

Review

Predictive Biomarkers of Antiangiogenic Therapy for Advanced Hepatocellular Carcinoma: Where Are We?

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Antiangiogenic therapy · Hepatocellular carcinoma · Predictive marker · Prognosis

Abstract

Antiangiogenic therapy, especially treatment with sorafenib, is the primary treatment for patients with advanced hepatocellular carcinoma (HCC). However, the efficacy of such therapy is modest, with low objective response rates and limited prolongation of survival times. Several researchers have investigated predictive biomarkers to help identify patients who can benefit most from antiangiogenic therapy. The largest study on this topic to date was based on the pivotal phase III study of sorafenib (the SHARP study) and did not find any plasma markers that could predict the efficacy of sorafenib. Other studies based on single-arm phase II clinical trials found some potential predictive markers, such as early alpha-fetoprotein response, the serum insulin-like growth factor-1 level at baseline, and the volume transfer constants of dynamic contrast-enhanced magnetic resonance imaging. These findings require validation by further studies. Identifying predictive biomarkers of antiangiogenic therapy for HCC remains challenging and warrants further investigations.

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Introduction

Advanced hepatocellular carcinoma (HCC), defined as metastatic or locally advanced disease not amenable to locoregional therapies such as surgery [1], local ablation [2], or transcatheter arterial chemoembolization (TACE) [3] is notorious for its extremely poor prognosis. Patients who received best supportive care for advanced HCC had a median overall survival (OS) of only 4.2 months in East Asia and 7.9 months in Western countries [4, 5]. Because HCC is refractory to cytotoxic chemotherapy [6–8], it has for decades been a disease with no proven therapy offering survival benefits.

Since 2007, sorafenib has become the standard of care for patients with advanced HCC. Two large randomized, placebo-controlled, double-blind phase III clinical trials have shown that sorafenib, compared to a placebo, significantly improved the OS of patients with advanced HCC [4, 5]. Sorafenib is a multi-kinase inhibitor of several cellular signaling pathways, including the vascular endothelial growth factor (VEGF) pathway [via the inhibition of VEGF receptors (VEGFR)] and the mitogen-activated protein kinase (MAPK) pathway (via Raf inhibition) [9]. Based on the success of sorafenib, several other targeted agents (e.g., bevacizumab and sunitinib) that produce antiangiogenic activity by inhibiting the VEGF/VEGFR pathway or other angiogenic pathways were also found to have clinical activities against HCC [10–14]. Thus, the treatment of advanced HCC has evolved into the era of antiangiogenic therapy.

However, the clinical effects of sorafenib and other antiangiogenic agents against HCC are modest. According to Response Evaluation Criteria In Solid Tumors (RECIST) [15], the objective tumor response rate to sorafenib for advanced HCC is only 2–3%; the disease stabilization rate for sorafenib is approximately 34–43%, and survival prolongation by sorafenib is no more than 3 months [4, 5]. It is imperative to find biomarkers that predict the efficacies of sorafenib and other antiangiogenic therapies for HCC. These markers could help identify the minority of patients with advanced HCC who are likely to benefit from antiangiogenic therapies, and identify the majority of patients who are unlikely to benefit from such treatments. To date, clinically useful predictive biomarkers of sorafenib and other antiangiogenic therapies for HCC remain undefined.

This review article presents a summary of the current understanding of predictive markers for antiangiogenic agents, especially for sorafenib, in advanced HCC. To avoid confusion, we exclude studies based on HCC of all stages (i.e., those not focusing on advanced HCC) and those based on patients receiving only cytotoxic chemotherapy for advanced HCC. Tables 1 and 2 present the key findings of the studies discussed in this review.

Prognostic and Predictive Markers

The characteristics of patients and their biospecimens can act as prognostic or predictive markers when they are significantly correlated with patient outcomes. Prognostic markers identify survival differences in a specific group of patients regardless of treatment [16]. A prognostic marker does not necessarily guide treatment because patients classified with a poor prognosis may still benefit from treatment. Conversely, predictive markers identify outcome differences as a result of certain treatments [16]. The outcomes of cancer therapies can be measured as objective tumor responses or survival benefits. Comparative studies involving a treatment group and a reference or control group of patients are required to find a predictive marker for survival benefits from a specific treatment. Otherwise, a marker associated with poor survival may be a prognostic marker but not necessarily a predictive marker for treatment.

Table 1. Studies on predictive and prognostic serum and plasma markers for advanced HCC

	Authors	Treatment	Results		
			Predictive markers	Prognostic markers	Others
Serum/plasma angiogenic factors					
VEGF-A	Llovet et al. [18]	Sorafenib vs. placebo	No predictive value	Low VEGF-A → better OS	
	Shao et al. [19]	Sorafenib plus UFT	No predictive value	Low VEGF-A → better OS	
	Kaseb et al. [20]	Varied	—	Low VEGF-A → better OS	
	Miyahara et al. [21]	Sorafenib	No predictive value	No prognostic value	
	Boige et al. [10]	Bevacizumab	No predictive value	No prognostic value	
	Siegel et al. [14]	Bevacizumab	No predictive value	No prognostic value	
IL-6 and IL-8	Boige et al. [10]	Bevacizumab	Low IL-8 → disease control	High IL-6 and IL-8 → poor OS	High IL-6 and IL-8 → poor PFS
	Zhu et al. [12]	Sunitinib	High IL-6 and IL-8 → poor TTP	High IL-6 and IL-8 → poor OS	
	Shao et al. [19]	Sorafenib plus UFT	—	High IL-6 and IL-8 → poor OS	High IL-6 and IL-8 → poor PFS
Ang2	Llovet et al. [18]	Sorafenib vs. placebo	No predictive value	Low Ang2 → better OS	
	Miyahara et al. [18]	Sorafenib			
Other serum/plasma factors					
IGF-1	Shao et al. [32]	Sorafenib plus UFT or bevacizumab plus capecitabine	High IGF-1 → better DCR	High IGF-1 → better OS	High IGF-1 → better PFS
AFP response	Shao et al. [47]	Various antiangiogenic therapies	AFP response → better ORR, DCR	AFP response → better OS	AFP response → better PFS
	Personeni et al., Yau et al., and Kuzuya et al. [48–50]	Sorafenib	AFP response → better DCR	AFP response → better OS	AFP response → better PFS
Circulating endothelial cells/progenitors	Shao et al. [61]	Sorafenib plus UFT	Increase in post-treatment total CEC or viable CEC → progressive disease*	High CEP → poorer OS	High CEP → poorer PFS
	Boige et al. [10]	Bevacizumab	High or increased post-treatment total CEC → better ORR or DCR	No prognostic value	
	Zhu et al. [12]	Sunitinib	Increase in post-treatment CEP levels → increased risk of disease progression	No prognostic value	

* Borderline significant. UFT = tegafur/uracil.

