limited.

Conversely, a post-treatment change in AFP levels has been proposed as a potential surrogate marker for treatment efficacy in advanced HCC. For patients who were enrolled in clinical trials and received antiangiogenic regimens including sorafenib, Shao *et al*[31] demonstrated the AFP response, defined as a 20% decline in AFP levels after treatment, was associated with a higher DCR and improved PFS and OS. Subsequently, similar findings were reported in several studies with patients receiving sorafenib alone[32-34]. Another study revealed that patients with post-treatment AFP elevation exhibited poorer outcomes to sorafenib treatment for HCC[35]. The usefulness of AFP response may not be restricted to sorafenib treatment. Before sorafenib became available, AFP response had been associated with radiologic tumor response and the survival of advanced HCC patients treated with conventional chemotherapy or other types of systemic agents[36-38].

However, several problems have been identified in using AFP response as a marker in clinical practice. First, AFP response cannot be used in determining treatment decisions before treatment. Second, AFP response is inapplicable for patients with normal pretreatment AFP levels. Third, no consensus exists regarding when post-treatment AFP levels should be examined. Finally, the clinical usefulness of integrating AFP response with sorafenib treatment has not yet been verified in prospective studies with adequate statistical power.

Circulating endothelial cells or progenitors

Circulating endothelial cells (CECs) and circulating endothelial progenitors (CEPs) are potential surrogate markers of angiogenesis activity. Increases of CECs and CEPs occur in various physiologic and pathologic conditions in which angiogenesis is critical[39]. Elevated CEC and CEP levels have been identified in patients with HCC and associated with tumor aggressiveness and advanced cancer stages[40-43].

Although a few other studies have examined CEC and CEP levels in patients with advanced HCC[44,45], only one study enrolled patients who received sorafenib-containing regimens[46]. Shao *et al*[46] evaluated 40 patients enrolled in a phase Ⅱ study testing first-line combination therapy with sorafenib and metronomic chemotherapy. The study reported increasing levels of total CECs or viable CECs after 4 wk of treatment in patients with progressive disease but not in patients with disease control. A high baseline CEP level predicted poorer PFS or OS.

Despite the sound rationale of using CECs or CEPs to predict the efficacy of antiangiogenic therapy, the findings of Shao *et al*[46] have not been validated. Enumeration of CECs or CEPs is highly technique-dependent, which might be a major obstacle for large validation studies and the clinical use of these markers.

Neutrophil-to-lymphocyte ratio

The blood neutrophil-to-lymphocyte ratio (NLR) has been proposed as a potential prognostic marker for HCC. A high NLR has been associated with poor prognosis in patients who received resection, TACE, or ablation[47-49], but negative results have also been reported[50].

For patients with advanced HCC who received sorafenib, the NLR has also been associated with prognosis[51]. In a retrospective study, a high pretreatment NLR was associated with decreased OS and TTP[52]. The research is the only study to demonstrate the potential predictive role of NLRs. Because NLRs have been demonstrated to be easily affected by numerous diseases such as acute coronary syndromes, diabetes mellitus, essential hypertension, and thyroid dysfunction[53], a large study adequately controlling these cofactors is necessary to study the predictive and prognostic roles of NLRs for advanced HCC.

Changes in activity of extracellular signal-regulated kinase

One of the targets of sorafenib is Raf[3], a member of the MAPK pathway. Extracellular signal-regulated

kinase (ERK) locates downstream to Raf in the signaling cascade[54]. Caraglia *et al*[55] examined ERK activity in peripheral blood mononuclear cells as a pharmacodynamic marker for sorafenib. Patients with disease progression after receiving sorafenib treatment for advanced HCC exhibited increased post-treatment ERK activity. By contrast, ERK activity declined in patients with disease control.

Like AFP response, ERK activity changes cannot be used as a pretreatment predictive marker. Unlike AFP response, which can be only for patients with elevated AFP levels, ERK activity changes, if a truly effective marker, can be used for all patients treated with sorafenib. Additional validation studies are anticipated.

Others

The potential of other molecule levels as predictive markers for sorafenib efficacy has also been explored. The study by Llovet *et al*[11] reported borderline statistical significance for soluble c-Kit levels in predicting sorafenib efficacy. A retrospective study showed high blood granulocyte-colony stimulating factor and leptin levels to be associated with disease progression and poor PFS[13]. Even lactate dehydrogenase has been associated with PFS and OS[56]. However, most of these findings were based on studies comprising a limited number of patients are thus pending validation. Some of the studies also lacked clear mechanisms for explaining the potential associations.

TISSUE BIOMARKERS

Direct examining tumor tissues is a more direct method for determining predictive biomarkers than are blood factors. However, studies that do so for advanced HCC are scarce. A crucial reason for this is that HCC diagnosis does not necessarily require tissue proof. We discuss this issue further in the "CHALLENGES IN BIOMARKER STUDIES FOR ADVANCED HCC" section.

Phospho-ERK expression

Because sorafenib inhibits both angiogenesis-related pathways and the MAPK pathway through Raf, several studies have examined the downstream signaling molecules of Raf, such as ERK. In a phase Ⅱ study of sorafenib for advanced HCC, Abou-Alfa *et al*[57] showed that patients with tumors expressing high phospho-(p-) ERK had a longer TTP. However, recent studies have reported conflicting results[58,59]. Because of the failure of other inhibitors against the MAPK pathway in treating HCC[60], the role of MAPK pathway inhibition in sorafenib efficacy for HCC treatment might not be as pivotal as expected. Whether p-ERK expression is useful in predicting sorafenib efficacy remains unclear.

Phospho-c-Jun expression

A study based on 39 patients treated with sorafenib for advanced HCC showed that the tumor expression of p-c-Jun was associated with a poor tumor response, TTP, and OS[61]. By demonstrating its association with CD-133 expression, p-c-Jun expression was associated with stem cell-like characteristics. The mechanism underlying the association between p-c-Jun and sorafenib efficacy requires further exploration.

CLINICAL PARAMETERS

Dynamic contrast-enhanced magnetic resonance imaging

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) measures changes in tumor blood flow, vascular permeability, and interstitial and intravascular volumes[62]. Therefore, DCE-MRI has the potential for monitoring antiangiogenic effects. Because sorafenib harbors antiangiogenic activity, Hsu *et al*[29] performed DCE-MRI on patients who were enrolled in a phase Ⅱ clinical trial that evaluated sorafenib with metronomic tegafur/uracil as first-line therapy for advanced HCC. DCE-MRI was performed before treatment and after 14 d of treatment[63]. The research selected the most contrast-enhanced region of the tumor and measured the K^{trans} parameter. A vascular response, defined as a $\geqslant$ 40% decrease in K^{trans} after treatment, was associated with improved PFS and OS.

