VOLUME 27 · NUMBER 18 · JUNE 20 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Efficacy, Safety, and Potential Biomarkers of Sunitinib Monotherapy in Advanced Hepatocellular Carcinoma: A Phase II Study

Andrew X. Zhu, Dushyant V. Sahani, Dan G. Duda, Emmanuelle di Tomaso, Marek Ancukiewicz, Onofrio A. Catalano, Vivek Sindhwani, Lawrence S. Blaszkowsky, Sam S. Yoon, Johanna Lahdenranta, Pankaj Bhargava, Jeffrey Meyerhardt, Jeffrey W. Clark, Eunice L. Kwak, Aram F. Hezel, Rebecca Miksad, Thomas A. Abrams, Peter C. Enzinger, Charles S. Fuchs, David P. Ryan, and Rakesh K. Jain

From the Division of Hematology/Oncology, Department of Radiology, Department of Radiation Oncology, Division of Surgical Oncology, Massachusettes General Hospital and Harvard Medical School; Department of Adult Oncology, Dana-Farber Cancer Institute and Harvard Medical School; and Division of Hematology/Oncology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.

Submitted November 10, 2008; accepted January 16, 2009; published online ahead of print at www.jco.org on May 26, 2009.

Supported in part by Pfizer (A.X.Z.) and by National Institutes of Health Grants No. P01CA80124 and R01CA115767 (R.K.J.) and M01-RR-01066, Harvard Clinical and Translational Science Center.

A.X.Z., D.V.S., and D.G.D. contributed equally to this work. A.X.Z. and R.K.J. are co-senior authors.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Andrew X. Zhu, MD, PhD, Massachusetts General Hospital, 55 Fruit St, LH/POB, Room 232, Boston, MA, 02114; e-mail: azhu@partners.org.

The Acknowledgment and Appendix are included in the full-text version of this article; they are available online at www.jco.org. They are not included in the PDF version (via Adobe® Reader®).

0732-183X/09/2718-3027/$20.00

DOI: 10.1200/JCO.2008.20.9908

ABSTRACT

Purpose
To assess the safety and efficacy of sunitinib in patients with advanced hepatocellular carcinoma (HCC) and explore biomarkers for sunitinib response.

Patients and Methods
We conducted a multidisciplinary phase II study of sunitinib, an antivascular endothelial growth factor receptor tyrosine kinase inhibitor, in advanced HCC. Patients received sunitinib 37.5 mg/d for 4 weeks followed by 2 weeks of rest per cycle. The primary end point was progression-free survival (PFS). We used functional magnetic resonance imaging to evaluate vascular changes in HCC after sunitinib treatment. Circulating molecular and cellular biomarkers were evaluated before and at six time points after sunitinib treatment.

Results
Thirty-four patients were enrolled. The objective response rate was 2.9%, and 50% of patients had stable disease. Median PFS was 3.9 months (95% CI, 2.6 to 6.9 months), and overall survival was 9.8 months (95% CI, 7.4 months to not available). Grade 3 or 4 toxicities included leukopenia/neutropenia, thrombocytopenia, elevation of aminotransferases, and fatigue. Sunitinib rapidly decreased vessel leakiness, and this effect was more pronounced in patients with delayed progression. When evaluated early (at baseline and day 14) as well as over three cycles of treatment, higher levels of inflammatory molecules (eg, interleukin-6, stromal-derived factor 1α, soluble c-KIT) and circulating progenitor cells were associated with a poor outcome.

Conclusion
Sunitinib shows evidence of modest antitumor activity in advanced HCC with manageable adverse effects. Rapid changes in tumor vascular permeability and circulating inflammatory biomarkers are potential determinants of response and resistance to sunitinib in HCC. Our study suggests that control of inflammation might be critical for improving treatment outcome in advanced HCC.

J Clin Oncol 27:3027-3035. © 2009 by American Society of Clinical Oncology

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer-related death.[1] The incidence of HCC is increasing in the United States and Europe.[2,3] Advanced HCC carries a poor prognosis, and systemic therapy with cytotoxic agents provides marginal benefit.[4]

Emerging data have supported the role of angiogenesis in hepatocarcinogenesis and suggested that inflammatory pathways and/or immune cells promote tumor angiogenesis.[5-8] Excessive and abnormal vasculature, presumably as a result of up-regulation of proangiogenic factors including vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF), is a hallmark of HCC.[9] Inflammation, which is induced by hepatitis and other etiologies,[10] is another key feature of HCC.[11]

The orally available multitargeted receptor tyrosine kinase inhibitor (TKI) sorafenib (Nexavar; Bayer, West Haven, CT and Onyx, Emeryville, CA) is the first agent to demonstrate significant improvement in median overall survival (OS) in two randomized phase III trials in advanced HCC patients[12,13] and has been approved by the US Food and Drug Administration. Sorafenib may exert its

antivascular effects by targeting receptors for VEGF (VEGFR2 and VEGFR3) and PDGF (PDGFRβ) and may block tumor cell proliferation by targeting the RAF/MEK/ERK signaling pathway.[14,15] Sunitinib (Sutent; Pfizer, New York, NY) is an oral multitargeted TKI with partially overlapping target inhibition profile with sorafenib. Sunitinib is approved for the treatment of renal cell carcinoma and imatinib-resistant GI stromal tumors.[16,17] Sunitinib inhibits VEGFR1, VEGFR2, VEGFR3, PDGFRα, PDGFRβ, stem-cell factor receptor (KIT), FMS-like tyrosine kinase 3, colony-stimulating factor receptor type 1, and the glial cell line–derived neurotrophic factor receptor (RET).[18] These pathways have been implicated in angiogenesis and inflammation.

Improving treatment outcomes in advanced HCC patients requires the development of other active agents/regimens with tolerable safety profiles and the identification of mechanism of drug action and biomarkers capable of predicting tumor response and/or resistance to treatment. To assess the efficacy and tolerability of sunitinib and to identify its mechanism of action and potential biomarkers, we conducted a multidisciplinary phase II study of sunitinib in patients with advanced HCC. We explored candidate biomarkers that might be correlated with clinical efficacy by comparing clinical outcome with dynamic contrast-enhanced (DCE) magnetic resonance imaging (MRI) parameters (eg, forward volume transfer constant [K^{trans}] at baseline and day 14 after treatment) and circulating biomarkers involved in angiogenic and inflammatory pathways (at baseline; changes after 2 weeks of treatment; and changes at six time points during the first three cycles of treatment).