There are inherent difficulties in identifying predictive biomarkers of sorafenib and other antiangiogenic agents for advanced HCC. Following two large placebo-controlled, randomized, phase III studies of sorafenib, several large randomized phase III studies comparing new compounds to sorafenib have been unsuccessful to improve OS. Most studies incorporating biomarker research in advanced HCC are single arm and small scale. The objective tumor response rates of sorafenib and other antiangiogenic agents in HCC are so low that these small studies are not robust enough to identify markers with consistent and significant predictive power.

Because of the low objective tumor response rates of sorafenib and other antiangiogenic agents for advanced HCC, many researchers have turned to other measurements to determine treatment efficacies. The disease control rate (DCR), defined as the percentage of objective tumor response and disease stabilization, commonly appears in clinical studies on advanced HCC. The time to tumor progression (TTP), defined as the interval from the start of treatment to when radiologic tumor progression is documented, has been proposed as a proper endpoint in clinical trials of interventional studies on HCC [17]. Unfortunately, both DCR and TTP are based on the non-progression of the targeted tumor lesions, which could be affected by less aggressive tumor behavior rather than the effectiveness of the investigational drugs. Many studies use progression-free survival (PFS), defined as the interval from the start of treatment to when either tumor progression or patient death occurs, to gauge the efficacy of HCC treatment. As an efficacy endpoint, PFS is heavily skewed when non-tumor-related death occurs. This commonly occurs in patients with advanced HCC, who frequently suffer from complications related to liver cirrhosis and chronic liver disease.

Despite these difficulties and limitations, many studies have attempted to identify biomarkers that either predict the efficacy of antiangiogenic therapy or are associated with the prognosis of patients who receive antiangiogenic therapy for advanced HCC. The following sections present the potential predictive or prognostic markers reported in the recent literature.

Serum/plasma Angiogenic Factors

Vascular Endothelial Growth Factor-A (VEGF-A)

VEGF-A, the most potent pro-angiogenic factor of tumor neovascularization and angiogenesis, has been the prime target in the development of antiangiogenic therapy as cancer treatment. All currently approved antiangiogenic agents, including sorafenib, have the ability to inhibit the VEGF-A/VEGFR pathway. Therefore, it is reasonable to assume predictive and prognostic values for the serum or plasma VEGF-A level in the antiangiogenic therapy of advanced HCC.

Based on the SHARP study, which tested sorafenib as first-line therapy for advanced HCC, Llovet et al. found that a low baseline plasma VEGF-A concentration was associated with better OS, both in patients who received a placebo and in the entire cohort [18]. The prognostic value of low baseline plasma VEGF-A concentration was independent of other clinicopathologic variables. However, comparing the OS of the placebo group and the treatment group showed that the VEGF-A level was not associated with sorafenib efficacy. Based on a single-arm phase II study testing the combination of sorafenib and metronomic tegafur/uracil in advanced HCC, Shao et al. found that low plasma VEGF-A predicted better OS, but was not associated with DCR or PFS [19]. Kaseb et al. analyzed patients with advanced HCC treated with supportive care only or with diverse chemotherapy regimens and confirmed the prognostic role of the plasma VEGF-A level [20].

Table 2. Studies of predictive and prognostic markers, other than serum or plasma markers, for advanced HCC

	Authors	Treatment	Results		
			Predictive markers	Prognostic markers	Others
Tumor characteristics					
Phospho-ERK expression	Abou-Alfa et al. [71]	Sorafenib	High p-ERK → longer TTP	—	
	Ozenne et al. [72]	Sorafenib	No predictive value	—	
Phospho-c-Jun expression	Hagiwara et al. [73]	Sorafenib	Phospho-c-Jun expression → poor response, TTP	Phospho-c-Jun expression → poor OS	
Functional imaging					
DCE-MRI	Hsu et al. [75]	Sorafenib plus UFT	High baseline K^{trans} or decreased K^{trans} after treatment → DCR	Vascular response ^a → better OS	Vascular response ^a → better PFS
Positron emission tomography	Lee et al. [83]	Sorafenib	—	Low SUV → better OS	Low SUV → better PFS
Treatment side effects					
Hypertension	Estfan et al. [87]	Sorafenib	Hypertension → better TTP (?) ^b	Hypertension → better OS	
	Otsuka et al. [88]	Sorafenib	No predictive value	No prognostic value	
Skin toxicity	Otsuka et al. [88]	Sorafenib	No predictive value	Skin toxicity → better OS	
	Vincenzi et al. [89]	Sorafenib	Early ^c skin toxicity → better DCR and TTP	Early skin toxicity → better OS ^d	

a=defined as $\geq 40\%$ decrease in K^{trans} after treatment; b=statistical values of the comparison not reported; c=within the first month of treatment; d=borderline statistical significance.

However, a small study of 30 patients who received sorafenib for advanced HCC showed no association between the serum VEGF-A level and PFS or OS [21]. Two phase II studies testing bevacizumab as first-line treatment for advanced HCC found a post-treatment decrease in the levels of plasma VEGF-A [10, 14]. However, neither study found an association between plasma VEGF-A levels and patient survival outcomes.

These findings show that plasma VEGF-A levels may be a prognostic marker for patients with advanced HCC. However, the current data fail to show a correlation between plasma VEGF-A levels and the efficacy of sorafenib or other antiangiogenic therapies in advanced HCC. The post-treatment plasma VEGF-A level might serve as a pharmacodynamic marker for inhibitors of the VEGF-A/VEGFR pathway; however, the predictive or prognostic role of plasma VEGF-A levels in advanced HCC patients treated with antiangiogenic therapy remains unclear.

