VOLUME 24 · NUMBER 26 · SEPTEMBER 10 2006

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Phase II Study of Sorafenib in Patients With Advanced Hepatocellular Carcinoma

Ghassan K. Abou-Alfa, Lawrence Schwartz, Sergio Ricci, Dino Amadori, Armando Santoro, Arie Figer, Jacques De Greve, Jean-Yves Douillard, Chetan Lathia, Brian Schwartz, Ian Taylor, Marius Moscovici, and Leonard B. Saltz

From the Memorial Sloan-Kettering Cancer Center, New York, NY; Bayer Pharmaceuticals Corporation, West Haven, CT; Ospedale S. Chiara, Pisa; Ospedale Morgagni Pierantoni, Forli; Istituto Clinico Humanitas, Rozzano (MI); Bayer S.p.A. PH/Medical Department, Milan, Italy; Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; AZ-VUB, Brussels, Belgium; and the Centre René Gauducheau, Nantes, France.

Submitted September 26, 2005; accepted May 4, 2006; published online ahead of print at www.jco.org on August 14, 2006.

Presented at the 4th International Meeting on Hepatocellular Carcinoma: Eastern and Western Experiences, Hong Kong, December 14-16, 2004; European Organisation for Research and Treatment of Cancer–National Cancer Institute–American Association for Cancer Research Meeting, September 28-October 1, 2004, Geneva, Switzerland.

Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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

Address reprint requests to Ghassan K. Abou-Alfa, MD, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10022; e-mail: abou-alg@mskcc.org.

0732-183X/06/2426-4293/$20.00

DOI: 10.1200/JCO.2005.01.3441

ABSTRACT

Purpose
This phase II study of sorafenib, an oral multikinase inhibitor that targets Raf kinase and receptor tyrosine kinases, assessed efficacy, toxicity, pharmacokinetics, and biomarkers in advanced hepatocellular carcinoma (HCC) patients.

Methods
Patients with inoperable HCC, no prior systemic treatment, and Child–Pugh (CP) A or B, received continuous, oral sorafenib 400 mg bid in 4-week cycles. Tumor response was assessed every two cycles using modified WHO criteria. Sorafenib pharmacokinetics were measured in plasma samples. Biomarker analysis included phosphorylated extracellular signal regulated kinase (pERK) in pretreatment biopsies (immunohistochemistry) and blood-cell RNA expression patterns in selected patients.

Results
Of 137 patients treated (male, 71%; median age, 69 years), 72% had CP A, and 28% had CP B. On the basis of independent assessment, three (2.2%) patients achieved a partial response, eight (5.8%) had a minor response, and 46 (33.6%) had stable disease for at least 16 weeks. Investigator-assessed median time to progression (TTP) was 4.2 months, and median overall survival was 9.2 months. Grade 3/4 drug-related toxicities included fatigue (9.5%), diarrhea (8.0%), and hand–foot skin reaction (5.1%). There were no significant pharmacokinetic differences between CP A and B patients. Pretreatment tumor pERK levels correlated with TTP. A panel of 18 expressed genes was identified that distinguished "nonprogressors" from "progressors" with an estimated 100% accuracy.

Conclusion
Although single-agent sorafenib has modest efficacy in HCC, the manageable toxicity and mechanisms of action support a role for combination regimens with other anticancer agents.

J Clin Oncol 24:4293-4300. © 2006 by American Society of Clinical Oncology

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide,[1] with approximately 500,000 new cases per year. Approximately 80% of cases arise in Asia and Africa,[2] mainly due to chronic hepatitis B virus (HBV) infection. The incidence of HCC is rising in the United States and Europe because of increased incidence of hepatitis C (HCV) infection.[3]

Surgical resection and liver transplantation are considered the only cures for HCC, but benefit only approximately 15% of patients.[4] Unresectable or metastatic disease patients have median survival of a few months.[5] There is a substantial need for novel treatments for advanced HCC, because systemic therapy induces relatively few responses and has no clear survival benefit.

Preclinical studies demonstrated that Raf/MAPK-ERK kinase (MEK)/extracellular signal regulated kinase (ERK) pathway has a role in HCC.[6] Furthermore, over-expression of activated MEK1 in HCC cell lines enhanced tumor growth and survival by preventing apoptosis. HCV core proteins elicit high basal Raf-1 activity in hepatocytes, increasing the risk of neoplastic transformation.[7,8] HCC tumors are highly vascularized, and vascular endothelial growth factor (VEGF) augments HCC development and metastasis.[9] Therefore, blocking signaling through Raf-1 may offer therapeutic benefits in HCC.[6]

Sorafenib, an oral multikinase inhibitor, blocks tumor cell proliferation by targeting Raf/MEK/ERK

signaling at the level of Raf kinase, and exerts an antiangiogenic effect by targeting vascular endothelial growth factor receptor-2/-3 (VEGFR-2/-3), and platelet derived growth factor receptor beta (PDGFR-β) tyrosine kinases.[10]

In a phase I trial of sorafenib, a confirmed partial response was observed in a metastatic HCC patient.[11] This response, and the importance of VEGF and Raf/MEK/ERK signaling in HCC, prompted this phase II study to evaluate further the efficacy, toxicity, and pharmacokinetics (PK) of sorafenib in advanced HCC. The predictive value of molecular biomarkers in determining time to progression (TTP) was also evaluated.

METHODS

This was a multicenter, international, uncontrolled phase II trial in advanced HCC patients. The trial was approved by a human investigation committee at each center, and conducted in accordance with the US Dept of Health and Human Services guidelines. Informed consent was obtained from each patient.

Patients' Eligibility

Patients with measurable, histologically proven, inoperable HCC who had not received prior systemic treatments for HCC were eligible for enrollment. Inclusion criteria included Eastern Cooperative Oncology Group performance status of 0 or 1; Child-Pugh (CP) score of A or B; life expectancy of at least 12 weeks; elevated alphafetoprotein (AFP) level and adequate hematologic, hepatic, and renal function. HBV or HCV infection status at baseline was collected from medical history or laboratory tests.