PATIENTS AND METHODS

Patients

The trial was approved by the Institutional Review Board (IRB) at Dana-Farber/Harvard Cancer Center (Boston, MA). All patients provided written informed consent before study participation. Eligibility criteria included histologically proven, measurable, locally advanced, recurrent or metastatic HCC; no more than one prior chemotherapy regimen; prior chemoembolization therapy only if performed more than 4 weeks before study entry and measurable disease present outside of the chemoembolization field; age $\geq$ 18 years; Eastern Cooperative Oncology Group performance status of 0 or 1; Cancer of the Liver Italian Program (CLIP) score $\leq$3; and adequate hepatic, renal, and bone marrow function. Exclusion criteria included concurrent malignancies; significant medical comorbidities; significant cardiovascular disease including uncontrolled hypertension, myocardial infarction, and unstable angina; New York Heart Association grade 2 or greater congestive heart failure; prolongation of QTc of more than 450 msec in screening ECG or history of familial long QT syndrome; history of bleeding; proteinuria at baseline (more than 2 g/d); pregnancy or lactation; CNS metastases; or an inability to provide written informed consent.

Study Treatment

Eligible patients received sunitinib at a dose of 37.5 mg daily by mouth for 28 days followed by 14 days of rest in 6-week cycles. Patients with grade 3 or 4 toxicities underwent dose reduction to 25 or 12.5 mg daily, respectively. Treatment was continued until progression, unacceptable toxicity, or withdrawal of consent.

Efficacy and Safety Assessments

Patients were observed once every 2 weeks for serial laboratory testing and physical examinations. Multiple-gated acquisition or echocardiogram measurement of left ventricular ejection fraction was performed at screening and at the end of even-numbered cycles. Computed tomography/MRI scans were performed at baseline and after each of the first three cycles and after every two cycles thereafter. Objective tumor response was assessed using RECIST (Response Evaluation Criteria in Solid Tumors).[19] Patients were observed after treatment discontinuation for survival status.

Evaluation of Biomarkers

Histology. Biopsies were available from 15 patients. Five-micrometer thick sections were cut from the formalin-fixed, paraffin-embedded blocks and stained with the following antibodies: CD31 (Dako, Carpinteria, CA); VEGFR2, PDGFRα, and PDGFRβ (Cell Signaling Technology, Danvers, MA); and c-KIT (Cell Marque, Rocklin, CA), as described.[20]

DCE-MRI. DCE-MRI of the liver was performed using a phased array body coil on a 1.5-T MRI system (Avento; Siemens, New York, NY) using the following protocol. First, T1-weighted images were obtained. Three-dimensional volume interpolated excitation coronal T1 sequence of varying flip angles of 10, 15, 30, 60, and 90 degrees were obtained in a breath hold before contrast media injection using the following parameters: TR = 5 msec, TE = 1.58 msec, 5-mm slice thickness, 0-mm interslice gap, 20 slices, 123 $\times$ 192 matrix, and field of view of 400 $\times$ 400 mm. Second, through the 20-guage peripheral intravenous line in the arm, 0.1 mmol/kg bodyweight of gadolinium-diethylenetriaminepentaacetic acid contrast was power injected at 2 mL/sec. Third, DCE acquisition was performed. A series of coronal T1-weighted three-dimensional volume interpolated excitation images were obtained after 5-second delay after the initiation of contrast media injection, and the scanning continued for up to 4 minutes and 34 seconds. The acquisition parameters included: TR = 5 msec, TE = 1.58 msec, 5-mm slice thickness, 0-mm interslice gap, 20 slices, 123 $\times$ 192 matrix, 15-degree flip angle, and field of view of 400 $\times$ 400 mm. Two consecutive 7-second acquisitions forming two different time points were repeated 10 times with a delay of 14 seconds between them. The scanning time in every acquisition was 14 seconds with a break of 14 seconds, and the patients were asked to hold their breath during acquisition. Finally, delayed postcontrast T1-weighted images were taken as follows: axial and coronal two-dimensional T1-weighted fat-saturated gradient echo (GRE) using TR = 150 msec, TE = 2.1 msec, 160 $\times$ 256 matrix, 20 slices, 5-mm thickness, and 0-mm interslice gap.

For tumor burden, an experienced radiologist, who was blinded to clinical details and treatment status, measured enhancing lesions on post–T1-weighted images.

To obtain permeability maps, DCE images were processed at pixel resolution by using a commercially available full time point (fTP) model (CAD Sciences, White Plains, NY) to analyze the time evolution of contrast enhancement. The fTP-pharmacokinetic (PK) image analysis platform implements the Tofts pharmacokinetic model to quantify vascular permeability (K^{trans} and reverse reflux rate constant between extracellular space and plasma [K_{ep}]).[21] Regions of interest were hand-drawn on postprocessed images in all the anatomic locations from the section in which the tumor was first visible to the last section in which the tumor was visible to enable whole tumor evaluation. For patients with multiple lesions, we drew regions of interest for all tumors and estimated a mean value for K^{trans} and K_{ep}.

Measurement of angiogenic proteins and inflammatory cytokines in plasma. Peripheral blood was obtained from all patients with advanced HCC enrolled onto this study at baseline and 14 days after the first dose of sunitinib. After obtaining IRB approval and informed consent, additional samples were collected in EDTA-containing vacutainers at days 28, 56, 84, and 112 after the first ingestion of the drug from 13 consecutive patients. Plasma analysis was carried out for circulating VEGF, placental-derived growth factor, soluble VEGFR1, basic fibroblast growth factor, interleukin (IL) -1β, IL-6, IL-8, and tumor necrosis factor α (TNF-α) using multiplex enzyme-linked immunosorbent assay plates from Meso-Scale Discovery (Gaithersburg, MD), as well as soluble VEGFR2, soluble VEGFR3, stromal-derived factor 1α (SDF1α), VEGF-C, and soluble c-KIT from R&D Systems (Minneapolis, MN).[20] Every sample was run in duplicate.