Interleukin (IL)-6 and IL-8

IL-6 and IL-8 are key inflammatory response mediators that can promote angiogenesis [22, 23]. In colorectal cancer, high plasma IL-6 and IL-8 levels may predict a poor tumor response to bevacizumab-containing chemotherapy regimens [24]. For advanced HCC, based on

a phase II trial testing bevacizumab, Boige et al. showed that high baseline plasma IL-6 and IL-8 levels were associated with poor PFS and OS [10]. They also found that low baseline plasma IL-8 predicted disease control.

Two other phase II clinical trials of advanced HCC investigated the significance of plasma IL-6 and IL-8 levels. Zhu et al. analyzed patients treated with sunitinib, and Shao et al. analyzed patients treated with sorafenib combined with metronomic tegafur/uracil [12, 19]. Although both studies found that high baseline plasma IL-6 or IL-8 levels were associated with poor OS, they found no association between DCR and plasma IL-6 or IL-8 levels [12, 19].

The consistent association of plasma IL-6 and IL-8 levels with the OS of patients with advanced HCC implies their prognostic roles, regardless of the treatment regimen. Conversely, only the study testing bevacizumab found some potential predictive value based on the correlation with DCR and PFS. The predictive value of plasma IL-6 or IL-8 levels in patients treated with sorafenib or other antiangiogenic therapies remain to be investigated.

Angiopoietin-2 (Ang2)

Ang2, by interacting with its receptor Tie2, cooperates with the VEGF/VEGFR pathway to maintain normal physiologic functions. In the presence of VEGF, Ang2 destabilizes blood vessels and promotes vascular sprouting [25]. In cancers, Ang2 is linked not only to angiogenesis but also to invasive and metastatic phenotypes [25]. Sorafenib has no significant activity against Tie2 [9].

Based on the SHARP study comparing sorafenib versus a placebo as first-line therapy for advanced HCC, Llovet et al. found that high baseline plasma Ang2 levels independently predicted poorer OS in both the sorafenib group and the placebo group [18]. However, the analysis of the interaction between the prognostic value of Ang2 and sorafenib treatment showed that Ang2 could not predict the efficacy of sorafenib. Conversely, based on 30 patients who received sorafenib for advanced HCC, Miyahara et al. found that high levels of Ang2 predicted poorer PFS [21].

Overall, these results indicate that the plasma Ang2 level is a prognostic factor for patients with advanced HCC. However, the plasma Ang2 level does not predict the efficacy of sorafenib in advanced HCC.

VEGF-C

Sunitinib, a kinase inhibitor against multiple receptors involved in angiogenesis, including VEGFR-2 and VEGFR-3, has been examined for its efficacy against advanced HCC. Based on a phase II study enrolling 37 patients who received sunitinib as first-line therapy for advanced HCC, Faivre et al. found that patients with high baseline plasma levels of VEGF-C, the ligand of VEGFR-3, were more likely to have disease control [13].

Sorafenib has limited efficacy against VEGFR-3 [9]. Based on a study of 64 patients treated with sorafenib and metronomic tegafur/uracil, Shao et al. failed to find any association between VEGF-C levels and treatment outcomes. Therefore, the value of VEGF-C levels as predictive or prognostic markers for sorafenib appears to be limited.

Other Serum/Plasma Factors

Insulin-like growth factor (IGF)-1

IGF signaling pathway is a key regulator of energy metabolism and growth [26]. This pathway plays an important role in the carcinogenesis of many cancers. Neoplastic tissues frequently express the ligands of the pathway, IGF-1 and IGF-2 [26]. The activation of IGF-1

signaling promotes mitogenesis and inhibits apoptosis in cancer cells [26]. The vast majority of IGF-1 and IGF-2 are synthesized by the liver [27]. Thus, IGF-1 levels appear to be associated with liver reserves. Low serum IGF-1 levels are associated with extensive liver involvement and vascular invasion in patients with HCC [28, 29].

Based on patients with all-stage HCC, Kaseb et al. found that lower plasma IGF-1 levels are significantly correlated with poor OS. They also found that incorporating plasma IGF-1 levels into HCC staging, such as the Barcelona Clinic Liver Cancer staging system, can significantly enhance the prognostic stratification of patients [30, 31]. However, based on patients enrolled in two phase II clinical trials testing first-line antiangiogenic therapy for advanced HCC, Shao et al. found that serum IGF-1 levels may be more than a prognostic marker [32]. Their study showed that high pre-treatment serum levels of IGF-1 were associated with not only better OS, but also improved DCR and PFS. Although no control arm was available in the study, the vast difference in DCR implies the potential of IGF-1 as a predictive marker for the treatment response of antiangiogenic therapy for advanced HCC. This result requires validation in large-scale studies and further exploration of its underlying mechanisms.

Conversely, blood IGF-2 levels have little association with the treatment efficacy of sorafenib for advanced HCC. Llovet et al., based on a phase III study testing sorafenib as first-line therapy for advanced HCC, did not find an association between IGF-2 levels and treatment outcomes [18]. The aforementioned study by Shao et al. also failed to find any such association [32].

Alpha-fetoprotein (AFP)

AFP is a glycoprotein expressed by HCC and secreted into the blood of approximately 70% of HCC patients. Thus, the blood AFP level is useful for early detection and differential diagnosis of HCC [33]. For patients who undergo curative hepatectomy for localized HCC, the AFP level is also helpful for recurrence detection and is associated with prognosis [34–37].

Whether the pre-treatment baseline AFP level is a prognostic or even a predictive marker for patients with advanced HCC receiving antiangiogenic therapy remains unclear. Whereas some studies have shown that the pre-treatment AFP level is associated with patient prognosis for advanced HCC [18, 38–41], other studies have shown no such association [32, 42]. Shim et al. found that a high pre-treatment AFP level (≥ 400 ng/ml) predicted shorter TTP [43], but they did not analyze the association between the baseline AFP level and objective response rates (ORR), DCR, or OS. Other studies failed to confirm the prediction of TTP based on the baseline AFP level [38, 39]. Overall, the pre-treatment baseline AFP level is likely a prognostic factor but not a predictive factor for patients with advanced HCC.