Because of its mechanism, DCE-MRI may not be a specific marker for sorafenib but for all kinds of antiangiogenic therapy. Another study examining sunitinib as a treatment for advanced HCC found similar results[45]. The currently available data suggest that DCE-MRI measurements, like post-treatment AFP response, may be used as an early surrogate marker for response to sorafenib treatment. However, the association between DCE-MRI parameters and tumor response awaits validation. In addition, whether this method is cost effective also requires careful evaluation.

Positron emission tomography

Although ^{18}F-fluorodeoxyglucose (FDG)-PET exhibits a low sensitivity (50%-55%) for HCC diagnosis[64,65], it may predict prognosis after resection or TACE for patients with HCC avid for labeled glucose[66,67]. Only one study examined FDG-PET in patients who received antiangiogenic therapy for advanced HCC. After studying 29 patients treated with sorafenib, Lee *et al*[68] showed that a low pretreatment standardized uptake value of FDG predicted improved PFS and OS but not a higher DCR. This result requires further confirmation.

Treatment side effects

The occurrence of treatment-related side effects can be a pharmacodynamic marker. Numerous studies have investigated whether the occurrence of certain treatment side effects can predict treatment efficacy. For example, a skin rash is associated with cetuximab efficacy in

patients with metastatic colorectal cancer[69,70].

Hypertension is a class-specific toxicity for anti-angiogenic therapy. Estfan *et al*[71] performed a retrospective review of 41 patients who received sorafenib for advanced HCC and showed that documented hypertension during treatment was associated with OS, regardless of baseline blood pressure status. However, another retrospective study based on patients treated with sorafenib for advanced HCC failed to identify associations between hypertension and DCR, PFS, or OS[72].

Skin toxicities, including hand-foot skin reactions and rashes, are relatively common in patients receiving sorafenib treatment. Two retrospective studies, one conducted in Japan and one in South Korea, revealed that the occurrence of skin toxicities during sorafenib treatment is associated with improved OS[72,73]. The South Korean study also demonstrated an association between hand-foot skin reactions and improved PFS[73]. However, a retrospective analysis of skin toxicities during the entire treatment period may have been confounded by an inherent observation bias because patients who are treated for longer periods may be at a greater risk of experiencing toxicities.

Vincenzi *et al*[74] examined associations between treatment outcomes and skin toxicities within only the first month of treatment. Sixty-five patients who received sorafenib for advanced HCC were enrolled, and early all-grade skin toxicities predicted a significantly improved DCR and TTP and prolonged OS with borderline significance.

Clearly, treatment side effects are not ideal biomarkers for dictating patient treatment. Like AFP response and parameter changes in DCE-MRI, these treatment side effects can serve only as surrogate markers.

Hepatitis etiology

HCC is a unique cancer because most patients had clear etiology. Common etiologic factors for HCC included hepatitis B virus, hepatitis C virus (HCV), and alcoholic liver disease. These various etiologic factors lead to different carcinogenesis processes and thus different tumor biologic signatures[75-77]. These variations may affect the efficacy of targeted therapies.

In a meta-analysis based on 4 randomized phase Ⅲ clinical trials that compared sorafenib with either a placebo or other treatments, Shao *et al*[78] revealed that patients with HCV infection may have better treatment outcomes than do patients with other etiologies. The synthesized hazard ratio for overall mortality, which compared patients who received sorafenib with patients who received other treatments, was 0.65 in HCV-positive patients and 0.87 in HCV-negative patients. The study was limited by the lack of a multivariate analysis to adjust for other confounding factors. However, the research indicated that other clinical factors, such as ethnicity and performance status, did not exert such a difference in hazard ratios as that between patients with or without HCV infection. This finding requires further validation and exploration. If true, the underlying mechanisms of HCV etiology associated with improved treatment outcomes by using sorafenib may assist in determining new treatment targets.

NOVEL APPROACHES

Most biomarker studies begin with a cohort of patients who receive a specific treatment. In our review, such studies enrolled patients who received sorafenib for advanced HCC with various responses. However, novel approaches have emerged. One approach is to begin with a few patients who exhibited dramatic response from sorafenib treatment for HCC. These super-responders have been argued as providing stronger signals, rather than noise, in biomarker detection. Another approach is combining mouse liver cancer models, which are intended to mimic human HCC more closely than do conventional human cancer cell lines, with cutting-edge, mass genetic screening strategies to examine critical molecular mechanisms underlying sensitivity and resistance to sorafenib treatment.

Super-responders

Arao *et al*[79] identified *FGF3/FGF4* amplification in a patient with advanced HCC who dramatically responded to sorafenib. Among 10 other sorafenib responders, 3 patients also exhibited *FGF3/FGF4* amplification. By contrast, no *FGF3/FGF4* amplification was found in 38 patients who exhibited stable or progressive disease. According to the literature, *FGF3/FGF4* amplification can be found in 0% to 7% of patients with HCC[80-82].

However, no validation studies have been performed and how *FGF3/FGF4* amplification is associated with sorafenib efficacy remains unclear. Located at 11q13, the *FGF3/FGF4* site is beside *FGF19* and *CCND1*, 2 genes also frequently reported to exert amplifications in HCC tissues[83,84]. Whether *FGF3/FGF4* amplification has a direct association with sorafenib or is just a bystander, which would suggest other underlying mechanisms, requires further study.

Mouse liver cancer models

After applying array-based, comparative genomic hybridization in *Mdr2*-deficient mice (*Mdr2*$^{-/-}$) that had developed chronic liver inflammation and liver tumors, Horwitz *et al*[85] found that 14% of the mouse liver tumors exhibited the genomic amplification of *VEGFA*, the VEGF-A coding gene. The researchers determined that the mouse liver tumors with *VEGFA* amplification exhibited a more distinctive sensitivity to sorafenib treatment than did those without *VEGFA* amplification. Among 54 patients who received sorafenib, *VEGFA* amplification was associated with improved OS. No

association with DCR, TTP, or PFS was reported. However, the finding with this mouse model indicated the potential of *VEGFA* amplification as a predictive marker in human HCC. Further validation is warranted.