Patients with tumors of mixed histology or fibrolamellar variant, pregnant or lactating women, or those requiring systemic anticancer therapy, biologic-response modifiers, or CYP34A inhibitors or with medical/psychological/social problems that might affect study participation or evaluations were excluded.

Treatment and Dose Modifications

Patients received sorafenib 400 mg bid, but were allowed up to two dose reductions (200 mg bid and 200 mg qd) for drug-related toxicities (National Cancer Institute [Bethesda, MD] Common Toxicity Criteria v2.0). Otherwise, treatment continued until disease progression (PD) or unacceptable drug-related toxicities.

Dose delays or modifications were required for drug-related toxicities. For grade 3/4 toxicities; patients received lower doses when toxicity improved to grade 2 or better, but therapy was discontinued if recovery time was 3 weeks or longer. A dose delay was introduced for grade 3 nonhematologic toxicities, until toxicity was grade 2 or better; patients were then treated at one dose level lower, and therapy was discontinued if recovery time was 3 weeks or longer. Patients with drug-related grade 4 nonhematologic toxicities were treated at two dose levels lower at the first appearance and withdrawn at the second. A modified scale was used for hand–foot skin reaction (HFS), to facilitate interpretation (Table 1), and specific dose modifications were implemented (Table 2).

Table 1. Skin Toxicity Grading

Grade	Description
Grade 1	Numbness, dysesthesia/paresthesia, tingling, painless swelling or erythema of the hands and/or feet, and/or discomfort that does not disrupt normal activities
Grade 2	Painful erythema and swelling of the hands and/or feet and/or discomfort affecting the patient's activities
Grade 3	Moist desquamation, ulceration, blistering or severe pain of the hands and/or feet, and/or severe discomfort that causes the patient to be unable to work or perform activities of daily living

Table 2. Skin Toxicity Criteria for Dose Delay and Dose Modification

Toxicity Grade	During a Course of Therapy	Dose for Next Cycle
Grade 1	Maintain dose level	Maintain dose level
Grade 2		
1st appearance	Interrupt until resolved	400 mg bid
2nd appearance	Interrupt until resolved to grade 0-1	200 mg bid
3rd appearance	Interrupt until resolved to grade 0-1	200 mg qd
4th appearance	Discontinue treatment permanently	
Grade 3		
1st appearance	Interrupt until resolved to grade 0-1	200 mg bid
2nd appearance	Interrupt until resolved to grade 0-1	200 qd
3rd appearance	Discontinue treatment permanently	

Response Assessment

Investigator-assessed bidimensional tumor measurements were performed at baseline and every 8 weeks (two cycles), according to modified WHO criteria. Independent radiologic assessment was also performed for patients who had baseline and at least one postbaseline imaging measurement. Stable disease (SD) was required to last at least 16 weeks. Throughout the study, lesions measured at baseline were evaluated using the same technique and, preferably, by the same investigator. Overall tumor response was scored as a complete response (CR), partial response (PR), or minor response (MR; a reduction in tumor size of $\geq$ 25% but $<$ 50% *v* baseline) if the response was confirmed at least 4 weeks later.

Secondary objectives were duration of response (first administration of study drug until PD in patients with objective responses); TTP (first administration of study drug until PD); duration of SD (first day of receiving sorafenib until PD or response); overall survival (first day of receiving study medication to death). Patients who received at least one dose of sorafenib and had post-treatment data available were assessable for overall response rate.

Assessing Tumor Necrosis

In addition to evaluation of tumor necrosis (TN) by independent radiologists, a semiautomated computerized technique quantified TN on intravenous contrast-enhanced scans in 11 of 16 patients accrued at Memorial Sloan-Kettering Cancer Center (New York, NY). A dominant hepatic mass(es) was selected at baseline and followed every two cycles. Response assessment was calculated with uni- and bidimensional tumor measurements, and TN was calculated at each time point by a radiologist blinded to the clinical data.

PK

Blood samples were collected on day 1 of cycle 2 for PK analysis of sorafenib plasma concentrations using a validated liquid chromatography/mass spectrometry/mass spectrometry assay, with a lower limit of quantification of 1 to 10 μg/L. PK was assessed in 22 patients on or after 28 days of dosing. Samples were collected at 0 hours (predose), and 0.5, 1, 2, 4, 8, and 12 hours postdose. Parameters included area under the curve (AUC), maximum concentration (C_{max}), and time to maximum concentration (t_{max}). AUC over 8 hours (AUC_{0-8}) was determined, because 12-hour samples were not collected for most patients. Noncompartmental PK parameters were calculated using KinCalc software (Bayer AG, Wuppertal, Germany).

Biomarker Evaluation: Tumor-Cell Phosphorylated Extracellular Signal Regulated Kinase Levels

Sections (5 to 6 μm thick) cut from paraffin-embedded tumor biopsies from 33 patients were analyzed with immunohistochemistry using a rabbit polyclonal antibody for phosphorylated extracellular signal regulated kinase (pERK) (phospho-p44/42 mitogen-activated protein kinase [Thr202/Tyr204]; Cell Signaling Technology Inc, Danvers, MA). Positive and negative

controls ensured that the integrity of all tumor samples and reagents was maintained. Positive controls, selected because they encompass a range of pERK staining, included human xenograft MDA-MB-231 (breast) and MiaPaCa (pancreas) cells, and a renal cell carcinoma biopsy. Slides from each biopsy were stained with a species, isotype (immunoglobulin G) and concentration-matched negative control antibody. A hematoxylin-eosin slide was also stained for each sample. Stained slides were evaluated independently by two pathologists. Localization of pERK staining to cell nuclei or cytoplasm was evaluated qualitatively. Nuclear pERK staining intensity was graded semi-quantitatively using a five-point scale: 0, no staining; 1+, weak; 2+, moderate; 3+, strong and 4+, intense. Tumor response and pERK staining were correlated using the Peto modification of the Gehan-Wilcoxon rank sum test.[12]