The post-treatment change of AFP level in patients with advanced HCC has gained much attention recently because it may predict the treatment response earlier than the scheduled imaging studies adopted in conventional tumor response assessments. Before the era of antiangiogenic therapy for advanced HCC, a few studies identified that a decline in the post-treatment AFP level was associated with the radiologic tumor response and with survival in patients receiving systemic therapy [44–46]. However, these studies were not in agreement on the definition of AFP response regarding the magnitude of AFP decline or the timing of AFP level evaluation.

After analyzing 72 advanced HCC patients enrolled in three phase II studies testing first-line antiangiogenic therapy, Shao et al. found that an early AFP response predicted the treatment response [47]. This study defined early AFP response as a $> 20\%$ decline from the baseline AFP level within 4 weeks of treatment initiation. Early AFP responders, compared with non-responders, had significantly improved ORR, DCR, PFS, and OS. These findings were confirmed in other studies with patients receiving sorafenib for advanced HCC, although the time of assessment of AFP response varied from 2 to 8 weeks in these studies [48–50].

The dynamic change of serum AFP level might represent tumor viability, thus making it a reasonable early marker of tumor response to interventions or treatment. Therefore, it is not a marker specific to sorafenib or to antiangiogenic therapy. Other caveats are that advanced HCC patients with normal baseline serum AFP levels cannot be assessed for AFP response, and the optimal definition of AFP response remains unclear. However, until researchers find useful pre-treatment markers with predictive power at the baseline, the early AFP response can be a good complement to help make early clinical decisions.

Other Serum/plasma factors

The largest study on predictive and prognostic markers for advanced HCC was based on a phase III clinical trial comparing sorafenib and placebo as first-line therapy. Llovet et al. found a trend that, although not statistically significant, patients with high serum c-Kit levels and a low serum hepatocyte growth factor (HGF) concentration at baseline had greater survival benefits from sorafenib. Sorafenib exhibits some activity against c-Kit but no activity against c-Met, the receptor for HGF. However, these findings have not yet been confirmed in other studies based on patients treated with sorafenib.

Circulating Endothelial Cells (CECs) / Circulating Endothelial Progenitors (CEPs)

CECs and CEPs are potential surrogate markers of angiogenesis activity. Increased numbers of CECs and CEPs appear in various physiologic and pathologic conditions in which angiogenesis plays a significant role [51]. Preclinical models have shown that antiangiogenic therapy suppressed viable CEPs and raised the number of apoptotic CECs [52–56]. Elevated CEC and CEP levels appeared in patients with HCC [57, 58], and were associated with tumor aggressiveness or advanced stage [58–60].

Despite the sound rationale of using CECs or CEPs to predict the efficacy of antiangiogenic therapy, the results of clinical studies have been conflicting and limited. One obstacle in using CECs or CEPs in clinical practice is that the enumeration of CECs and CEPs remains highly technique-dependent. The time from blood sampling to examination is crucial. Therefore, it is difficult to examine CEC or CEP levels in large clinical trials across continents.

Only three studies have attempted to investigate the predictive or prognostic values of CEC or CEP levels in patients undergoing antiangiogenic therapy for advanced HCC. Shao et al. evaluated 40 patients enrolled in a phase II study testing first-line combination therapy with sorafenib and metronomic tegafur/uracil [61]. This study found increasing trends in the levels of total CECs and viable CECs after 4 weeks of treatment in patients with progressive disease. No such trends appeared in patients who had disease control. A high baseline CEP level predicted poorer PFS or OS.

In a phase II study investigating bevacizumab as first-line therapy for advanced HCC, Boige et al. evaluated the significance of CECs. Their study showed that high or increased CEC counts at 15 days after the start of treatment were associated with better ORR or DCR [10]. However, the change in CEC count was not associated with either PFS or OS. CEPs were not examined in this study. Based on nine patients enrolled in a phase II study using sunitinib for advanced HCC, Zhu et al. found that an increase in post-treatment CEP levels was associated with an increased risk of disease progression [12].

The conflicting results of these three studies are not surprising, considering the limited number of patients involved and the diverse treatment regimens. Therefore, whether CEC or CEP levels can serve as a predictive or prognostic marker for antiangiogenic therapy against advanced HCC requires further research.

Tumor Characteristics

The characteristics of cancer cells, including protein expression or genetic alterations, are frequently used as predictive or prognostic markers in an increasing number of human cancers. The expression of estrogen receptors predicts the efficacy of hormone therapy for breast cancer; *EGFR* mutations in lung adenocarcinoma predict the efficacy of gefitinib and erlotinib [62, 63]. Similar studies are few for advanced HCC.

One of the main reasons is that the diagnosis of HCC does not necessarily require histologic examination [64]. Another possibility is that the methods of tissue procurement may have significant impact on the stability of certain markers and their expression levels. Shao et al. compared the immunohistochemical (IHC) staining results of paired HCC tissues acquired by pre-operative biopsy and by hepatectomy from the same patients. Although some markers such as p53 and beta-catenin resulted in similar expression levels between paired HCC tissues, the staining results of phospho-Akt and phospho-ERK showed marked dissimilarities [65]. This confounding factor needs to be taken into consideration when interpreting IHC-detected biomarker studies of HCC employing tissues procured by different methods.

Antiangiogenic therapy is currently the main treatment for advanced HCC, and several markers are associated with angiogenic activity. Microvessel density has been used as a surrogate to evaluate angiogenic activity [66–68]. The expression of VEGF-A, hypoxia inducible factor-1 α , and Ang2 can predict microvessel density [69, 70]. However, whether these angiogenic markers are useful for predicting the efficacy of sorafenib for advanced HCC remains unclear.

Because sorafenib not only inhibits angiogenesis-related pathways but also the MAPK pathway via 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. found that patients with tumors expressing high levels of phospho-ERK had a longer TTP [71]; however, another study did not find this association [72].

A study based on 39 patients treated with sorafenib for advanced HCC showed that the tumor expression of phospho-c-Jun (p-c-Jun) was associated with a poor tumor response and shorter TTP and OS [73]. p-c-Jun expression is associated with stem cell-like characteristics, as demonstrated by CD-133 expression; however, the mechanism underlying the association between p-c-Jun and the efficacy of sorafenib remains unclear.