Using a focused short hairpin RNA (shRNA) library targeting genes located within the focal genomic amplifications of human HCC in a transposon-based, mouse liver cancer model, Rudalska *et al*[86] intended to identify target genes, the inhibition of which would increase sorafenib efficacy. The researchers determined that the shRNA-mediated downregulation or chemical inhibition of Mapk14 (p38α) sensitized mouse liver tumors to sorafenib treatment. The study also reported that, among 16 HCC patients treated with sorafenib, high tumor expression of p-Mapk14 and its downstream target p-Atf2 before treatment was associated with shorter OS. The study suggests that Mapk14 signaling could be a critical mechanism in sorafenib resistance. Whether Mapk14 signaling could serve as a predictive biomarker for sorafenib treatment in advanced HCC requires additional validation studies.

CHALLENGES IN BIOMARKER STUDIES FOR ADVANCED HCC

Despite the clinical need for a predictive marker for sorafenib efficacy, a lack of progress in this search has persisted over the past few years. A major factor contributing to this lack of progress is the scarcity of HCC tissues, particularly those obtained at advanced stages and before sorafenib treatment. As a result, some studies failed to validate their *in vitro* findings about potential predictive biomarkers in adequate clinical cohorts[87].

HCC diagnosis does not necessarily require a histology diagnosis[88], which is extremely rare among malignant diseases. Routinely obtaining tissues from patients with advanced HCC for biomarker research may not be ethically sound. Even when tissue samples are available, the methods of tissue procurement may considerably affect the stability of certain markers and their expression levels. For example, Shao *et al*[89] compared the immunohistochemical staining results of paired HCC tissues acquired through pre-operative biopsies and hepatectomies performed on the same patients. Although markers such as p53 and beta-catenin exhibited in similar expression levels between paired HCC tissues, the staining results of p-Akt and p-ERK revealed marked differences. In summary, collecting HCC tissues properly from patients with advanced HCC is imperative for the success of future biomarker research. Novel clinical trial designs such as allocating patients with distinctive molecular derangement into matched targeted therapy might justify and facilitate obtaining HCC tissues in patients with advanced HCC.

Prognoses of advanced HCC can be extremely heterogeneous. Future biomarker research should address the etiological factors of HCC, which might affect carcinogenetic processes and patient sensitivity to various types of drug therapy[75-78]. Several scoring systems have sustained their prognosis prediction abilities when only patients with advanced HCC were enrolled[21,90-92]. When biomarker research is based on single-arm studies, an adequate adjustment for these prognostic factors must be included in the statistical analysis.

The possibility for several biomarkers synergistically predictive of sorafenib efficacy should be kept in mind. Investigators have found several potential predictive biomarkers in separate studies, but a study examining all of them together or even attempting to combine them is lacking. Future studies should consider addressing this issue.

A group effort is essential for success in biomarker studies. An adequate patient sampling for sufficient statistical power is a basic requirement for a satisfactory biomarker study. Currently, several biomarkers with potential for predicting sorafenib efficacy exist but are pending validation. An international multi-institution patient cohort with adequate adjustments for etiological factors could assist in validating these biomarkers definitely.

CONCLUSION

A considerable, unmet need in clinical practice exists for a predictive marker for sorafenib efficacy. Although sorafenib was approved as first-line therapy for advanced HCC, not all patients benefit from it. The largest biomarker study based on SHARP study was the most useful research for us in determining such biomarkers because it included an adequate number of participants and a placebo-controlled group. However, the study demonstrated the potential of Ang-2 and HGF with only borderline statistical significance. Other studies exploring predictive markers for sorafenib have been based on small, single-arm studies, and their results, though instructive, awaits validation. For example, serum IGF-1 levels and the gene amplification of *FGF3/FGF4* and *VEGFA* showed promise. However, validation studies are required to confirm these findings. A group effort that considers the various prognostic factors and proper collection of tumor tissues before treatment is imperative for the success of future biomarker research.

REFERENCES

1 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514 DOI: 10.1056/NEJMoa0708857]

2 **Cheng AL**, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak

WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. *Lancet Oncol* 2009; **10**: 25-34 [PMID: 19095497 DOI: 10.1016/S1470-2045(08)70285-7]

3 **Wilhelm S**, Carter C, Lynch M, Lowinger T, Dumas J, Smith RA, Schwartz B, Simantov R, Kelley S. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Discov* 2006; **5**: 835-844 [PMID: 17016424 DOI: 10.1038/nrd2130]

4 **Hsu CH**, Shen YC, Shao YY, Hsu C, Cheng AL. Sorafenib in advanced hepatocellular carcinoma: current status and future perspectives. *J Hepat Carcino* 2014; **1**: 85-99 [DOI: 10.2147/JHC.S45040]

5 **Johnson PJ**, Qin S, Park JW, Poon RT, Raoul JL, Philip PA, Hsu CH, Hu TH, Heo J, Xu J, Lu L, Chao Y, Boucher E, Han KH, Paik SW, Robles-Aviña J, Kudo M, Yan L, Sobhonslidsuk A, Komov D, Decaens T, Tak WY, Jeng LB, Liu D, Ezzeddine R, Walters I, Cheng AL. Brivanib versus sorafenib as first-line therapy in patients with unresectable, advanced hepatocellular carcinoma: results from the randomized phase III BRISK-FL study. *J Clin Oncol* 2013; **31**: 3517-3524 [PMID: 23980084 DOI: 10.1200/JCO.2012.48.4410]

6 **Cheng AL**, Kang YK, Lin DY, Park JW, Kudo M, Qin S, Chung HC, Song X, Xu J, Poggi G, Omata M, Pitman Lowenthal S, Lanzalone S, Yang L, Lechuga MJ, Raymond E. Sunitinib versus sorafenib in advanced hepatocellular cancer: results of a randomized phase III trial. *J Clin Oncol* 2013; **31**: 4067-4075 [PMID: 24081937 DOI: 10.1200/JCO.2012.45.8372]

7 **Cainap C**, Qin S, Huang WT, Chung IJ, Pan H, Cheng Y, Kudo M, Kang YK, Chen PJ, Toh HC, Gorbunova V, Eskens FA, Qian J, McKee MD, Ricker JL, Carlson DM, El-Nowiem S. Linifanib versus Sorafenib in patients with advanced hepatocellular carcinoma: results of a randomized phase III trial. *J Clin Oncol* 2015; **33**: 172-179 [PMID: 25488963 DOI: 10.1200/JCO.2013.54.3298]