Biomarker Evaluation: Blood-Cell RNA Expression Patterns

Blood samples were taken at baseline (within 7 days before treatment), on day 15 of cycle 1, day 1 of every second cycle (cycle 3, 5, 7 and so on) and at the final visit, and stored at −80°C. A modified Affymetrix (Santa Clara, CA) microarray technique was used to identify blood-cell RNA expression patterns that may predict clinical benefit from treatment.[13] Total RNA was isolated from blood samples using Qiagen QIAamp RNA Blood Mini kit and protocol (Qiagen Inc, Valencia, CA), and stored at −80°C. cDNA synthesis was performed with 500 ng–5 μg RNA using Superscript Choice System (Invitrogen, Carlsbad, CA). In vitro transcription of cDNA using the BioArray HighYield Transcription kit (Enzo Diagnostics, Farmingdale, NY) generated cRNA for array analyses. HG-U133 Plus 2.0 arrays (containing > 60,000 probe sets, which represent 50,000 RNA transcripts) from Affymetrix were hybridized, washed, and stained with 6 μg phycoerythrin-streptavidin (Molecular Probes, Carlsbad, CA). Affymetrix GeneChips were scanned at 488 nm by GeneChip Scanner 3000 and analyzed using Affymetrix MicroArray Suite 5.0 software. GeneChip data were used to develop mathematical models based on algorithms to predict TTP with sorafenib. Models were evaluated for predictive (estimated) accuracy using either 10-fold or leave-one-out cross validation.[14] The gene selection process was based on a data-mining technique called Support Vector Machine using investigator-assessed SD at 8 weeks, rather than 16 weeks used for efficacy end points, due to technical limitations.

Quality-control procedures ensured that deviations in sample collection, freezing, storage, and shipping were minimized, and that resulting cRNA was of suitable quality for analysis on Affymetrix GeneChips. From a total of 240 whole-blood samples, only 52 (representing 32 patients) yielded cRNA of sufficient quality.

Statistical Analysis

The study progressed using a three-stage design, recruiting a total of 26 stage 1, 71 stage 2, and 135 stage 3 patients. Two planned interim analyses were conducted after the availability of at least 3-month tumor response data from stages 1 and 2. Accrual was not held during interim analyses, and continued during the 3-month maturation of response data, thus accounting for the larger-than-planned accrual. Under the null hypothesis, the regimen would be rejected as a cytoreductive agent if confirmed response rate was 7% or less.

The first interim analysis considered the following: (a) 1 confirmed CR/PR; (b) 2 confirmed MRs; (c) 2 patients with at least 50% reduction in AFP; (d) 3 patients with either confirmed MRs or more than 50% reduction in elevated AFP or SD for 12 weeks. If none of these were met, the null hypothesis was accepted. If (a) was met, stage 2 could proceed. If (a) was not met but at least one of the other three conditions was met, stage 2 could proceed due to potential clinical benefit in the form of MRs, tumor marker reduction, and cytostatic and/or biomarker reduction. The first interim analysis suggested potential activity; therefore, patient enrollment was permitted to continue.

The second interim analysis considered the following: (a) If five or fewer patients had confirmed CR or PR, the null hypothesis was accepted; (b) If at least 11 patients had confirmed CR or PR, the null hypothesis was rejected; (c) If six to 10 patients responded, accrual proceeded to stage 3. Stage 3 would accrue a cumulative total of 135 and consider the following: (a) If 14 or fewer patients had confirmed PR/CR, the null hypothesis was accepted. Patient recruitment was placed on hold, at the outset of the second interim analysis, to complete statistical analysis; however, investigators were permitted to enroll patients who were already in the screening phase. Due to rapid multicenter accrual, at the accrual hold, 147 patients had been screened and were eligible for enrollment.

Table 3. Patients' Baseline Characteristics (N = 137)

Characteristic	No.	%
Age, years		
Median	69	
Range	28-86	
>65	84	61
≤65	53	39
Sex		
Male	97	71
Female	40	29
ECOG performance status		
0	68	50
1	69	50
Child-Pugh status		
A	98	72
B	38	28
Missing	1	< 1
AFP > ULN		
Yes	104	76
No	33	24
Positive hepatitis status*		
Hepatitis B	23	17
Hepatitis C	66	48
Disease stage at study entry (TNM classification)		
II	4	3
IIIA/IIIB	42	31
IV	91	66
Grading (AJCC) at initial diagnosis		
Not applicable†	23	17
Well-differentiated	35	26
Moderately well-differentiated	36	26
Poorly differentiated	21	15
Undifferentiated	1	< 1
Not assessable‡	21	15

Abbreivations: AFP, alphafetoprotein; AJCC, American Joint Committee on Cancer; ULN, upper limit of normal; ECOG, Eastern Cooperative Oncology Group.
*Hepatitis B or C status was missing in 30 and 7 patients, respectively.
†No tissue available for examination.
‡Tissue available, but pathology result was inconclusive.

RESULTS

Demographics

The study enrolled 147 patients in Belgium, France, Italy, Israel, and the United States from August 2002 until June 2003, and all results

Table 4. Best Response (N = 137) Based on Independent Assessment

Best Response	No.	%
Partial response	3	2.2
Minor response	8	5.8
Stable disease*	46	33.6
Progressive disease (by radiologic assessment)	48	35.0
Not available for independent review	32	23.4

*To be classified as stable disease, patients needed to have stable disease for ≥ 16 weeks.

are based on the treated population of 137 patients (Table 3). Ten patients did not meet the inclusion/exclusion criteria, and were registered as screening failures. Twelve patients were still receiving treatment as of May 16, 2004. Sixty-five percent had hepatitis B or C.