Studies employing pre-treatment HCC tissues to analyze for protein marker expression and/or genetic alterations in tumor cells as predictive and prognostic markers for advanced HCC are relatively few. This dearth warrants further studies evaluating the markers of angiogenic activity in pre-treatment HCC tissues of patients treated with sorafenib or other antiangiogenic agent.

Functional Imaging

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI)

Because substantial anatomic tumor shrinkage rarely occurs after antiangiogenic therapy for advanced HCC, investigators are keen to find other methods of evaluating antiangiogenic therapy. DCE-MRI measures changes in tumor blood flow, vascular permeability, and interstitial and intravascular volumes [74]. Therefore, DCE-MRI has gained considerable interest for its potential predictive and prognostic values for antiangiogenic therapy for HCC.

Based on the patient cohort of a phase II study testing sorafenib combined with metronomic tegafur/uracil as first-line therapy for advanced HCC [11], Hsu et al. performed DCE-

MRI before treatment and after 14 days of treatment [75]. They selected the most contrast-enhanced region of the tumor and measured the volume transfer constant, K^{trans} . Compared to patients with progressive disease, patients with disease control had a significantly higher baseline K^{trans} , which significantly decreased after treatment. A vascular response, defined as $\geq 40\%$ decreased in K^{trans} after treatment, was associated with improved PFS and OS. Another study examining sunitinib as treatment for advanced HCC found similar results. Zhu et al. found a significant decrease in K^{trans} in all patients, but the extent of the decrease was significantly greater in patients who had disease control compared to those with progressive disease.

These two studies demonstrated compatible results, supporting the potential use of DEC-MRI or other functional imaging studies as predictive and/or prognostic markers of antiangiogenic therapy for advanced HCC. Further studies are warranted to confirm these findings.

Positron emission tomography (PET)

^{18}F -fluorodeoxyglucose (FDG)-PET can be used to evaluate the increased uptake and accumulation of radiolabeled glucose as a surrogate of viable malignancies. Although FDG-PET can help with staging of several cancers, its sensitivity for HCC diagnosis is not sufficiently high (50–55%) [76, 77]. However, for patients with HCC that avidly takes up labeled glucose, FDG-PET may predict prognosis after resection [78–80]. In patients with unresectable HCC receiving a hepatic arterial infusion of chemotherapy or transcatheter arterial chemoembolization, several studies have shown the potential role of FDG-PET in predicting prognosis [81, 82].

Only one study has examined FDG-PET in patients who received antiangiogenic therapy for advanced HCC. Based on 29 patients treated with sorafenib, Lee et al. found that low pre-treatment standardized uptake values (SUV) predicted improved PFS and OS, but not DCR [83]. This early result requires further confirmation.

Treatment Side Effects

The occurrence of treatment-related side effects can be a pharmacokinetic and pharmacodynamic marker. Many studies have attempted to investigate whether the occurrence of certain treatment side effects can predict treatment efficacy. For example, a skin rash is associated with the efficacy of cetuximab in patients with metastatic colorectal cancer [84, 85]. Several studies have evaluated whether the occurrence of sorafenib-specific side effects are correlated with its efficacy.

Hypertension

Hypertension is a class-specific toxicity for antiangiogenic therapy and is associated with the efficacy of bevacizumab-containing chemotherapy for colorectal cancer [86]. Based on a retrospective review of 41 patients who received sorafenib for advanced HCC, Estfan et al. found that documented hypertension during treatment was associated with OS, regardless of the baseline blood pressure status [87]. However, another retrospective study based on patients treated with sorafenib for advanced HCC failed to identify associations between hypertension and DCR, PFS, or OS [88].

Skin toxicities

Skin toxicities, including hand-foot skin reactions and rashes, are relatively common in patients undergoing sorafenib treatment. In Western countries, approximately 20% of patients receiving sorafenib for advanced HCC experience skin toxicities [4]. In East Asia, the occurrence rate is higher: approximately 45% of patients may develop hand-foot skin reactions [5]. In a retrospective study, Otsuka et al. found that the occurrence of skin toxicities during sorafenib treatment for advanced HCC was associated with improved OS, but not with DCR or TTP [88]. A retrospective analysis of skin toxicities during the whole treatment period may be 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. prospectively examined 65 patients who received sorafenib for advanced HCC by evaluating and scoring early skin toxicities (within the first month of treatment) [89]. Early all-grade skin toxicities predicted significantly improved DCR and TTP, and prolonged OS with borderline significance.

Because of their association with pharmacodynamic effects, treatment-specific side effects may be used to predict the treatment efficacy of sorafenib and other antiangiogenic agents in patients with advanced HCC. However, prospective studies with well-controlled treatment schedules and toxicity evaluation intervals are warranted to verify this concept.

Genetic Polymorphisms

Genetic polymorphisms of VEGF-A, one of the key regulators of angiogenesis, have been associated with survival after the resection of early cancers, including colon cancer, non-small-cell lung cancer, breast cancer, and HCC [90–93]. For patients with colon cancer who received bevacizumab-containing chemotherapy, VEGF-A polymorphisms may also predict the efficacy of bevacizumab [94, 95].

No study has investigated VEGF-A polymorphisms in HCC patients treated with sorafenib. Only one study based on a phase III clinical trial comparing sorafenib and axitinib for advanced renal cell carcinoma examined VEGF-A polymorphism. However, this study found no association between VEGF-A polymorphisms and sorafenib efficacy [96]. Because sorafenib targets the VEGF receptor, not VEGF itself, investigators should consider focusing on VEGFR polymorphisms rather than VEGF-A polymorphisms.

Summary

No predictive markers for the efficacy of antiangiogenic therapy for advanced HCC have yet been confirmed. The largest biomarker study of antiangiogenic therapy for HCC evaluated ten plasma angiogenic factors incorporated in the pivotal phase III study of sorafenib (i.e., the SHARP study). Unfortunately, none of the tested biomarkers significantly predicted the response to sorafenib. Small-scale single-arm studies have found that an early AFP response, the baseline IGF-1 level, and DCE-MRI findings showed some promise as potential predictive markers of antiangiogenic therapy for patients with advanced HCC. However, these preliminary data require validation by large studies.

The heterogeneous etiology of advanced HCC is another challenge for studies on predictive markers. The various etiologic factors of HCC, including hepatitis B virus infection, hepatitis C virus infection, and alcoholic liver cirrhosis, can lead to different carcinogenesis process-

es and may influence different signaling pathways. This confounding factor may complicate studies attempting to find predictors of treatment outcomes.