8 **Zhu AX**, Rosmorduc O, Evans TR, Ross PJ, Santoro A, Carrilho FJ, Bruix J, Qin S, Thuluvath PJ, Llovet JM, Leberre MA, Jensen M, Meinhardt G, Kang YK. SEARCH: a phase III, randomized, double-blind, placebo-controlled trial of sorafenib plus erlotinib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2015; **33**: 559-566 [PMID: 25547503 DOI: 10.1200/JCO.2013.53.7746]

9 **Coate LE**, John T, Tsao MS, Shepherd FA. Molecular predictive and prognostic markers in non-small-cell lung cancer. *Lancet Oncol* 2009; **10**: 1001-1010 [PMID: 19796752 DOI: 10.1016/S1470-2045(09)70155-X]

10 **Shao YY**, Chang YL, Huang CY, Hsu CH, Cheng AL. The germline BIM deletion polymorphism is not associated with the treatment efficacy of sorafenib in patients with advanced hepatocellular carcinoma. *Oncology* 2013; **85**: 312-316 [PMID: 24217400 DOI: 10.1159/000356019]

11 **Llovet JM**, Peña CE, Lathia CD, Shan M, Meinhardt G, Bruix J. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2012; **18**: 2290-2300 [PMID: 22374331 DOI: 10.1158/1078-0432.CCR-11-2175]

12 **Shao YY**, Hsu CH, Huang CC, Cheng AL. Use of plasma angiogenesis-related factors to investigate the association of interleukin 8 and interleukin 6 levels with efficacy of sorafenib-based antiangiogenic therapy in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2011; **29** suppl 4: Abst 199

13 **Miyahara K**, Nouso K, Tomoda T, Kobayashi S, Hagihara H, Kuwaki K, Toshimori J, Onishi H, Ikeda F, Miyake Y, Nakamura S, Shiraha H, Takaki A, Yamamoto K. Predicting the treatment effect of sorafenib using serum angiogenesis markers in patients with hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011; **26**: 1604-1611 [PMID: 22011296 DOI: 10.1111/j.1440-1746.2011.06887.x]

14 **Guo Y**, Xu F, Lu T, Duan Z, Zhang Z. Interleukin-6 signaling pathway in targeted therapy for cancer. *Cancer Treat Rev* 2012; **38**: 904-910 [PMID: 22651903 DOI: 10.1016/j.ctrv.2012.04.007]

15 **Waugh DJ**, Wilson C. The interleukin-8 pathway in cancer. *Clin Cancer Res* 2008; **14**: 6735-6741 [PMID: 18980965 DOI: 10.1158/1078-0432.ccr-07-4843]

16 **Hu B**, Cheng SY. Angiopoietin-2: development of inhibitors for cancer therapy. *Curr Oncol Rep* 2009; **11**: 111-116 [PMID: 19216842]

17 **Pollak M**. Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 2008; **8**: 915-928 [PMID: 19029956 DOI: 10.1038/nrc2536]

18 **Stuver SO**, Kuper H, Tzonou A, Lagiou P, Spanos E, Hsieh CC, Mantzoros C, Trichopoulos D. Insulin-like growth factor 1 in hepatocellular carcinoma and metastatic liver cancer in men. *Int J Cancer* 2000; **87**: 118-121 [PMID: 10861461]

19 **Kaseb AO**, Morris JS, Hassan MM, Siddiqui AM, Lin E, Xiao L, Abdalla EK, Vauthey JN, Aloia TA, Krishnan S, Abbruzzese JL. Clinical and prognostic implications of plasma insulin-like growth factor-1 and vascular endothelial growth factor in patients with hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 3892-3899 [PMID: 21911725 DOI: 10.1200/JCO.2011.36.0636]

20 **Shao YY**, Huang CC, Lin SD, Hsu CH, Cheng AL. Serum insulin-like growth factor-1 levels predict outcomes of patients with advanced hepatocellular carcinoma receiving antiangiogenic therapy. *Clin Cancer Res* 2012; **18**: 3992-3997 [PMID: 22623732 DOI: 10.1158/1078-0432.CCR-11-2853]

21 **Kaseb AO**, Xiao L, Hassan MM, Chae YK, Lee JS, Vauthey JN, Krishnan S, Cheung S, Hassabo HM, Aloia T, Conrad C, Curley SA, Vierling JM, Jalal P, Raghav K, Wallace M, Rashid A, Abbruzzese JL, Wolff RA, Morris JS. Development and validation of insulin-like growth factor-1 score to assess hepatic reserve in hepatocellular carcinoma. *J Natl Cancer Inst* 2014; **106**: [PMID: 24815863 DOI: 10.1093/jnci/dju088]

22 **Song BC**, Chung YH, Kim JA, Choi WB, Suh DD, Pyo SI, Shin JW, Lee HC, Lee YS, Suh DJ. Transforming growth factor-beta1 as a useful serologic marker of small hepatocellular carcinoma. *Cancer* 2002; **94**: 175-180 [PMID: 11815974]

23 **Lin TH**, Shao YY, Chan SY, Huang CY, Hsu CH, Cheng AL. High Serum Transforming Growth Factor-β1 Levels Predict Outcome in Hepatocellular Carcinoma Patients Treated with Sorafenib. *Clin Cancer Res* 2015; **21**: 3678-3684 [PMID: 25977342]

24 **Trusolino L**, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. *Nat Rev Mol Cell Biol* 2010; **11**: 834-848 [PMID: 21102609 DOI: 10.1038/nrm3012]

25 **Chen W**, Wu J, Shi H, Wang Z, Zhang G, Cao Y, Jiang C, Ding Y. Hepatic stellate cell coculture enables sorafenib resistance in Huh7 cells through HGF/c-Met/Akt and Jak2/Stat3 pathways. *Biomed Res Int* 2014; **2014**: 764981 [PMID: 25057499 DOI: 10.1155/2014/764981]

26 **McIntire KR**, Vogel CL, Princler GL, Patel IR. Serum alpha-fetoprotein as a biochemical marker for hepatocellular carcinoma. *Cancer Res* 1972; **32**: 1941-1946 [PMID: 4116292]

27 **Ijichi M**, Takayama T, Matsumura M, Shiratori Y, Omata M, Makuuchi M. alpha-Fetoprotein mRNA in the circulation as a predictor of postsurgical recurrence of hepatocellular carcinoma: a prospective study. *Hepatology* 2002; **35**: 853-860 [PMID: 11915031 DOI: 10.1053/jhep.2002.32100]