Circulating cell biomarker evaluation. Blood circulating cells were enumerated in fresh samples using a standard flow cytometry protocol.[22] The quantitative analysis end point was the change in the fraction of $CD31^{bright}CD34^{+}CD45^{-}$ circulating endothelial cells (CECs) and $CD133^{+}CD34^{+}CD45^{dim}$ circulating progenitor cells (CPCs) among blood mononuclear cells after sunitinib treatment. Percent values were obtained

pretreatment and at 14, 28, 56, 84, and 112 days after the first ingestion of sunitinib from 13 consecutive patients, after obtaining IRB approval and informed consent.

Data and Statistical Analyses

The primary objective of this study was progression-free survival (PFS). This study used a two-stage design. The planned accrual was for 34 patients. If at least 20 patients (59%) were observed to survive 3 months progression free, this regimen would be considered worthy of further testing. If no more than eight patients (47%) were observed to survive 3 months progression free among the initial 17 patients, the study would have been terminated. This design yielded at least 90% power to detect a true 3-month PFS rate of 69%. It yielded at least 0.90 probability of a negative result if the true 3-month PFS rate was less than 47%. Secondary end points included radiographic responses, toxicity, OS, and biologic and imaging biomarkers.

Biomarker changes from baseline were tested using the exact paired Wilcoxon test[23] and reported as the on-study to baseline ratios. The ratios were compared between partial response/stable disease (PR/SD) and progressive disease (PD) groups using two-sample exact Wilcoxon test. Associations of log-transformed biomarker levels with PFS and OS were tested in the Cox proportional hazards models,[24] after stratifying patients by the CLIP score. One variant of such analysis involved all serial measurements and used assumption of time-dependent covariates (defined by last measurement carried forward); others were with fixed covariates, at baseline and at 14 days, adjusted for baseline. The analysis with time-dependent covariates used robust sandwich variance estimators[25] to account for within-patient correlations. We report *P* values for the robust score test for time-dependent Cox model and for Wald test otherwise. Missing measurements of biomarkers were excluded from all analyses. We chose the parameters measured based on their known implication in the pathogenesis of disease and were interested in the results separately for each biomarker. Hence, we did not adjust *P* values with respect to multiple biomarkers. However, in multiple comparisons of on-study versus baseline biomarker levels, we adjusted *P* values using the false discovery control method of Genovese et al,[26] with weights proportional to the square root of paired measurements.

Table 1. Patient Characteristics

Patient Demographics	No. of Patients (N = 34)	%
Sex		
Male	29	85
Female	5	15
Age, years		
Median	64	
Range	30-82	
ECOG performance status		
0	15	44
1	19	56
CLIP score		
1	13	38
2	12	35
3	9	27
Child-Pugh class		
A	33	97
B	1	3
BCLC stage		
B (intermediate)	5	15
C (advanced)	29	85
Macroscopic vascular invasion	11	32
Extrahepatic spread	17	50
Previous therapy		
Surgical resection	3	9
Chemoembolization	2	6
Radiofrequency ablation	2	6
Radiation	1	3
Systemic therapy	6	18
Cause of disease		
Alcohol	10	29
Hepatitis C	7	21
Hepatitis B	4	12
Hepatitis C/B coinfection	4	12
Autoimmune hepatitis	1	3
Unknown	8	24
Baseline laboratory values		
α-fetoprotein, ng/mL		
Median	377	
Range	1.1-242,000	
Total bilirubin, mg/dL		
Median	0.6	
Range	0.2-2.4	
Serum AST, U/L		
Median	65	
Range	16-144	
Albumin, mg/dL		
Median	3.8	
Range	2.5-4.7	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; CLIP, Cancer of the Liver Italian Program; BCLC, Barcelona Clinic Liver Cancer.

RESULTS

Patient Characteristics

The 34 patients enrolled had histologically confirmed advanced HCC with a CLIP score of 1 (n = 13, 38%), 2 (n = 12, 35%),

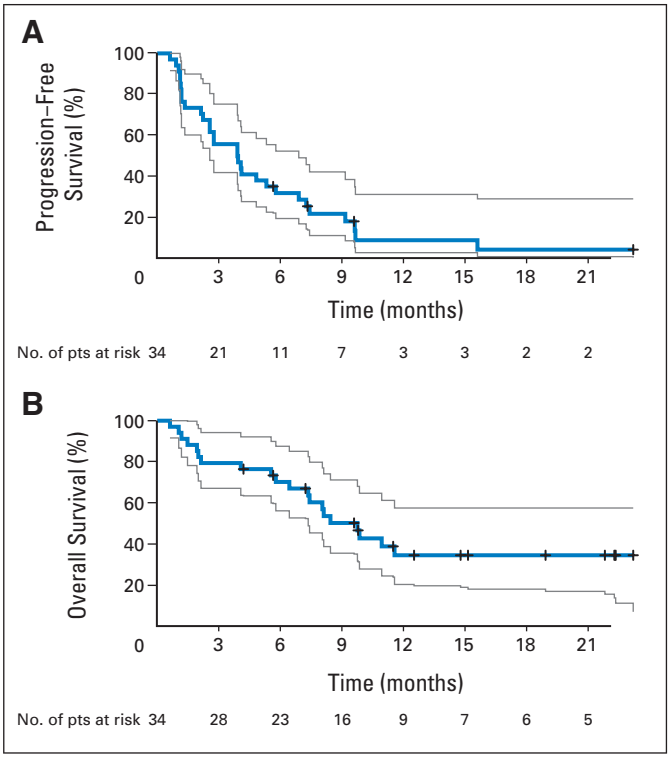

Fig 1. Kaplan-Meier survival distributions. (A) Progression-free survival and (B) overall survival in 34 advanced hepatocellular carcinoma patients receiving sunitinib. On the *x*-axis is the number of patients at risk at each time point.

or 3 (n = 9, 27%). The median age was 64 years, with 29 male patients (85%; Table 1). Twenty-nine patients (85%) had Barcelona Clinic Liver Cancer (BCLC) stage C, and five patients (15%) had BCLC stage B. The majority of patients (n = 28, 82%) had no prior systemic treatments.