Dose and Duration of Therapy

Median study duration was 3.4 months (range, 0 to 17.4 months), median number of treatment cycles was four (range, 1 to 19), and 72% of patients (92 of 128 patients) received six or fewer treatment cycles. Of the 132 patients who discontinued, 79 were because of disease progression, 27 because of adverse events, and 11 died.

Efficacy

Independently assessed responses were as follows: three patients (2.2%) achieved PR, eight (5.8%) had MR, and 46 (33.6%) had SD (≥ 16 weeks; Table 4). Of the three patients who had independently confirmed PR, the duration of response ranged from 12.0 to 14.5 months. Responses based on investigator assessment were as follows: eight (5.8%) achieved PR, six (4.4%) had MRs, and 50 (36.5%) had SD (≥ 16 weeks).

Tumor Necrosis

Despite tumors' appearing to have grown, many patients' scans displayed central TN. TN was assessed more rigorously in 11 patients (Fig 1). Tumors that increased in size (diameter and cross-product) demonstrated increases in TN. Baseline mean TN was 9.8% (range 0.4% to 33.5%), tumor diameter was 6.4 cm (range 2.5 to 14.2 cm), and cross-product was 28.9 cm^2 (range 5.3 to 91.3 cm^2). Follow-up mean TN was 27% (0.7% to 75%), tumor diameter was 7.2 cm (1.7 to 16.0 cm), and cross-product was 36.9 cm^2 (2.1 to 162.5 cm^2).

TTP and Survival

On the basis of investigator assessment, median TTP was 4.2 months (Fig 2). On the basis of independent assessment, median TTP was 5.5 months (Fig 3), and median overall survival was 9.2 months (Fig 4).

Toxicity

The most common drug-related adverse events (any grade) were dermatologic, constitutional, and GI (Table 5). Grade 3 toxicities included fatigue (9.5%), diarrhea (8.0%), and HFS (5.1%).

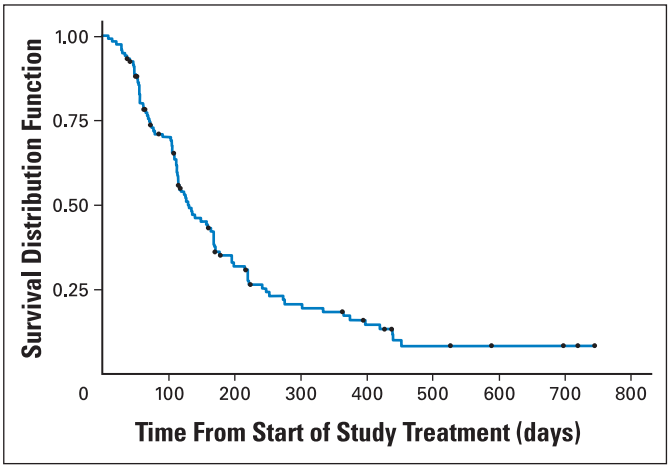

Fig 2. Median investigator assessed time to progression for the treated population (N = 137) was 4.2 months.

Sixty-day mortality was 10% of patients; 13 of 14 deaths within the first 60 days from starting therapy were related to PD. One death was secondary to an intracranial hemorrhage, but it is unclear whether it was drug related.

PK Data

There was some variability in AUC and C_{max} values, which were slightly greater in CP B than in CP A groups (Table 6; Fig 5). These differences were not considered significant.

Biomarker Evaluation: Tumor-Cell pERK Levels

Thirty-three patients had tissue available for tumor-cell pERK staining and comparative analyses. In the majority of tumor samples, staining was generally most intense within the nucleus of tumor cells (Fig 6), and regional differences in the amount of staining were observed. There was a significant difference in TTP between patients with higher (2 to 4+, n = 18) tumor-cell pERK staining intensity, in archived specimens obtained before study treatment, versus those with lower intensity (0 to 1+, n = 15; $P = .00034$; Figs 6 and 7). Patients with tumors expressing higher pERK staining intensity had a longer TTP.