28 **Shao YY**, Chen PJ, Lin ZZ, Huang CC, Ding YH, Lee YH, Hsu CH, Cheng AL. Impact of baseline hepatitis B viral DNA levels on survival of patients with advanced hepatocellular carcinoma. *Anticancer Res* 2011; **31**: 4007-4011 [PMID: 22110234]

29 **Hsu CH**, Shen YC, Lin ZZ, Chen PJ, Shao YY, Ding YH, Hsu C, Cheng AL. Phase II study of combining sorafenib with metronomic tegafur/uracil for advanced hepatocellular carcinoma. *J Hepatol* 2010; **53**: 126-131 [PMID: 20416968 DOI: 10.1016/j.jhep.2010.01.035]

30 **Lin ZZ**, Hsu C, Hu FC, Shao YY, Chang DY, Yang CH, Hong RL, Hsu CH, Cheng AL. Factors impacting prognosis prediction in BCLC stage C and Child-Pugh class A hepatocellular carcinoma

patients in prospective clinical trials of systemic therapy. *Oncologist* 2012; **17**: 970-977 [PMID: 22673633 DOI: 10.1634/theoncologist.2011-0411]

31 **Shao YY**, Lin ZZ, Hsu C, Shen YC, Hsu CH, Cheng AL. Early alpha-fetoprotein response predicts treatment efficacy of antiangiogenic systemic therapy in patients with advanced hepatocellular carcinoma. *Cancer* 2010; **116**: 4590-4596 [PMID: 20572033 DOI: 10.1002/cncr.25257]

32 **Personeni N**, Bozzarelli S, Pressiani T, Rimassa L, Tronconi MC, Sclafani F, Carnaghi C, Pedicini V, Giordano L, Santoro A. Usefulness of alpha-fetoprotein response in patients treated with sorafenib for advanced hepatocellular carcinoma. *J Hepatol* 2012; **57**: 101-107 [PMID: 22414760 DOI: 10.1016/j.jhep.2012.02.016]

33 **Yau T**, Yao TJ, Chan P, Wong H, Pang R, Fan ST, Poon RT. The significance of early alpha-fetoprotein level changes in predicting clinical and survival benefits in advanced hepatocellular carcinoma patients receiving sorafenib. *Oncologist* 2011; **16**: 1270-1279 [PMID: 21885876 DOI: 10.1634/theoncologist.2011-0105]

34 **Kuzuya T**, Asahina Y, Tsuchiya K, Tanaka K, Suzuki Y, Hoshioka T, Tamaki S, Kato T, Yasui Y, Hosokawa T, Ueda K, Nakanishi H, Itakura J, Takahashi Y, Kurosaki M, Izumi N. Early decrease in α-fetoprotein, but not des-γ-carboxy prothrombin, predicts sorafenib efficacy in patients with advanced hepatocellular carcinoma. *Oncology* 2011; **81**: 251-258 [PMID: 22116493 DOI: 10.1159/000334454]

35 **Nakazawa T**, Hidaka H, Takada J, Okuwaki Y, Tanaka Y, Watanabe M, Shibuya A, Minamino T, Kokubu S, Koizumi W. Early increase in α-fetoprotein for predicting unfavorable clinical outcomes in patients with advanced hepatocellular carcinoma treated with sorafenib. *Eur J Gastroenterol Hepatol* 2013; **25**: 683-689 [PMID: 23395995 DOI: 10.1097/MEG.0b013e32835d913b]

36 **Chen LT**, Liu TW, Chao Y, Shiah HS, Chang JY, Juang SH, Chen SC, Chuang TR, Chin YH, Whang-Peng J. alpha-fetoprotein response predicts survival benefits of thalidomide in advanced hepatocellular carcinoma. *Aliment Pharmacol Ther* 2005; **22**: 217-226 [PMID: 16091059 DOI: 10.1111/j.1365-2036.2005.02547.x]

37 **Vora SR**, Zheng H, Stadler ZK, Fuchs CS, Zhu AX. Serum alpha-fetoprotein response as a surrogate for clinical outcome in patients receiving systemic therapy for advanced hepatocellular carcinoma. *Oncologist* 2009; **14**: 717-725 [PMID: 19581525 DOI: 10.1634/theoncologist.2009-0038]

38 **Chan SL**, Mo FK, Johnson PJ, Hui EP, Ma BB, Ho WM, Lam KC, Chan AT, Mok TS, Yeo W. New utility of an old marker: serial alpha-fetoprotein measurement in predicting radiologic response and survival of patients with hepatocellular carcinoma undergoing systemic chemotherapy. *J Clin Oncol* 2009; **27**: 446-452 [PMID: 19064965 DOI: 10.1200/JCO.2008.18.8151]

39 **Blann AD**, Woywodt A, Bertolini F, Bull TM, Buyon JP, Clancy RM, Haubitz M, Hebbel RP, Lip GY, Mancuso P, Sampol J, Solovey A, Dignat-George F. Circulating endothelial cells. Biomarker of vascular disease. *Thromb Haemost* 2005; **93**: 228-235 [PMID: 15711737 DOI: 10.1267/THRO05020228]

40 **Sieghart W**, Fellner S, Reiberger T, Ulbrich G, Ferlitsch A, Wacheck V, Peck-Radosavljevic M. Differential role of circulating endothelial progenitor cells in cirrhotic patients with or without hepatocellular carcinoma. *Dig Liver Dis* 2009; **41**: 902-906 [PMID: 19501032 DOI: 10.1016/j.dld.2009.04.013]

41 **Ho JW**, Pang RW, Lau C, Sun CK, Yu WC, Fan ST, Poon RT. Significance of circulating endothelial progenitor cells in hepatocellular carcinoma. *Hepatology* 2006; **44**: 836-843 [PMID: 17006919 DOI: 10.1002/hep.21353]

42 **Goon PK**, Lip GY, Stonelake PS, Blann AD. Circulating endothelial cells and circulating progenitor cells in breast cancer: relationship to endothelial damage/dysfunction/apoptosis, clinicopathologic factors, and the Nottingham Prognostic Index. *Neoplasia* 2009; **11**: 771-779 [PMID: 19649207]

43 **Naik RP**, Jin D, Chuang E, Gold EG, Tousimis EA, Moore AL, Christos PJ, de Dalmas T, Donovan D, Rafii S, Vahdat LT. Circulating endothelial progenitor cells correlate to stage in patients with invasive breast cancer. *Breast Cancer Res Treat* 2008; **107**: 133-138 [PMID: 18043899 DOI: 10.1007/s10549-007-9519-6]