Efficacy

Eleven (65%) of 17 patients in the first stage were progression free after 3 months, and therefore, the study proceeded to complete accrual. Sunitinib induced a PR (of 20 months) in one patient (2.9%; 95% CI, 0.2% to 14.9%) and achieved SD in 17 patients (50%; 95% CI, 34.1% to 65.9%). Three patients (8.8%) showed a greater than 50% decrease in α-fetoprotein (AFP). With a median follow-up time of 8.1 months, the PFS of this cohort was 3.9 months (95% CI, 2.6 to 6.9 months), the time to progression (TTP) was 4.1 months (95% CI, 2.8 to 9.2 months), the 3-month PFS rate was 56%, and the median OS was 9.8 months (95% CI, 7.4 months to not available; Figs 1A and 1B).

Safety

Adverse events were generally manageable, and the most common adverse events included hematologic toxicities, fatigue, and transaminase elevation (Table 2). Of note, grade 3 or 4 adverse events occurred in less than 20% of the patients in any category. Two patients died during the first 4 weeks, likely as a result of rapid PD and hepatic failure.

DCE-MRI and Biomarker Analyses

All analyzable tumor samples showed endothelial cell expression of VEGFR2, PDGFRα, and PDGFRβ (14 of 14 samples, 100%), but not c-KIT (Appendix Fig A1, online only). Of these markers, PDGFRα was often detected in cancer cells, whereas VEGFR2, PDGFRβ, and c-KIT were mostly seen in stromal cells.

In patients with valid pre- and post-treatment DCE-MRI measurements, we found significant decreases in K^{trans} and K_{ep} to approximately half ($P < .0001$, Fig 2A). An example of significant decreases in K^{trans} and K_{ep} is shown in Figure 2B. Moreover, the extent of decrease in K^{trans} in patients who experienced PR/SD (n = 17) was significantly greater (two-fold on average) compared with patients with PD or who died (n = 8) during the first two cycles of therapy (ie, after 3 months; $P < .05$; Fig 2C).

Sunitinib treatment induced significant and sustained increases in VEGF, placental-derived growth factor, and SDF1α and decreases in sVEGFR2, sVEGFR3 and CPCs (Table 3), but not other angiogenic and inflammatory biomarkers (basic fibroblast growth factor, VEGF-C, sVEGFR1, TNF-α, IL-1β, IL-6, IL-8, soluble c-KIT, or CECs; Appendix Table A1, online only). We later tested whether these changes in circulating proangiogenic and proinflammatory factors were associated with PFS or OS in HCC patients, after stratifying the patients by their disease stage using the CLIP score. We found significantly higher baseline serum levels of AFP and plasma levels of the inflammatory cytokines IL-8, IL-6, SDF1α, and TNF-α in patients

Table 2. Adverse Events After Sunitinib Treatment in Advanced Hepatocellular Carcinoma Patients

	Any Grade		Grade 3		Grade 4	
Toxicity	No. of Patients	%	No. of Patients	%	No. of Patients	%
Leukopenia	29	85	6	18		
Thrombocytopenia	22	65	2	6	2	6
Fatigue	21	62	4	12		
Neutropenia	21	62	6	18		
AST	20	59	6	18		
Anemia	20	59	1	3		
Lymphopenia	20	59	6	18		
Diarrhea	16	47				
ALT	15	44	3	9		
Nausea	15	44	2	6		
Anorexia	13	38	2	6		
Total bilirubin	11	32	2	6		
Alkaline phosphatase	9	26				
Constipation	9	26				
Hypophosphatemia	8	24	1	3		
Dysgeusia	8	24				
Vomiting	7	21	1	3		
Stomatitis	6	18	1	3		
Epistaxis	6	18				
Hand-foot syndrome	5	15	2	6		
Dyspnea	4	12				
Hypertension	4	12	2	6		
Rash	4	12	1	3		
Cough	3	9				
Dry skin	3	9				
Hyponatremia	3	9	1	3		
Pulmonary embolism	1	3			1	3
Upper GI bleeding	2	6	2	6		
Ataxia	1	3	1	3		