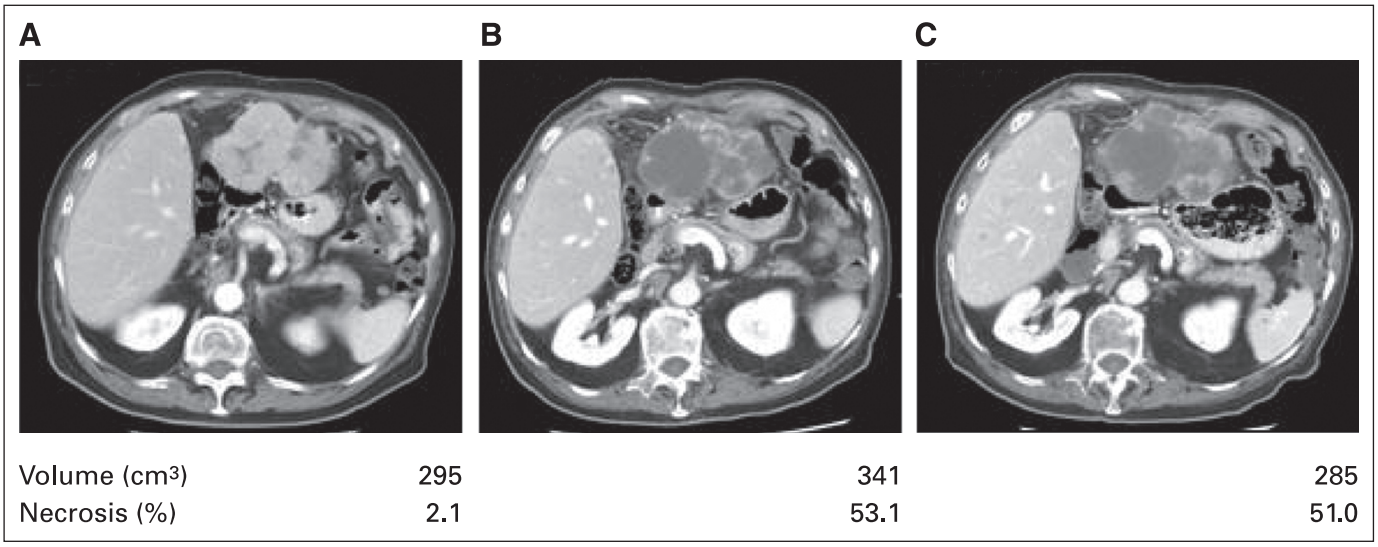

Fig 1. A representative example of baseline and serial follow-up scans demonstrating tumor necrosis in a hepatocellular carcinoma patient.

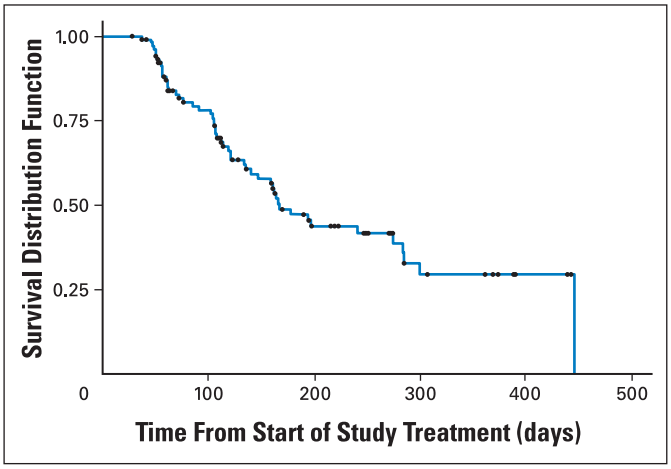

Fig 3. Median independently assessed time to progression for the treated population (N = 137) was 5.5 months.

Levels of nonspecific background staining were very low, and were attributable to endogenous biotin, pigments, or residual peroxidase. There was also some evidence of pERK staining within most tumor samples, predominantly localized to the nuclei, in non-neoplastic cells including endothelial cells, fibroblasts, and lymphocytes.

Biomarker Evaluation: Blood-Cell RNA Expression Patterns

Twenty-one patients were initially evaluated for blood-cell RNA expression levels. A panel of 18 genes was identified whose expression distinguished "nonprogressors" (investigator assessed) from "progressors" with an estimated accuracy of 100% (Table 7). This panel of 18 genes was then used to predict, in a blinded manner, the status of an additional 10 patients (eight nonprogressors and two progressors) receiving sorafenib. The predictive accuracy of the panel was 100%, because the status of all 10 patients was predicted correctly.

DISCUSSION

In this phase II trial, sorafenib was generally well tolerated and demonstrated antitumor activity in advanced HCC patients. HCC is a highly resistant solid tumor, and HCC cells have been shown to overexpress the multidrug resistance gene[15] and gene product P-glycoprotein.[16] HCC is associated with upregulation of dihydropyrimidine dehydrogenase, and thus is potentially resistant to chemotherapies such as fluorouracil.[17,18] With the development of novel targeted therapies, there is an opportunity to evaluate these agents in HCC.[19] Sorafenib demonstrated a median overall survival of 9.2 months, with 34% of patients achieving SD for at least 16 weeks and 8% achieving PRs or MRs. These data compare favorably with single-arm studies evaluating combination therapy (cisplatin, interferon, doxorubicin, and fluorouracil [PIAF] or doxorubicin plus cisplatin) in HCC patients.[20,21] Median overall survival rates of 8.9 and 7.3 months and SD rates of 28% and 16%, respectively, for PIAF and doxorubicin/cisplatin regimens were reported.[20,21] Despite differences in study designs, our results are not unlike median overall survival results from a recent randomized phase III trial by Yeo et al[22] in HCC of doxorubicin versus PIAF; 6.8 and 8.7 months for doxorubicin and PIAF arms, respectively). A notable difference in the Yeo et al trial is the higher rate of HBV, which is consistent with an Asian population.

Table 5. Grade 3 and 4 Drug-Related Adverse Events in ≥ 10% of All 137 Patients

Adverse Event	All Grades		Grade 3		Grade 4	
	No.	%	No.	%	No.	%
Dermatology						
Hand-foot skin reaction	42	30.7	7	5.1	0	0
Rash/desquamation	23	16.8	1	0.7	0	0
Alopecia	14	10.2	0	0	0	0
Constitutional symptoms						
Fatigue	41	29.9	13	9.5	0	0
Gastrointestinal						
Diarrhea (without colostomy)	59	43.1	11	8.0	0	0
Nausea	22	16.1	0	0	0	0
Anorexia	19	13.9	2	1.5	0	0
Stomatitis	15	10.9	1	0.7	0	0
Vomiting	14	10.2	0	0	0	0

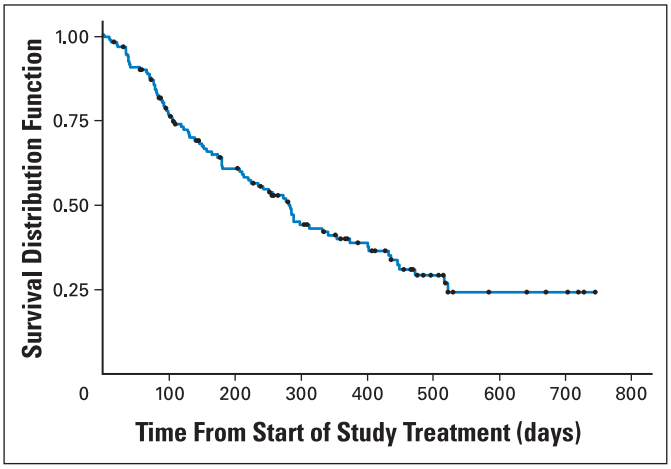

Fig 4. Median investigator-assessed overall survival for the treated population (N = 137) was 9.2 months.