44 **Boige V**, Malka D, Bourredjem A, Dromain C, Baey C, Jacques N, Pignon JP, Vimond N, Bouvet-Forteau N, De Baere T, Ducreux M, Farace F. Efficacy, safety, and biomarkers of single-agent bevacizumab therapy in patients with advanced hepatocellular carcinoma. *Oncologist* 2012; **17**: 1063-1072 [PMID: 22707516 DOI: 10.1634/theoncologist.2011-0465]

45 **Zhu AX**, Sahani DV, Duda DG, di Tomaso E, Ancukiewicz M, Catalano OA, Sindhwani V, Blaszkowsky LS, Yoon SS, Lahdenranta J, Bhargava P, Meyerhardt J, Clark JW, Kwak EL, Hezel AF, Miksad R, Abrams TA, Enzinger PC, Fuchs CS, Ryan DP, Jain RK. Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: a phase II study. *J Clin Oncol* 2009; **27**: 3027-3035 [PMID: 19470923 DOI: 10.1200/JCO.2008.20.9908]

46 **Shao YY**, Lin ZZ, Chen TJ, Hsu C, Shen YC, Hsu CH, Cheng AL. High circulating endothelial progenitor levels associated with poor survival of advanced hepatocellular carcinoma patients receiving sorafenib combined with metronomic chemotherapy. *Oncology* 2011; **81**: 98-103 [PMID: 21986371 DOI: 10.1159/000331684]

47 **Mano Y**, Shirabe K, Yamashita Y, Harimoto N, Tsujita E, Takeishi K, Aishima S, Ikegami T, Yoshizumi T, Yamanaka T, Maehara Y. Preoperative neutrophil-to-lymphocyte ratio is a predictor of survival after hepatectomy for hepatocellular carcinoma: a retrospective analysis. *Ann Surg* 2013; **258**: 301-305 [PMID: 23774313 DOI: 10.1097/SLA.0b013e318297ad6b]

48 **Chen TM**, Lin CC, Huang PT, Wen CF. Neutrophil-to-lymphocyte ratio associated with mortality in early hepatocellular carcinoma patients after radiofrequency ablation. *J Gastroenterol Hepatol* 2012; **27**: 553-561 [PMID: 21913982 DOI: 10.1111/j.1440-1746.2011.06910.x]

49 **Huang ZL**, Luo J, Chen MS, Li JQ, Shi M. Blood neutrophil-to-lymphocyte ratio predicts survival in patients with unresectable hepatocellular carcinoma undergoing transarterial chemoembolization. *J Vasc Interv Radiol* 2011; **22**: 702-709 [PMID: 21514523 DOI: 10.1016/j.jvir.2010.12.041]

50 **Sullivan KM**, Groeschl RT, Turaga KK, Tsai S, Christians KK, White SB, Rilling WS, Pilgrim CH, Gamblin TC. Neutrophil-to-lymphocyte ratio as a predictor of outcomes for patients with hepatocellular carcinoma: a Western perspective. *J Surg Oncol* 2014; **109**: 95-97 [PMID: 24122764 DOI: 10.1002/jso.23448]

51 **da Fonseca LG**, Barroso-Sousa R, Bento Ada S, Blanco BP, Valente GL, Pfiffer TE, Hoff PM, Sabbaga J. Pre-treatment neutrophil-to-lymphocyte ratio affects survival in patients with advanced hepatocellular carcinoma treated with sorafenib. *Med Oncol* 2014; **31**: 264 [PMID: 25273866 DOI: 10.1007/s12032-014-0264-5]

52 **Zheng YB**, Zhao W, Liu B, Lu LG, He X, Huang JW, Li Y, Hu BS. The blood neutrophil-to-lymphocyte ratio predicts survival in patients with advanced hepatocellular carcinoma receiving sorafenib. *Asian Pac J Cancer Prev* 2013; **14**: 5527-5531 [PMID: 24175853]

53 **Tanoglu A**, Karagoz E. Predictive role of the neutrophil-to-lymphocyte ratio in patients with advanced hepatocellular carcinoma receiving sorafenib. *Asian Pac J Cancer Prev* 2014; **15**: 1063 [PMID: 24568452]

54 **Johnson GL**, Lapadat R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science* 2002; **298**: 1911-1912 [PMID: 12471242 DOI: 10.1126/science.1072682]

55 **Caraglia M**, Giuberti G, Marra M, Addeo R, Montella L, Murolo M, Sperlongano P, Vincenzi B, Naviglio S, Prete SD, Abbruzzese A, Stiuso P. Oxidative stress and ERK1/2 phosphorylation as predictors of outcome in hepatocellular carcinoma patients treated with sorafenib plus octreotide LAR. *Cell Death Dis* 2011; **2**: e150 [PMID: 21525937 DOI: 10.1038/cddis.2011.34]

56 **Faloppi L**, Scartozzi M, Bianconi M, Svegliati Baroni G, Toniutto P, Giampieri R, Del Prete M, De Minicis S, Bitetto D, Loretelli C, D'Anzeo M, Benedetti A, Cascinu S. The role of LDH serum

levels in predicting global outcome in HCC patients treated with sorafenib: implications for clinical management. *BMC Cancer* 2014; **14**: 110 [PMID: 24552144 DOI: 10.1186/1471-2407-14-110]

57 **Abou-Alfa GK**, Schwartz L, Ricci S, Amadori D, Santoro A, Figer A, De Greve J, Douillard JY, Lathia C, Schwartz B, Taylor I, Moscovici M, Saltz LB. Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2006; **24**: 4293-4300 [PMID: 16908937 DOI: 10.1200/JCO.2005.01.3441]

58 **Ozenne V**, Paradis V, Pernot S, Castelnau C, Vullierme MP, Bouattour M, Valla D, Farges O, Degos F. Tolerance and outcome of patients with unresectable hepatocellular carcinoma treated with sorafenib. *Eur J Gastroenterol Hepatol* 2010; **22**: 1106-1110 [PMID: 20300004 DOI: 10.1097/MEG.0b013e3283386053]

59 **Chen D**, Zhao P, Li SQ, Xiao WK, Yin XY, Peng BG, Liang LJ. Prognostic impact of pERK in advanced hepatocellular carcinoma patients treated with sorafenib. *Eur J Surg Oncol* 2013; **39**: 974-980 [PMID: 23845703 DOI: 10.1016/j.ejso.2013.06.018]