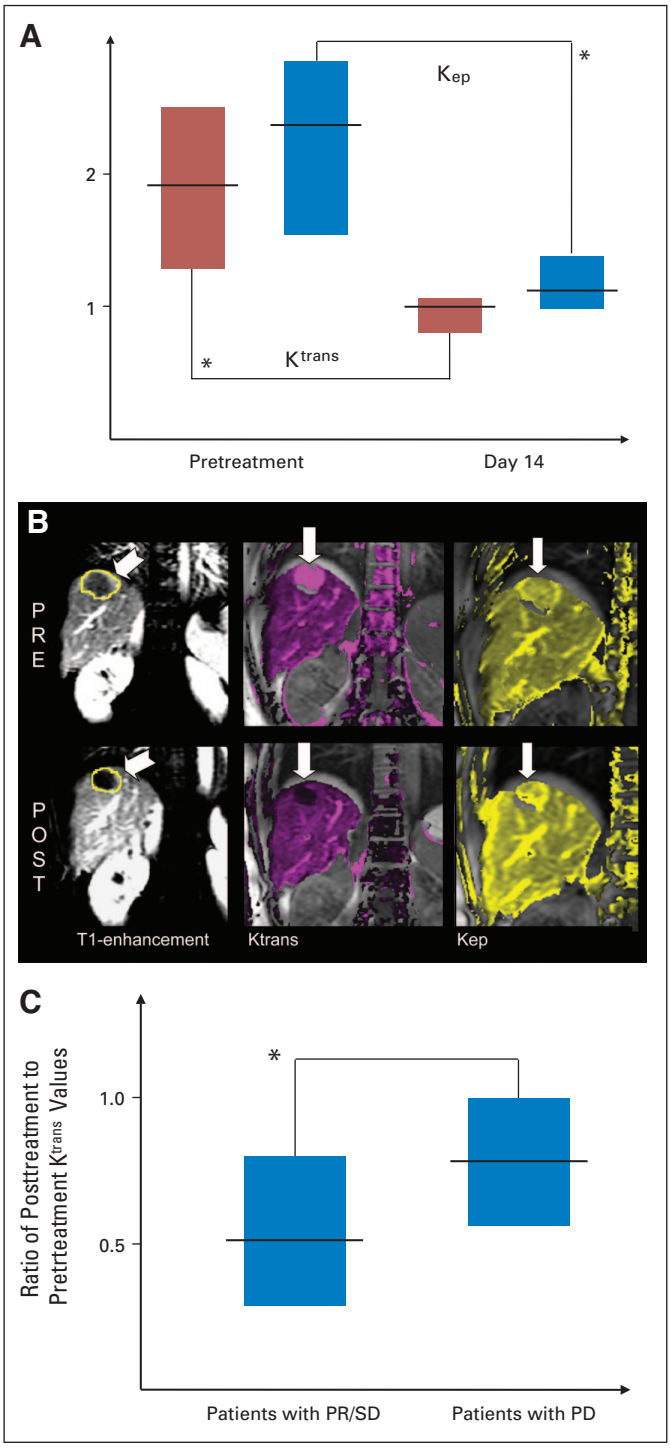

Fig 2. Measurement of the effects of sunitinib using dynamic contrast-enhanced magnetic resonance imaging (MRI). (A) Sunitinib significantly decreased forward volume transfer constant (K^{trans}; red boxes) and reverse reflux rate constant between extracellular space and plasma (K_{ep}; blue boxes) in advanced hepatocellular carcinoma (HCC) patients (*$P < .0001$, data shown as medians with 95% CIs). (B) Representative MRI images of T1-weighted tumor enhancement (left) and maps of K^{trans} (center) and K_{ep} (right, two measures of tumor vessel permeability) before and after sunitinib demonstrating a dramatic radiographic tumor response within 2 weeks of treatment. (C) Correlation between the extent of K^{trans} decrease at day 14 in HCC patients with partial response (PR) or stable disease (SD) versus patients with progressive disease (PD) after sunitinib (*$P < .05$).

with rapid tumor progression and/or mortality after sunitinib ($P < .05$, Table 4). Moreover, patients with decreases in plasma IL-6 and soluble c-KIT after 14 days of sunitinib treatment had significantly improved PFS and OS ($P < .05$; Table 4). Finally, analysis performed in a time-dependent proportional hazards model showed that patients with more elevated AFP, IL-6, soluble c-KIT, SDF1α, sVEGFR1, and CPCs at any time point during sunitinib treatment were associated with higher hazard of immediate progression or mortality ($P < .05$, Table 4).

DISCUSSION

In two randomized, placebo-controlled, phase III studies, sorafenib monotherapy yielded an OS of 10.7 months and TTP of 5.5 months (SHARP [Sorafenib HCC Assessment Randomized Protocol] study[13]) and OS of 6.5 months and TTP of 2.8 months (Asian-Pacific study[12]) in advanced HCC. In our study, although the PFS rate of 56% at 3 months failed to meet the predefined targeted end point of 59%, sunitinib treatment showed a response rate of 2.9%, SD in 50% of the patients, PFS of 3.9 months, TTP of 4.1 months, and OS of 9.8 months in advanced HCC. The results are consistent with data preliminarily reported from another phase II study of sunitinib in advanced HCC with a response rate of 2.7%, a median TTP of 21 weeks, and median OS of 45 weeks.[27] The modest single-agent activity of sunitinib observed would support further testing of sunitinib in combination with chemotherapeutic or other targeted agents. Given the single-arm nature of these studies and potential patient selection bias, the initial experience with sunitinib in HCC underscores the importance of randomized studies and the difficulty of selecting optimal primary end points in phase II studies in HCC.[28]

Sunitinib was well tolerated by most patients when administered in the current dose schedule with close monitoring. The major toxicities encountered included myelosuppression, fatigue, and transaminase elevation. Of note, two patients died during the first cycle likely as a result of rapid disease progression and hepatic failure. An increased incidence of toxicity, including hepatic failure, was seen with sunitinib at higher doses (50 mg/d) in HCC patients.[27] Although the safety profiles of sunitinib in HCC should be determined in larger populations, it seems that the 37.5-mg dose schedule has a favorable safety profile and should be used for future development of sunitinib in HCC.

Our results of target validation by immunohistochemistry are consistent with previous reports of these markers in HCC.[29-31] However, we detected PDGFRβ expression in HCC endothelial cells and thus hypothesized that sunitinib may induce similar antivascular and antipermeability effects in HCC as seen with cediranib in glioblastomas, consistent with vascular normalization.[20,32] To measure the changes in HCC vessel function, we used DCE-MRI, the most widely used technique for evaluating vessel leakiness. Parameters such as K^{trans} depend on vascular permeability and are being considered as biomarker candidates because they can detect functional changes in tumor vasculature after treatment with anti-VEGF agents.[20,33-35] The extent of the decrease in K^{trans} was greater in patients with delayed progression, suggesting that control of vessel leakiness may be a determinant of HCC response to sunitinib. A decrease in K^{trans} after 1 day of treatment with cediranib, another VEGFR TKI, has been shown to be