Table 6. Sorafenib Plasma Pharmacokinetic Parameters in Hepatically Impaired Cancer Patients

Status	$AUC_{0\text{-}8}$ (mg · h/L)	C_{max} (mg/L)	t_{max} (hours)
Child-Pugh A (n = 14)			
Geometric mean	25.4	4.9	
Approx. CV%	38.4%	38.7%	
Median			1.0
Range			0-12
Child-Pugh B (n = 8)			
Geometric mean	30.3	6.0	
Approx. CV%	82.1%	73.8%	
Median			0.5
Range			0-8

NOTE. $AUC_{0\text{-}8}$ was reported because plasma samples were collected only up to 8 hours in all patients.
Abbreviations: $AUC_{0\text{-}8}$, area under the curve over 8 hours; C_{max}, maximum concentration; t_{max}, time to maximum concentration; CV, coefficient of variation.

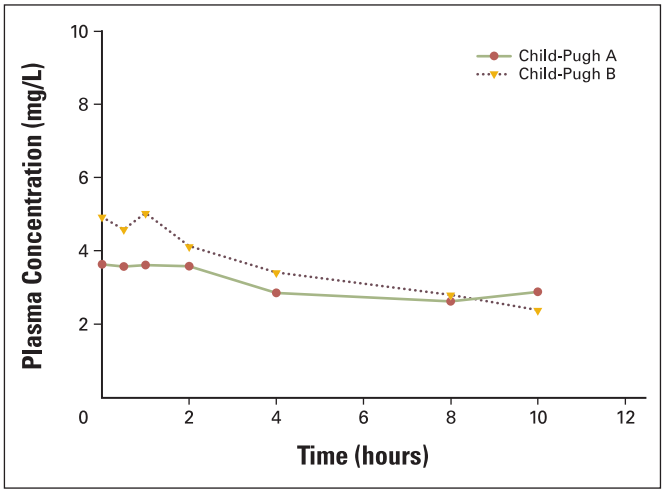

Fig 5. Geometric mean plasma concentrations of sorafenib following administration of 400 mg bid in hepatocellular carcinoma patients with either Child-Pugh A or B hepatic impairment.

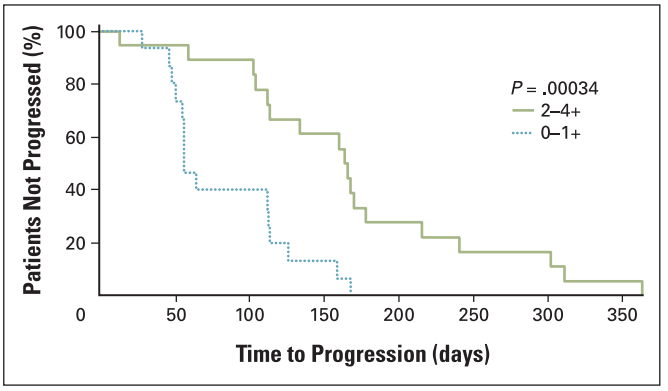

Fig 7. Percentage of patients not progressed plotted as a function of time to progression in patient tumors with a maximum phosphorylated extracellular signal regulated kinase staining intensity of either 0 and 1+ or 2+ through 4+ (n = 33).

There is also evidence that sorafenib may be combined successfully with other agents in HCC on the basis of a strong preclinical rationale and a favorable toxicity profile. Efficacy was demonstrated in a phase I combination study with sorafenib and doxorubicin in advanced HCC patients (one PR, one unconfirmed PR, and 61% SD).[23]

There is poor correlation between TN and conventional methods of response assessment, which poses questions of how best to quantify efficacy of sorafenib. Despite tumors' increasing in size, the observation of TN in this study is intriguing. Although the usefulness of TN in assessing efficacy of anticancer agents in HCC remains to be established, it is a potentially significant clinical end point that warrants further investigation. However, the relationship between tumor necrosis and clinical outcome remains to be determined.

Relatively infrequent dose-limiting toxicities were observed. Notable grade 3/4 adverse events included fatigue, diarrhea, and HFS, which are commonly associated with sorafenib.[11] The interpatient PK differences between CP A and B patients were not clinically relevant, because sorafenib was equally well tolerated by these two subgroups, and exposure values were similar to those reported in phase I studies that showed no relationship between PK variability and toxicity.[11] Importantly, it is unlikely that dose adjustment would be necessary when administering sorafenib to patients with mild (CP A) or moderate (CP B) hepatic insufficiency.

Several biomarkers have been shown to have potential predictive significance in HCC.[24,25] Because the Raf/MEK/ERK pathway has a role in HCC, and is targeted by sorafenib, pERK may be a useful biomarker. Staining was most intense in nuclei of tumor cells in this study, consistent with translocalization of pERK to the nucleus after activation.[26] Furthermore, HCC patients whose tumors expressed higher baseline pERK levels had a longer TTP following treatment with sorafenib. These data suggest that tumors containing higher levels of pERK are more sensitive, or responsive, to sorafenib.

WBCs and peripheral blood mononuclear cells, the main sources of RNA isolated from whole blood, are considered a "surrogate tissue" relative to a primary tumor or metastasis.[25] Therefore, gene-expression patterns of WBCs and peripheral blood mononuclear cells can be a molecular signature of a tumor that provides information on histologic stage or potential to respond to treatment. Although the RNA expression data from this study are encouraging, functional roles remain to be elucidated for the panel of genes that distinguished nonprogressors from progressors.