Other than the different etiologies, advanced HCC is a heterogeneous disease by definition. Because advanced HCC is defined as a disease not amenable to locoregional therapy, it may include metastatic diseases, localized diseases failing prior locoregional therapy, and locally advanced diseases involving major vessels. Several clinical trials have shown that even in patients with advanced HCC, staging systems such as the Cancer of the Liver Italian Program (CLIP) score can still differentiate different prognosis [39, 97]. These findings should be considered in clinical trial designs and biomarker studies.

Despite these many difficulties, the search for a useful predictive marker for the efficacy of antiangiogenic therapy for HCC should continue. Such predictive markers could help physicians form improved treatment decisions and shed light on the future development of targeted therapy for advanced HCC. International cooperation is the key to success because large patient numbers are necessary to validate these predictive and prognostic markers.

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References

- 1 Belghiti J, Fuks D, et al: Liver resection and transplantation in hepatocellular carcinoma. *Liver Cancer* 2012;1:71–82.
- 2 Lin S, Hoffmann K, Schemmer P, et al: Treatment of hepatocellular carcinoma: a systematic review. *Liver Cancer* 2012;1:144–158.
- 3 Lencioni R, et al: Chemoembolization in patients with hepatocellular carcinoma. *Liver Cancer* 2012;1:41–50.
- 4 Llovet JM, Ricci S, Mazzaferro V, et al: Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008;359:378–390.
- 5 Cheng AL, Kang YK, Chen Z, et al: 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.
- 6 Llovet JM, Bruix J: Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003;37:429–442.
- 7 Lopez PM, Villanueva A, Llovet JM: Systematic review: Evidence-based management of hepatocellular carcinoma – an updated analysis of randomized controlled trials. *Aliment Pharmacol Ther* 2006;23:1535–1547.
- 8 Yeo W, Mok TS, Zee B, et al: A randomized phase III study of doxorubicin versus cisplatin/interferon alpha-2b/doxorubicin/fluorouracil (PIAF) combination chemotherapy for unresectable hepatocellular carcinoma. *J Natl Cancer Inst* 2005;97:1532–1538.
- 9 Wilhelm S, Carter C, Lynch M, et al: Discovery and development of sorafenib: A multikinase inhibitor for treating cancer. *Nat Rev Drug Discov* 2006;5:835–844.
- 10 Boige V, Malka D, Bourredjem A, et al: Efficacy, safety, and biomarkers of single-agent bevacizumab therapy in patients with advanced hepatocellular carcinoma. *Oncologist* 2012.
- 11 Hsu CH, Yang TS, Hsu C, et al: Efficacy and tolerability of bevacizumab plus capecitabine as first-line therapy in patients with advanced hepatocellular carcinoma. *Br J Cancer* 2010;102:981–986.
- 12 Zhu AX, Sahani DV, Duda DG, et al: Efficacy, safety, and potential biomarkers of sunitinib monotherapy in advanced hepatocellular carcinoma: A phase II study. *J Clin Oncol* 2009;27:3027–3035.
- 13 Faivre S, Raymond E, Boucher E, et al: Safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma: An open-label, multicentre, phase II study. *Lancet Oncol* 2009;10:794–800.
- 14 Siegel AB, Cohen EI, Ocean A, et al: Phase II trial evaluating the clinical and biologic effects of bevacizumab in unresectable hepatocellular carcinoma. *J Clin Oncol* 2008;26:2992–2998.
- 15 Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000;92:205–216.

- 16 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.
- 17 Llovet JM, Di Bisceglie AM, Bruix J, et al: Design and endpoints of clinical trials in hepatocellular carcinoma. *J Natl Cancer Inst* 2008;100:698–711.
- 18 Llovet JM, Pena CE, Lathia CD, et al: Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2012;18:2290–2300.
- 19 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:abstr 199.
- 20 Kaseb AO, Hassan MM, Lin E, et al: V-CLIP: Integrating plasma vascular endothelial growth factor into a new scoring system to stratify patients with advanced hepatocellular carcinoma for clinical trials. *Cancer* 2011;117:2478–2488.
- 21 Miyahara K, Nouse K, Tomoda T, et al: Predicting the treatment effect of sorafenib using serum angiogenesis markers in patients with hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011;26:1604–1611.
- 22 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.
- 23 Waugh DJ, Wilson C: The interleukin-8 pathway in cancer. *Clin Cancer Res* 2008;14:6735–6741.
- 24 Abajo A, Boni V, Lopez I, et al: Identification of predictive circulating biomarkers of bevacizumab-containing regimen efficacy in pre-treated metastatic colorectal cancer patients. *Br J Cancer* 2012;107:287–290.
- 25 Hu B, Cheng SY: Angiopoietin-2: Development of inhibitors for cancer therapy. *Curr Oncol Rep* 2009;11:111–116.
- 26 Pollak M: Insulin and insulin-like growth factor signalling in neoplasia. *Nat Rev Cancer* 2008;8:915–928.
- 27 Stuver SO, Kuper H, Tzonou A, et al: Insulin-like growth factor 1 in hepatocellular carcinoma and metastatic liver cancer in men. *Int J Cancer* 2000;87:118–121.
- 28 Masoodi M, Aghazadeh R, Somi MH, Shavakhi A, Shabestari AA, Zali MR: Serum insulin-like growth factor-I and tumor size in patients with metastatic liver cancer. *Hep Mon* 2008;8:179–183.
- 29 Kaseb AO, Morris JS, Hassan MM, et al: 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.
- 30 Kaseb AO, Morris JS, Hassan MM, et al: 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.
- 31 Kaseb AO, Abbruzzese JL, Vauthey JN, et al: I-CLIP: Improved stratification of advanced hepatocellular carcinoma patients by integrating plasma IGF-1 into CLIP score. *Oncology* 2011;80:373–381.
- 32 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.
- 33 Snowberger N, Chinnakotla S, Lepe RM, et al: Alpha fetoprotein, ultrasound, computerized tomography and magnetic resonance imaging for detection of hepatocellular carcinoma in patients with advanced cirrhosis. *Aliment Pharmacol Ther* 2007;26:1187–1194.
- 34 Minata M, Nishida N, Komeda T, et al: Postoperative detection of alpha-fetoprotein mRNA in blood as a predictor for metastatic recurrence of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2001;16:445–451.
- 35 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.
- 36 Peng SY, Chen WJ, Lai PL, Jeng YM, Sheu JC, Hsu HC: High alpha-fetoprotein level correlates with high stage, early recurrence and poor prognosis of hepatocellular carcinoma: Significance of hepatitis virus infection, age, p53 and beta-catenin mutations. *Int J Cancer* 2004;112:44–50.
- 37 Furihata T, Sawada T, Kita J, et al: Serum alpha-fetoprotein level per tumor volume reflects prognosis in patients with hepatocellular carcinoma after curative hepatectomy. *Hepatogastroenterology* 2008;55:1705–1709.
- 38 Shao YY, Chen PJ, Lin ZZ, et al: Impact of baseline hepatitis B viral DNA levels on survival of patients with advanced hepatocellular carcinoma. *Anticancer Res* 2011;31:4007–4011.
- 39 Hsu CH, Shen YC, Lin ZZ, et al: Phase II study of combining sorafenib with metronomic tegafur/uracil for advanced hepatocellular carcinoma. *J Hepatol* 2010;53:126–131.
- 40 Zhang BH, Wang XH, Yue HY, Ling CQ: A new staging system is more discriminant than conventional staging systems for unresectable hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2010;136:821–827.
- 41 Baek KK, Kim JH, Uhm JE, et al: Prognostic factors in patients with advanced hepatocellular carcinoma treated with sorafenib: A retrospective comparison with previously known prognostic models. *Oncology* 2011;80:167–174.
- 42 Lin ZZ, Hsu C, Hu FC, et 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.
- 43 Shim JH, Park JW, Choi JI, Park BJ, Kim CM: Practical efficacy of sorafenib monotherapy for advanced hepatocellular carcinoma patients in a hepatitis B virus-endemic area. *J Cancer Res Clin Oncol* 2009;135:617–625.
- 44 Chen LT, Liu TW, Chao Y, et al: Alpha-fetoprotein response predicts survival benefits of thalidomide in advanced hepatocellular carcinoma. *Aliment Pharmacol Ther* 2005;22:217–226.