60 **O'Neil BH**, Goff LW, Kauh JS, Strosberg JR, Bekaii-Saab TS, Lee RM, Kazi A, Moore DT, Learoyd M, Lush RM, Sebti SM, Sullivan DM. Phase II study of the mitogen-activated protein kinase 1/2 inhibitor selumetinib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 2350-2356 [PMID: 21519015 DOI: 10.1200/JCO.2010.33.9432]

61 **Hagiwara S**, Kudo M, Nagai T, Inoue T, Ueshima K, Nishida N, Watanabe T, Sakurai T. Activation of JNK and high expression level of CD133 predict a poor response to sorafenib in hepatocellular carcinoma. *Br J Cancer* 2012; **106**: 1997-2003 [PMID: 22596232 DOI: 10.1038/bjc.2012.145]

62 **O'Connor JP**, Jackson A, Parker GJ, Jayson GC. DCE-MRI biomarkers in the clinical evaluation of antiangiogenic and vascular disrupting agents. *Br J Cancer* 2007; **96**: 189-195 [PMID: 17211479 DOI: 10.1038/sj.bjc.6603515]

63 **Hsu CY**, Shen YC, Yu CW, Hsu C, Hu FC, Hsu CH, Chen BB, Wei SY, Cheng AL, Shih TT. Dynamic contrast-enhanced magnetic resonance imaging biomarkers predict survival and response in hepatocellular carcinoma patients treated with sorafenib and metronomic tegafur/uracil. *J Hepatol* 2011; **55**: 858-865 [PMID: 21338641 DOI: 10.1016/j.jhep.2011.01.032]

64 **Khan MA**, Combs CS, Brunt EM, Lowe VJ, Wolverson MK, Solomon H, Collins BT, Di Bisceglie AM. Positron emission tomography scanning in the evaluation of hepatocellular carcinoma. *J Hepatol* 2000; **32**: 792-797 [PMID: 10845666]

65 **Trojan J**, Schroeder O, Raedle J, Baum RP, Herrmann G, Jacobi V, Zeuzem S. Fluorine-18 FDG positron emission tomography for imaging of hepatocellular carcinoma. *Am J Gastroenterol* 1999; **94**: 3314-3319 [PMID: 10566736 DOI: 10.1111/j.1572-0241.1999.01544.x]

66 **Seo S**, Hatano E, Higashi T, Hara T, Tada M, Tamaki N, Iwaisako K, Ikai I, Uemoto S. Fluorine-18 fluorodeoxyglucose positron emission tomography predicts tumor differentiation, P-glycoprotein expression, and outcome after resection in hepatocellular carcinoma. *Clin Cancer Res* 2007; **13**: 427-433 [PMID: 17255262 DOI: 10.1158/1078-0432.CCR-06-1357]

67 **Shiomi S**, Nishiguchi S, Ishizu H, Iwata Y, Sasaki N, Tamori A, Habu D, Takeda T, Kubo S, Ochi H. Usefulness of positron emission tomography with fluorine-18-fluorodeoxyglucose for predicting outcome in patients with hepatocellular carcinoma. *Am J Gastroenterol* 2001; **96**: 1877-1880 [PMID: 11419843 DOI: 10.1111/j.1572-0241.2001.03888.x]

68 **Lee JH**, Park JY, Kim do Y, Ahn SH, Han KH, Seo HJ, Lee JD, Choi HJ. Prognostic value of 18F-FDG PET for hepatocellular carcinoma patients treated with sorafenib. *Liver Int* 2011; **31**: 1144-1149 [PMID: 21745288 DOI: 10.1111/j.1478-3231.2011.02541.x]

69 **Jonker DJ**, O'Callaghan CJ, Karapetis CS, Zalcberg JR, Tu D, Au HJ, Berry SR, Krahn M, Price T, Simes RJ, Tebbutt NC, van Hazel G, Wierzbicki R, Langer C, Moore MJ. Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007; **357**: 2040-2048 [PMID: 18003960 DOI: 10.1056/NEJMoa071834]

70 **Van Cutsem E**, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 1408-1417 [PMID: 19339720 DOI: 10.1056/NEJMoa0805019]

71 **Estfan B**, Byrne M, Kim R. Sorafenib in advanced hepatocellular carcinoma: hypertension as a potential surrogate marker for efficacy. *Am J Clin Oncol* 2013; **36**: 319-324 [PMID: 22547010 DOI: 10.1097/COC.0b013e3182468039]

72 **Otsuka T**, Eguchi Y, Kawazoe S, Yanagita K, Ario K, Kitahara K, Kawasoe H, Kato H, Mizuta T. Skin toxicities and survival in advanced hepatocellular carcinoma patients treated with sorafenib. *Hepatol Res* 2012; **42**: 879-886 [PMID: 22469363 DOI: 10.1111/j.1872-034X.2012.00991.x]

73 **Lee S**, Kim BK, Kim SU, Park SY, Kim JK, Lee HW, Park JY, Kim do Y, Ahn SH, Tak WY, Kweon YO, Lee JI, Lee KS, Kim HJ, Han KH. Clinical outcomes and prognostic factors of patients with advanced hepatocellular carcinoma treated with sorafenib as first-line therapy: a Korean multicenter study. *J Gastroenterol Hepatol* 2014; **29**: 1463-1469 [PMID: 25273508 DOI: 10.1111/jgh.12542]

74 **Vincenzi B**, Santini D, Russo A, Addeo R, Giuliani F, Montella L, Rizzo S, Venditti O, Frezza AM, Caraglia M, Colucci G, Del Prete S, Tonini G. Early skin toxicity as a predictive factor for tumor control in hepatocellular carcinoma patients treated with sorafenib. *Oncologist* 2010; **15**: 85-92 [PMID: 20051477 DOI: 10.1634/theoncologist.2009-0143]

75 **Laurent-Puig P**, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F, Monges G, Thomas G, Bioulac-Sage P, Zucman-Rossi J. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. *Gastroenterology* 2001; **120**: 1763-1773 [PMID: 11375957]

76 **Lambert MP**, Paliwal A, Vaissière T, Chemin I, Zoulim F, Tommasino M, Hainaut P, Sylla B, Scoazec JY, Tost J, Herceg Z. Aberrant DNA methylation distinguishes hepatocellular carcinoma associated with HBV and HCV infection and alcohol intake. *J Hepatol* 2011; **54**: 705-715 [PMID: 21146512 DOI: 10.1016/j.jhep.2010.07.027]