Table 3. Plasma Cytokine and Circulating Cell Changes After Sunitinib Treatment

Plasma	Before Treatment	Day 14	Day 28	Day 56	Day 84*	Day 112
VEGF						
Median, pg/mL	126	268	217	243	226	269
Interquartile range, pg/mL	90-213	181-457	150-295	161-282	80-240	180-329
No. of patients	33	30	8	10	8	6
P†	NA	< .0001‡	.039	.13	.84	.43
P§	NA	.0001‡	.11	.23	1.0	.75
PIGF						
Median, pg/mL	17 (N = 33)	52	81	41	20	50
Interquartile range, pg/mL	12-21	23-96	25-114	30-102	16-23	37-63
No. of patients	33	30	8	10	8	6
P†	NA	< .0001‡	.039	.0039‡	.11	.062
P§	NA	< .0001‡	.077	.010‡	.13	.11
sVEGFR2						
Median, pg/mL	6,181	4,421	4,346	3,745	5,124	2,567
Interquartile range, pg/mL	4,854-7,811	3,574-5,216	3,300-4,503	3,206-5,244	4,438-5,922	2,136-2,754
No. of patients	29	27	6	7	6	4
P†	NA	< .0001‡	.062	.031	.15	.12
P§	NA	< .0001‡	.13	.087	.19	.19
sVEGFR3						
Median, pg/mL	3.35	2.57	2.04	2.54	4.34	2.92
Interquartile range, pg/mL	1.79-4.56	1.70-3.39	1.30-3.12	1.34-4.02	3.03-7.60	2.12-3.56
No. of patients	30	22	8	10	8	6
P†	NA	.0008‡	.0078‡	.064	.46	.31
P§	NA	.0028‡	.022‡	.12	.52	.51
SDF1α						
Median, pg/mL	2,721	3,024	3,436	2,861	2,611	2,683
Interquartile range, pg/mL	2,303-3,037	2,725-3,626	2,795-3,580	2,685-3,096	2,332-2,869	2,630-2,969
No. of patients	32	29	8	10	8	6
P†	NA	.0007‡	.0078‡	.0039‡	.078	.062
P§	NA	.0023‡	.015‡	.010‡	.092	.092
CPCs						
Median, % of PBMC	0.096	0.032	0.046	0.045	0.076	0.025
Interquartile range, % of PBMC	0.076-0.100	0.030-0.040	0.030-0.060	0.026-0.045	0.068-0.083	0.020-0.042
No. of patients	9	8	7	7	7	4
P†	NA	.015†	.015‡	.12	.25	.50
P§	NA	.032‡	.032‡	.22	.33	.76

NOTE. *P* values are shown with and without adjustment for multiple variable analysis.

Abbreviations: VEGF, vascular endothelial growth factor; NA, not applicable; CPCs, circulating progenitor cells; PIGF, placental-derived growth factor; VEGFR, vascular endothelial growth factor receptor; SDF1α, stromal-derived factor 1α; PBMC, percent of peripheral-blood mononuclear cell.

*This time point corresponds to the beginning of the third cycle of treatment and is after a 2-week treatment break.

†*P* values are from the paired exact Wilcoxon test, unadjusted.

‡Significant change.

§*P* values are from the paired exact Wilcoxon test, adjusted to control the false discovery rate over time, with weights proportional to the square root of number of the measurements.

associated with PFS and OS in recurrent glioblastoma.[36] Despite these promising leads, DCE-MRI has not been integrated in any of the previous phase III studies of anti-VEGF agents. Moreover, a range of MRI techniques have been reported in the literature, and there is currently no consensus on what are the most appropriate parameters to be used for anti-VEGF agents. Thus, the predictive value of imaging biomarkers remains to be standardized and validated in larger studies, and RECIST criteria remain the standard for response assessment in HCC.

Some blood circulating proangiogenic and proinflammatory molecules are often elevated in patients with tumors and are currently being evaluated as potential biomarkers of response or resistance to anti-VEGF therapy.[37] Here, we show that sunitinib treatment significantly changed multiple angiogenic biomarkers in HCC patients. Nevertheless, delayed tumor progression after sunitinib correlated with a decrease in the circulating inflammatory molecules IL-6 and soluble c-KIT. Moreover, we found that higher levels of IL-6, soluble c-KIT, SDF1α, and CPCs at any time point during sunitinib treatment in HCC patients was associated with rapid progression or mortality. These data emphasize the potential role of IL-6 and soluble c-KIT modulation during sunitinib treatment in HCC. They also underscore the potential role of upregulation of inflammatory pathways such as IL-6 and SDF1α (a chemokine significantly upregulated by sunitinib throughout the treatment) in tumor refractoriness to this therapy, as well as potential novel targets for this disease. Counterintuitively, higher sVEGFR1 correlated with rapid progression, and there were no correlations between outcome and other VEGF members measured in this study in plasma. However, it is important to note that these

Table 4. Blood Biomarkers Significantly Associated With Time to Tumor Progression and Mortality in Patients With Advanced Hepatocellular Carcinoma (stratified by CLIP score) Who Received Sunitinib Therapy

	Pretreatment Measurement*		Change at Day 14*		Time-Dependent Change†	
Biomarker	Tumor Progression	Mortality	Tumor Progression	Mortality	Tumor Progression	Mortality
AFP						
HR	1.22	1.37	NA	NA	1.25	1.24
95% CI	1.00 to 1.49	0.98 to 1.90			1.09 to 1.43	1.08 to 1.42
No. of patients	26	26			23/26‡	13/26‡
P	.048	.055			.0012	.012
IL-6						
HR	1.70	1.82	2.46	1.96	3.28	2.77
95% CI	1.12 to 2.57	1.12 to 2.96	1.27 to 4.76	1.14 to 3.35	2.09 to 5.13	1.78 to 4.30
No. of patients	29	29	27	27	26/29‡	18/29‡
P	.013	.016	.008	.027	.0026	.0072
Soluble c-KIT						
HR	0.74	1.92	1.95	4.13	1.30	2.54
95% CI	0.39 to 1.40	0.88 to 4.17	0.75 to 5.06	1.13 to 15.02	0.82 to 2.09	1.03 to 6.28
No. of patients	33	33	30	30	30/33‡	20/33‡
P	NS	.099	.099	.032	NS	.019
IL-8						
HR	1.68	1.84	0.67	0.58	1.31	1.43
95% CI	1.04 to 2.71	1.07 to 3.17	0.31 to 1.45	0.25 to 1.36	0.69 to 2.48	0.70 to 2.91
No. of patients	28	28	26	26	25/28‡	17/28‡
P	.035	.028	NS	NS	NS	NS
SDF1α						
HR	1.11	5.41	2.76	1.82	1.87	15.98
95% CI	0.42 to 2.94	1.51 to 19.32	0.45 to 16.86	0.18 to 18.42	0.62 to 5.61	3.21 to 79.64
No. of patients	32	32	29	29	29/32‡	19/32‡
P	NS	.009	NS	NS	NS	.0065
TNF-α						
HR	1.88	4.83	1.03	0.49	2.15	2.05
95% CI	0.71 to 5.03	1.33 to 17.53	0.35 to 3.01	0.13 to 1.82	0.87 to 5.34	0.91 to 4.65
No. of patients	29	29	27	27	26/29‡	18/29‡
P	NS	.017	NS	NS	.061	.075
sVEGFR1						
HR	1.29	0.98	1.31	1.09	1.39	1.16
95% CI	0.87 to 1.91	0.65 to 1.47	0.57 to 3.01	0.34 to 3.47	1.07 to 1.80	0.80 to 1.67
No. of patients	33	33	30	30	30/33‡	20/33‡
P	NS	NS	NS	NS	.021	NS
VEGF						
HR	1.60	1.17	1.05	0.82	1.21	1.38
95% CI	1.08 to 2.36	0.77 to 1.77	0.62 to 1.79	0.45 to 1.49	0.91 to 1.62	0.93 to 2.04
No. of patients	33	33	30	30	30/33‡	20/33‡
P	.018	NS	NS	NS	NS	.082
CPCs						
HR	0.78	0.99	NA	NA	1.01	4.83
95% CI	0.04 to 15.28	0.00 to 695.7			0.48 to 2.12	0.92 to 25.42
No. of patients	9	9			11/13‡	6/13‡
P	NS	NS			NS	.035