- 45 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.
- 46 Chan SL, Mo FK, Johnson PJ, et al: 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.
- 47 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.
- 48 Personeni N, Bozzarelli S, Pressiani T, et al: Usefulness of alpha-fetoprotein response in patients treated with sorafenib for advanced hepatocellular carcinoma. *J Hepatol* 2012;57:101–107.
- 49 Yau T, Yao TJ, Chan P, et al: 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.
- 50 Kuzuya T, Asahina Y, Tsuchiya K, et al: Early decrease in alpha-fetoprotein, but not des-gamma-carboxy prothrombin, predicts sorafenib efficacy in patients with advanced hepatocellular carcinoma. *Oncology* 2011;81:251–258.
- 51 Blann AD, Woywodt A, Bertolini F, et al: Circulating endothelial cells. Biomarker of vascular disease. *Thromb Haemost* 2005;93:228–235.
- 52 Capillo M, Mancuso P, Gobbi A, et al: Continuous infusion of endostatin inhibits differentiation, mobilization, and clonogenic potential of endothelial cell progenitors. *Clin Cancer Res* 2003;9:377–382.
- 53 Schuch G, Heymach JV, Nomi M, et al: Endostatin inhibits the vascular endothelial growth factor-induced mobilization of endothelial progenitor cells. *Cancer Res* 2003;63:8345–8350.
- 54 Bertolini F, Paul S, Mancuso P, et al: Maximum tolerable dose and low-dose metronomic chemotherapy have opposite effects on the mobilization and viability of circulating endothelial progenitor cells. *Cancer Res* 2003;63:4342–4346.
- 55 Shaked Y, Bertolini F, Man S, et al: Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis; implications for cellular surrogate marker analysis of antiangiogenesis. *Cancer Cell* 2005;7:101–111.
- 56 Shaked Y, Ciarrocchi A, Franco M, et al: Therapy-induced acute recruitment of circulating endothelial progenitor cells to tumors. *Science* 2006;313:1785–1787.
- 57 Sieghart W, Fellner S, Reiberger T, et al: Differential role of circulating endothelial progenitor cells in cirrhotic patients with or without hepatocellular carcinoma. *Dig Liver Dis* 2009;41:902–906.
- 58 Ho JW, Pang RW, Lau C, et al: Significance of circulating endothelial progenitor cells in hepatocellular carcinoma. *Hepatology* 2006;44:836–843.
- 59 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.
- 60 Naik RP, Jin D, Chuang E, et al: Circulating endothelial progenitor cells correlate to stage in patients with invasive breast cancer. *Breast Cancer Res Treat* 2008;107:133–138.
- 61 Shao YY, Lin ZZ, Chen TJ, et 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.
- 62 Goldhirsch A, Wood WC, Gelber RD, Coates AS, Thurlimann B, Senn HJ: Meeting highlights: Updated international expert consensus on the primary therapy of early breast cancer. *J Clin Oncol* 2003;21:3357–3365.
- 63 Mok TS, Wu YL, Thongprasert S, et al: Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. *N Engl J Med* 2009;361:947–957.
- 64 Bruix J, Sherman M: Management of hepatocellular carcinoma. *Hepatology* 2005;42:1208–1236.
- 65 Shao YY, Chen CL, Ho MC, et al: Dissimilar immunohistochemical expression of ERK and AKT between paired biopsy and hepatectomy tissues of hepatocellular carcinoma. *Anticancer Res* 2012; (in press).
- 66 Sun HC, Tang ZY, Li XM, Zhou YN, Sun BR, Ma ZC: Microvessel density of hepatocellular carcinoma: Its relationship with prognosis. *J Cancer Res Clin Oncol* 1999;125:419–426.
- 67 Kimura H, Nakajima T, Kagawa K, et al: Angiogenesis in hepatocellular carcinoma as evaluated by CD34 immunohistochemistry. *Liver* 1998;18:14–19.
- 68 El-Assal ON, Yamanoi A, Soda Y, et al: Clinical significance of microvessel density and vascular endothelial growth factor expression in hepatocellular carcinoma and surrounding liver: Possible involvement of vascular endothelial growth factor in the angiogenesis of cirrhotic liver. *Hepatology* 1998;27:1554–1562.
- 69 Wada H, Nagano H, Yamamoto H, et al: Expression pattern of angiogenic factors and prognosis after hepatic resection in hepatocellular carcinoma: Importance of angiopoietin-2 and hypoxia-induced factor-1 alpha. *Liver Int* 2006;26:414–423.
- 70 Huang GW, Yang LY, Lu WQ: Expression of hypoxia-inducible factor 1alpha and vascular endothelial growth factor in hepatocellular carcinoma: Impact on neovascularization and survival. *World J Gastroenterol* 2005;11:1705–1708.
- 71 Abou-Alfa GK, Schwartz L, Ricci S, et al: Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. *J Clin Oncol* 2006;24:4293–4300.
- 72 Ozenne V, Paradis V, Pernot S, et al: Tolerance and outcome of patients with unresectable hepatocellular carcinoma treated with sorafenib. *Eur J Gastroenterol Hepatol* 2010;22:1106–1110.