77 **Ura S**, Honda M, Yamashita T, Ueda T, Takatori H, Nishino R, Sunakozaka H, Sakai Y, Horimoto K, Kaneko S. Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. *Hepatology* 2009; **49**: 1098-1112 [PMID: 19173277 DOI: 10.1002/hep.22749]

78 **Shao YY**, Shau WY, Chan SY, Lu LC, Hsu CH, Cheng AL. Treatment efficacy differences of sorafenib for advanced hepatocellular carcinoma: a meta-analysis of randomized clinical trials. *Oncology* 2015; **88**: 345-352 [PMID: 25572912 DOI: 10.1159/000369559]

79 **Arao T**, Ueshima K, Matsumoto K, Nagai T, Kimura H, Hagiwara S, Sakurai T, Haji S, Kanazawa A, Hidaka H, Iso Y, Kubota K, Shimada M, Utsunomiya T, Hirooka M, Hiasa Y, Toyoki Y, Hakamada K, Yasui K, Kumada T, Toyoda H, Sato S, Hisai H, Kuzuya T, Tsuchiya K, Izumi N, Arii S, Nishio K, Kudo M. FGF3/FGF4 amplification and multiple lung metastases in responders to sorafenib in hepatocellular carcinoma. *Hepatology* 2013; **57**: 1407-1415 [PMID: 22890726 DOI: 10.1002/hep.25956]

80 **Chochi Y**, Kawauchi S, Nakao M, Furuya T, Hashimoto K, Oga A, Oka M, Sasaki K. A copy number gain of the 6p arm is linked with advanced hepatocellular carcinoma: an array-based comparative genomic hybridization study. *J Pathol* 2009; **217**: 677-684 [PMID: 19097070 DOI: 10.1002/path.2491]

81 **Takeo S**, Arai H, Kusano N, Harada T, Furuya T, Kawauchi S, Oga A, Hirano T, Yoshida T, Okita K, Sasaki K. Examination of oncogene amplification by genomic DNA microarray in hepatocellular carcinomas: comparison with comparative genomic hybridization analysis. *Cancer Genet Cytogenet* 2001; **130**: 127-132 [PMID: 11675133]

82 **Nishida N**, Fukuda Y, Komeda T, Kita R, Sando T, Furukawa M, Amenomori M, Shibagaki I, Nakao K, Ikenaga M. Amplification and overexpression of the cyclin D1 gene in aggressive human hepatocellular carcinoma. *Cancer Res* 1994; **54**: 3107-3110 [PMID: 8205525]

83 **Ahn SM**, Jang SJ, Shim JH, Kim D, Hong SM, Sung CO, Baek D, Haq F, Ansari AA, Lee SY, Chun SM, Choi S, Choi HJ, Kim J, Kim S, Hwang S, Lee YJ, Lee JE, Jung WR, Jang HY, Yang E, Sung WK, Lee NP, Mao M, Lee C, Zucman-Rossi J, Yu E, Lee HC, Kong G. Genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. *Hepatology* 2014; **60**: 1972-1982 [PMID: 24798001 DOI: 10.1002/hep.27198]

84 **Wang K**, Lim HY, Shi S, Lee J, Deng S, Xie T, Zhu Z, Wang Y, Pocalyko D, Yang WJ, Rejto PA, Mao M, Park CK, Xu J. Genomic landscape of copy number aberrations enables the identification of oncogenic drivers in hepatocellular carcinoma. *Hepatology* 2013; **58**: 706-717 [PMID: 23505090 DOI: 10.1002/hep.26402]

85 **Horwitz E**, Stein I, Andreozzi M, Nemeth J, Shoham A, Pappo O, Schweitzer N, Tornillo L, Kanarek N, Quagliata L, Zreik F, Porat RM, Finkelstein R, Reuter H, Koschny R, Ganten T, Mogler C, Shibolet O, Hess J, Breuhahn K, Grunewald M, Schirmacher P, Vogel A, Terracciano L, Angel P, Ben-Neriah Y, Pikarsky E. Human and mouse VEGFA-amplified hepatocellular carcinomas are highly sensitive to sorafenib treatment. *Cancer Discov* 2014; **4**: 730-743 [PMID: 24687604 DOI: 10.1158/2159-8290.CD-13-0782]

86 **Rudalska R**, Dauch D, Longerich T, McJunkin K, Wuestefeld T, Kang TW, Hohmeyer A, Pesic M, Leibold J, von Thun A, Schirmacher P, Zuber J, Weiss KH, Powers S, Malek NP, Eilers M, Sipos B, Lowe SW, Geffers R, Laufer S, Zender L. In vivo RNAi screening identifies a mechanism of sorafenib resistance in liver cancer. *Nat Med* 2014; **20**: 1138-1146 [PMID: 25216638 DOI: 10.1038/nm.3679]

87 **Ou DL**, Shyue SK, Lin LI, Feng ZR, Liou JY, Fan HH, Lee BS, Hsu C, Cheng AL. Growth arrest DNA damage-inducible gene 45 gamma expression as a prognostic and predictive biomarker in hepatocellular carcinoma. *Oncotarget* 2015; Epub ahead of print [PMID: 26172295]

88 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

89 **Shao YY**, Chen CL, Ho MC, Huang CC, Tu HC, Hsu CH, Cheng AL. Dissimilar immunohistochemical expression of ERK and AKT between paired biopsy and hepatectomy tissues of hepatocellular carcinoma. *Anticancer Res* 2012; **32**: 4865-4870 [PMID: 23155253]

90 **Shao YY**, Lu LC, Lin ZZ, Hsu C, Shen YC, Hsu CH, Cheng AL. Prognosis of advanced hepatocellular carcinoma patients enrolled in clinical trials can be classified by current staging systems. *Br J Cancer* 2012; **107**: 1672-1677 [PMID: 23059748 DOI: 10.1038/bjc.2012.466]

91 **Lu LC**, Shao YY, Chan SY, Hsu CH, Cheng AL. Clinical characteristics of advanced hepatocellular carcinoma patients with prolonged survival in the era of anti-angiogenic targeted-therapy. *Anticancer Res* 2014; **34**: 1047-1052 [PMID: 24511053]

92 **Shao YY**, Wu CH, Lu LC, Chan SY, Ma YY, Yen FC, Hsu CH, Cheng AL. Prognosis of patients with advanced hepatocellular carcinoma who failed first-line systemic therapy. *J Hepatol* 2014; **60**: 313-318 [PMID: 24036008 DOI: 10.1016/j.jhep.2013.08.027]

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