Cell-based and genomic analyses will undoubtedly advance the discovery of new biomarkers for HCC, and help refine inclusion criteria and patient selection. Analysis with a larger number of patients in a placebo-controlled trial is required to validate whether components identified in this study may be used prospectively to predict response to sorafenib. Evaluation of sorafenib in combination with cytotoxic agents in HCC is ongoing.

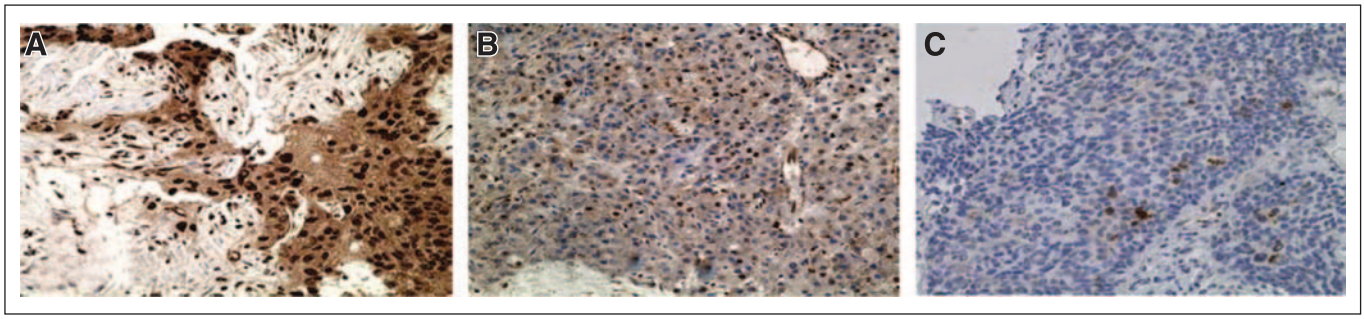

Fig 6. Pretreatment nuclear phosphorylated extracellular signal regulated kinase (pERK) maximum staining intensity (MSI) levels of hepatocellular carcinoma biopsies from three patients. (A) MSI = 4+ (76% to 100% of nuclei stained positively for pERK); time to progression (TTP) = 178 days; minor response. (B) MSI = 3+ (50% to 75% of nuclei stained positively for pERK); TTP = 134 days; stable disease. (C) MSI = 1+ (6% to 25% of nuclei stained positively for pERK); TTP = 46 days; progressive disease.

Table 7. Panel of 18 Genes Identified in Blood-Cell RNA That Were Differentially Expressed in Nonprogressors and Progressors

ProbeSet	Gene Description	Gene Symbol	Unigene ID
243570_at	KIAA0102 gene product	*KIAA0102*	Hs.87095
218287_s_at	Eukaryotic translation initiation factor 2C, 1	*EIF2C1*	Hs.309452
213957_s_at	Centrosome-associated protein 350	*CAP350*	Hs.413045
225469_at	Hypothetical protein LOC144363	*LOC144363*	Hs.447488
226154_at	Dynamin 1-like	*DNM1L*	Hs.180628
53720_at	Angiopoietin-related protein 5	*ARP5*	Hs.306971
202509_s_at	Tumor necrosis factor alpha-induced protein 2	*TNFAIP2*	Hs.101382
241408_at	Hypothetical protein FLJ34443	*FLJ34443*	Hs.26410
200732_s_at	Protein tyrosine phosphatase type IVA, member 1	*PTP4A1*	Hs.227777
228329_at	LOC343202	—	Hs.4204
225028_at	*H. sapiens* transcribed sequences	—	Hs.99676
214440_at	*N*-acetyltransferase 1 (arylamine *N*-acetyltransferase)	*NAT1*	Hs.458430
203405_at	Down syndrome critical region gene 2	*DSCR2*	Hs.5198
213379_at	Hypothetical protein CL640	*CL640*	Hs.144304
214370_at	S100 calcium binding protein A8 (calgranulin A)	*S100A8*	Hs.416073
221190_s_at	Colon cancer-associated protein Mic1	*MIC1*	Hs.287633
210357_s_at	Chromosome 20 open reading frame 16	*C20orf16*	Hs.433337
32209_at	Mouse mammary tumor virus receptor homolog 1	*MTVR1*	Hs.25723

REFERENCES

1. Parkin DM, Pisani P, Ferlay J: Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cancer 80:827-841, 1999

2. McGlynn KA, Tsao L, Hsing AW, et al: International trends and patterns of primary liver cancer. Int J Cancer 94:290-296, 2001

3. El Serag HB, Mason AC: Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 340:745-750, 1999

4. Llovet JM, Bruix J, Gores GJ: Surgical resection versus transplantation for early hepatocellular carcinoma: Clues for the best strategy. Hepatology 31:1019-1021, 2000

5. Abou-Alfa GK: Current and novel therapeutics for hepatocellular carcinoma. Am Soc Clin Oncol Educational Book, Alexandria, VA, 2004, pp 192-197

6. Huynh H, Nguyen TT, Chow KH, et al: Overexpression of the mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK in hepatocellular carcinoma: Its role in tumor progression and apoptosis. BMC Gastroenterol 3:19, 2003

7. Giambartolomei S, Covone F, Levrero M, et al: Sustained activation of the Raf/MEK/Erk pathway in response to EGF in stable cell lines expressing the Hepatitis C Virus (HCV) core protein. Oncogene 20:2606-2610, 2001

8. Shimotohno K, Watashi K, Tsuchihara K, et al: Hepatitis C virus and its roles in cell proliferation. J Gastroenterol 37:50-54, 2002 (suppl)

9. Yoshiji H, Kuriyama S, Yoshii J, et al: Halting the interaction between vascular endothelial growth factor and its receptors attenuates liver carcinogenesis in mice. Hepatology 39:1517-1524, 2004