NOTE. Biomarker were evaluated at baseline, early after sunitinib (day 14), and at six time points before and after treatment. $P < .05$ is considered significant.
Abbreviations: CLIP, Cancer of the Liver Italian Program; AFP, α-fetoprotein; HR, hazard ratio; NA, not applicable; IL, interleukin; NS, not significant; SDF1α, stromal-derived factor 1α; TNF-a, tumor necrosis factor α; VEGFR, vascular endothelial growth factor receptor; VEGF, vascular endothelial growth factor; CPCs, circulating progenitor cells.
*P values are from the Wald test in proportional hazards model.
†P values are from the robust score test in time-dependent proportional hazards model for patients with higher biomarker values at any time point.
‡Total number of measurements per number of patients.

changes occurred in the context of continued VEGF signaling inhibition by sunitinib and that polymorphisms in VEGF or VEGFR2 and not just total plasma protein concentration may be determinants for tumor responsiveness to anti-VEGF therapy.[38]

In summary, although the PFS rate at 3 months fell just short of the targeted 59% rate, we provided initial evidence of antitumor activity of sunitinib in advanced HCC with manageable safety profiles. Sunitinib rapidly reduces tumor vessel leakiness, as estimated by MRI, indicating a direct effect on HCC vasculature that might be associated with clinical benefit. Our circulating biomarker data suggest a critical role for the balance between angiogenic and inflammatory pathways in HCC response and resistance to sunitinib treatment. Successful

modulation of these inflammatory markers might be critical for achieving treatment response with sunitinib and potentially other antiangiogenic agents. The findings of this hypothesis-generating study should be validated in large prospective trials. It will be particularly important to further explore these potential biomarkers for other targeted agents, such as sorafenib or bevacizumab, to better understand the significance of these findings for anti-VEGF therapy and improve the outcome of treatment in HCC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory Role:** Andrew X. Zhu, Genentech (C), Bayer Pharmaceuticals (C); Dan G. Duda, Takeda Pharmaceutical (C); Charles S. Fuchs, Bristol-Myers Squibb Co (C), Genentech (C), sanofi-aventis (C), Genentech (C), Amgen (C), Roche (C), Pfizer Oncology (C); Rakesh K. Jain, AstraZeneca (C), Millennium Pharmaceuticals (C), Dyax (C), SynDevRx (U) **Stock Ownership:** None **Honoraria:** Jeffrey Meyerhardt, Genentech; Peter C. Enzinger, Roche; David P. Ryan, Genentech; Rakesh K. Jain, Pfizer, Roche **Research Funding:** Lawrence S. Blaszkowsky, Genentech; Charles S. Fuchs, Pfizer Oncology; Rakesh K. Jain, AstraZeneca, Dyax **Expert Testimony:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Andrew X. Zhu, Dushyant V. Sahani, Dan G. Duda, Emmanuelle di Tomaso, Marek Ancukiewicz, Rakesh K. Jain
Financial support: Andrew X. Zhu, Rakesh K. Jain
Administrative support: Andrew X. Zhu, Charles S. Fuchs, David P. Ryan, Rakesh K. Jain
Provision of study materials or patients: Andrew X. Zhu, Dushyant V. Sahani, Lawrence S. Blaszkowsky, Sam S. Yoon, Pankaj Bhargava, Jeffrey Meyerhardt, Jeffrey W. Clark, Eunice L. Kwak, Aram F. Hezel, Rebecca Miksad, Thomas A. Abrams, Peter C. Enzinger, Charles S. Fuchs, David P. Ryan, Rakesh K. Jain
Collection and assembly of data: Andrew X. Zhu, Dushyant V. Sahani, Dan G. Duda, Emmanuelle di Tomaso, Onofrio A. Catalano, Vivek Sindhwani, Johanna Lahdenranta, Rebecca Miksad, Thomas A. Abrams, Peter C. Enzinger
Data analysis and interpretation: Andrew X. Zhu, Dushyant V. Sahani, Dan G. Duda, Emmanuelle di Tomaso, Marek Ancukiewicz, Onofrio A. Catalano, Vivek Sindhwani, Sam S. Yoon, Johanna Lahdenranta, Rakesh K. Jain
Manuscript writing: Andrew X. Zhu, Dushyant V. Sahani, Dan G. Duda, Rakesh K. Jain
Final approval of manuscript: Andrew X. Zhu, Dushyant V. Sahani, Dan G. Duda, Emmanuelle di Tomaso, Marek Ancukiewicz, Onofrio A. Catalano, Vivek Sindhwani, Lawrence S. Blaszkowsky, Sam S. Yoon, Johanna Lahdenranta, Pankaj Bhargava, Jeffrey Meyerhardt, Jeffrey W. Clark, Eunice L. Kwak, Aram F. Hezel, Rebecca Miksad, Thomas A. Abrams, Peter C. Enzinger, Charles S. Fuchs, David P. Ryan, Rakesh K. Jain