- 73 Hagiwara S, Kudo M, Nagai T, et al: Activation of JNK and high expression level of CD133 predict a poor response to sorafenib in hepatocellular carcinoma. *Br J Cancer* 2012.
- 74 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.
- 75 Hsu CY, Shen YC, Yu CW, et al: 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.
- 76 Khan MA, Combs CS, Brunt EM, et al: Positron emission tomography scanning in the evaluation of hepatocellular carcinoma. *J Hepatol* 2000;32:792–797.
- 77 Trojan J, Schroeder O, Raedle J, et al: Fluorine-18 FDG positron emission tomography for imaging of hepatocellular carcinoma. *Am J Gastroenterol* 1999;94:3314–3319.
- 78 Hatano E, Ikai I, Higashi T, et al: Preoperative positron emission tomography with fluorine-18-fluorodeoxyglucose is predictive of prognosis in patients with hepatocellular carcinoma after resection. *World J Surg* 2006;30:1736–1741.
- 79 Seo S, Hatano E, Higashi T, et al: 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.
- 80 Shiomi S, Nishiguchi S, Ishizu H, et al: Usefulness of positron emission tomography with fluorine-18-fluorodeoxyglucose for predicting outcome in patients with hepatocellular carcinoma. *Am J Gastroenterol* 2001;96:1877–1880.
- 81 Higashi T, Hatano E, Ikai I, et al: FDG PET as a prognostic predictor in the early post-therapeutic evaluation for unresectable hepatocellular carcinoma. *Eur J Nucl Med Mol Imaging* 2010;37:468–482.
- 82 Kim BK, Kang WJ, Kim JK, et al: 18F-fluorodeoxyglucose uptake on positron emission tomography as a prognostic predictor in locally advanced hepatocellular carcinoma. *Cancer* 2011;117:4779–4787.
- 83 Lee JH, Park JY, Kim do Y, et al: Prognostic value of 18F-FDG PET for hepatocellular carcinoma patients treated with sorafenib. *Liver Int* 2011;31:1144–1149.
- 84 Van Cutsem E, Kohne CH, Hitre E, et al: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;360:1408–1417.
- 85 Jonker DJ, O'Callaghan CJ, Karapetis CS, et al: Cetuximab for the treatment of colorectal cancer. *N Engl J Med* 2007;357:2040–2048.
- 86 Österlund P, Soveri LM, Isoniemi H, Poussa T, Alanko T, Bono P: Hypertension and overall survival in metastatic colorectal cancer patients treated with bevacizumab-containing chemotherapy. *Br J Cancer* 2011;104:599–604.
- 87 Estfan B, Byrne M, Kim R: Sorafenib in advanced hepatocellular carcinoma: Hypertension as a potential surrogate marker for efficacy. *Am J Clin Oncol* 2012; (accepted).
- 88 Otsuka T, Eguchi Y, Kawazoe S, et al: Skin toxicities and survival in advanced hepatocellular carcinoma patients treated with sorafenib. *Hepatol Res* 2012;42:879–886.
- 89 Vincenzi B, Santini D, Russo A, et al: Early skin toxicity as a predictive factor for tumor control in hepatocellular carcinoma patients treated with sorafenib. *Oncologist* 2010;15:85–92.
- 90 Kong SY, Park JW, Lee JA, et al: Association between vascular endothelial growth factor gene polymorphisms and survival in hepatocellular carcinoma patients. *Hepatology* 2007;46:446–455.
- 91 Heist RS, Zhai R, Liu G, et al: VEGF polymorphisms and survival in early-stage non-small-cell lung cancer. *J Clin Oncol* 2008;26:856–862.
- 92 Kim JG, Chae YS, Sohn SK, et al: Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with colorectal cancer. *Clin Cancer Res* 2008;14:62–66.
- 93 Knechtel G, Hofmann G, Gerger A, et al: Analysis of common germline polymorphisms as prognostic factors in patients with lymph node-positive breast cancer. *J Cancer Res Clin Oncol* 2010;136:1813–1819.
- 94 Loupakis F, Ruzzo A, Salvatore L, et al: Retrospective exploratory analysis of VEGF polymorphisms in the prediction of benefit from first-line FOLFIRI plus bevacizumab in metastatic colorectal cancer. *BMC Cancer* 2011;11:247–255.
- 95 Pander J, Wessels JA, Gelderblom H, van der Straaten T, Punt CJ, Guchelaar HJ: Pharmacogenetic interaction analysis for the efficacy of systemic treatment in metastatic colorectal cancer. *Ann Oncol* 2011;22:1147–1153.
- 96 Escudier B, Loomis AK, Kaprin A, et al: Association of single nucleotide polymorphisms in VEGF pathway genes with progression-free survival and blood pressure in metastatic renal cell carcinoma in phase 3 axis trial of axitinib versus sorafenib (AXIS trial). *Eur J Cancer* 2011;47 suppl 1:abstr 7103.
- 97 Shao YY, Lin ZZ, Hsu C, et al: Efficacy, safety, and potential biomarkers of thalidomide plus metronomic chemotherapy for advanced hepatocellular carcinoma. *Oncology* 2012;82:59–66.