REFERENCES

1. Parkin DM: Global cancer statistics in the year 2000. Lancet Oncol 2:533-543, 2001
2. El-Serag HB, Rudolph KL: Hepatocellular carcinoma: Epidemiology and molecular carcinogenesis. Gastroenterology 132:2557-2576, 2007
3. McGlynn KA, London WT: Epidemiology and natural history of hepatocellular carcinoma. Best Pract Res Clin Gastroenterol 19:3-23, 2005
4. Zhu AX: Systemic therapy of advanced hepatocellular carcinoma: How hopeful should we be? Oncologist 11:790-800, 2006
5. de Visser KE, Eichten A, Coussens LM: Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 6:24-37, 2006
6. Murdoch C, Muthana M, Coffelt SB, et al: The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer 8:618-631, 2008
7. De Palma M, Venneri MA, Roca C, et al: Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. Nat Med 9:789-795, 2003
8. Shojaei F, Wu X, Malik AK, et al: Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. Nat Biotechnol 25: 911-920, 2007
9. Suzuki K, Hayashi N, Miyamoto Y, et al: Expression of vascular permeability factor/vascular endothelial growth factor in human hepatocellular carcinoma. Cancer Res 56:3004-3009, 1996
10. Brun P, Castagliuolo I, Pinzani M, et al: Exposure to bacterial cell wall products triggers an inflammatory phenotype in hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 289:G571-G578, 2005
11. Naugler WE, Karin M: The wolf in sheep's clothing: The role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med 14:109-119, 2008
12. 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 10:25-34, 2008
13. Llovet JM, Ricci S, Mazzaferro V, et al: Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 359:378-390, 2008
14. Carlomagno F, Anaganti S, Guida T, et al: BAY 43-9006 inhibition of oncogenic RET mutants. J Natl Cancer Inst 98:326-334, 2006
15. Wilhelm SM, Carter C, Tang L, et al: BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 64:7099-7109, 2004
16. Demetri GD, van Oosterom AT, Garrett CR, et al: Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: A randomised controlled trial. Lancet 368:1329-1338, 2006
17. Motzer RJ, Hutson TE, Tomczak P, et al: Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N Engl J Med 356:115-124, 2007
18. Chow LQ, Eckhardt SG: Sunitinib: From rational design to clinical efficacy. J Clin Oncol 25:884-896, 2007
19. 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 92:205-216, 2000
20. Batchelor TT, Sorensen AG, di Tomaso E, et al: AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell 11:83-95, 2007
21. Tofts PS, Brix G, Buckley DL, et al: Estimating kinetic parameters from dynamic contrast-enhanced T(1)-weighted MRI of a diffusable tracer: Standardized quantities and symbols. J Magn Reson Imaging 10:223-232, 1999
22. Duda DG, Cohen KS, Scadden DT, et al: A protocol for phenotypic detection and enumeration of circulating endothelial cells and circulating progenitor cells in human blood. Nat Protoc 2:805-810, 2007
23. Hollander M, Wolfe DA: Nonparametric Statistical Inference. New York, NY, John Wiley & Sons, 1973, pp 27-33
24. Kalbfleisch JD, Prentice RL: The Statistical Analysis of Failure Time Data. New York, NY, Wiley, 1980
25. Lin DY, Wei LJ: The robust inference for the proportional hazards model. J Am Stat Assoc 84: 1074-1078, 1989
26. Genovese T, Mazzon E, Crisafulli C, et al: False discovery control with p-value weighting. Biometrika 93:509-524, 2006
27. Faivre SJ, Raymond E, Douillard J, et al: Assessment of safety and drug-induced tumor necrosis with sunitinib in patients (pts) with unresectable hepatocellular carcinoma (HCC). J Clin Oncol 25:149s, 2007 (suppl; abstr 3546)

28. Llovet JM, Di Bisceglie AM, Bruix J, et al: Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 100:698-711, 2008

29. Becker G, Schmitt-Graeff A, Ertelt V, et al: CD117 (c-kit) expression in human hepatocellular carcinoma. Clin Oncol (R Coll Radiol) 19:204-208, 2007

30. Eckel F, von Delius S, Mayr M, et al: Pharmacokinetic and clinical phase II trial of imatinib in patients with impaired liver function and advanced hepatocellular carcinoma. Oncology 69:363-371, 2005

31. von Marschall Z, Cramer T, Hocker M, et al: Dual mechanism of vascular endothelial growth factor upregulation by hypoxia in human hepatocellular carcinoma. Gut 48:87-96, 2001

32. Jain RK: Normalization of tumor vasculature: An emerging concept in antiangiogenic therapy. Science 307:58-62, 2005

33. Galbraith SM: Antivascular cancer treatments: Imaging biomarkers in pharmaceutical drug development. Br J Radiol 76:S83–S86, 2003

34. Morgan B, Thomas AL, Drevs J, et al: Dynamic contrast-enhanced magnetic resonance imaging as a biomarker for the pharmacological response of PTK787/ZK 222584, an inhibitor of the vascular endothelial growth factor receptor tyrosine kinases, in patients with advanced colorectal cancer and liver metastases: Results from two phase I studies. J Clin Oncol 21:3955-3964, 2003

35. Wedam SB, Low JA, Yang SX, et al: Antiangiogenic and antitumor effects of bevacizumab in patients with inflammatory and locally advanced breast cancer. J Clin Oncol 24:769-777, 2006

36. Batchelor TT, Sorensen AG, di Tomaso E, et al: A multidisciplinary phase II study of AZD2171 (cediranib), an oral pan-VEGF receptor tyrosine kinase inhibitor, in patients with recurrent glioblastoma. 99th Annual Meeting of the American Association for Cancer Research, San Diego, CA, April 12-16, 2008 (abstr 5531)

37. Jain RK, Duda DG, Clark JW, et al: Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol 3:24-40, 2006

38. Schneider BP, Wang M, Radovich M, et al: Association of vascular endothelial growth factor and vascular endothelial growth factor receptor-2 genetic polymorphisms with outcome in a trial of paclitaxel compared with paclitaxel plus bevacizumab in advanced breast cancer: ECOG 2100. J Clin Oncol 26:4672-4678, 2008