10. Wilhelm SM, Carter C, Tang L, et al: BAY 43-9006 exhibits broad spectrum oral anti-tumor activity and targets the Raf/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res 64:7099-7109, 2004

11. Strumberg D, Richly H, Hilger RA, et al: Phase I clinical and pharmacokinetic study of the novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43-9006 in patients with advanced refractory solid tumors. J Clin Oncol 23:965-972, 2005

12. Pero R, Peto J: Asymptotically efficient rank-invariant test procedures. J R Stat Soc A 135:185-206, 1972

13. Hofmann WK, de Vos S, Elashoff D, et al: Relation between resistance of Philadelphia-chromosome-positive acute lymphoblastic leukaemia to the tyrosine kinase inhibitor STI571 and gene-expression profiles: A gene-expression study. Lancet 359:481-486, 2002

14. Kurokawa Y, Matoba R, Nagano H, et al: Molecular prediction of response to 5-fluorouracil and interferon-{alpha} combination chemotherapy in advanced hepatocellular carcinoma. Clin Cancer Res 10:6029-6038, 2004

15. Chenivesse X, Franco D, Brechot C: MDR1 (multidrug resistance) gene expression in human primary liver cancer and cirrhosis. J Hepatol 18:168-172, 1993s

16. Soini Y, Virkajarvi N, Raunio H, et al: Expression of P-glycoprotein in hepatocellular carcinoma: A potential marker of prognosis. J Clin Pathol 49:470-473, 1996

17. DeVita VT Jr, Abou-Alfa GK: Therapeutic implications of the new biology. Cancer J 6 Suppl 2:S113–S120, 2000

18. Jiang W, Lu Z, He Y, et al: Dihydropyrimidine dehydrogenase activity in hepatocellular carcinoma: Implication in 5-fluorouracil-based chemotherapy. Clin Cancer Res 3:395-399, 1997

19. Abou-Alfa GK, Morse M: New small molecules for liver tumors, in Clavien P (ed): Malignant Liver Tumors, Current and Emerging Therapies (ed 2). Sudbury, MA, Jones and Bartlett, 2004, pp 307-314

20. Leung TW, Patt YZ, Lau WY, et al: Complete pathological remission is possible with systemic combination chemotherapy for inoperable hepatocellular carcinoma. Clin Cancer Res 5:1676-1681, 1999

21. Lee J, Park JO, Kim WS, et al: Phase II study of doxorubicin and cisplatin in patients with metastatic hepatocellular carcinoma. Cancer Chemother Pharmacol 54:385-390, 2004

22. 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 97:1532-1538, 2005

23. Richly H, Kupsch P, Passage K, et al: Results of a phase I trial of BAY 43-9006 in combination with doxorubicin in patients with primary hepatic cancer. Int J Clin Pharmacol Ther 42:650-651, 2004

24. DePrimo SE, Wong LM, Khatry DB, et al: Expression profiling of blood samples from an SU5416 phase III metastatic colorectal cancer clinical trial: A novel strategy for biomarker identification. BMC Cancer 3:3, 2003

25. Qin LX, Tang ZY: Recent progress in predictive biomarkers for metastatic recurrence of human hepatocellular carcinoma: A review of the literature. J Cancer Res Clin Oncol 130:497-513, 2004

26. Khokhlatchev AV, Canagarajah B, Wilsbacher J, et al: Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. Cell 93:605-615, 1998

Acknowledgment

We thank Douglas Bigwood, MD, for his contribution to the biomarker analyses.

Authors' Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. 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.

Authors	Employment	Leadership	Consultant	Stock	Honoraria	Research Funds	Testimony	Other
Ghassan K. Abou-Alfa						Bayer (B)		
Chetan Lathia	Bayer Healthcare Pharmaceuticals (N/R)							
Ian Taylor	Bayer Healthcare Pharmaceuticals Division (N/R)							
Marius Moscovici	Bayer S.p.A Socio-Unico, Italy (N/R)							
Brian Schwartz	Bayer Pharmaceuticals (N/R)							
Leonard B. Saltz						Bayer (B)		

Dollar Amount Codes (A) < $10,000 (B) $10,000-99,999 (C) ≥ $100,000 (N/R) Not Required

Author Contributions

Conception and design: Ghassan K. Abou-Alfa, Lawrence Schwartz, Chetan Lathia, Brian Schwartz, Leonard B. Saltz
Administrative support: Jean-Yves Douillard
Provision of study materials or patients: Ghassan K. Abou-Alfa, Lawrence Schwartz, Sergio Ricci, Dino Amadori, Armando Santoro, Arie Figer, Jacques De Greve, Jean-Yves Douillard
Collection and assembly of data: Lawrence Schwartz, Jacques De Greve, Chetan Lathia, Ian Taylor, Brian Schwartz
Data analysis and interpretation: Ghassan K. Abou-Alfa, Lawrence Schwartz, Jacques De Greve, Chetan Lathia, Ian Taylor, Marius Moscovici, Brian Schwartz, Leonard B. Saltz
Manuscript writing: Ghassan K. Abou-Alfa, Leonard B. Saltz
Final approval of manuscript: Ghassan K. Abou-Alfa, Jacques De Greve, Jean-Yves Douillard, Chetan Lathia, Brian Schwartz, Ian Taylor, Leonard B. Saltz

GLOSSARY

Antiangiogenic: A process involved blocking the generation of new blood vessels in a tumor, which disrupts the blood supply thereby preventing tumor growth.

Blood-cell RNA expression: Provides a snapshot of the genes expressed in blood cells at specific points in time. This snapshot can be acquired by screening throughout the treatment period. Using mathematical algorithms, the extent of a particular gene expression can be used to predict response to a certain therapy.

Child-Pugh: A set of five independent parameters first developed by Pugh and later revised by Child, which help in predicting prognostic outcome of patients with liver cirrhosis. The five parameters are albumin, bilirubin, prothrombin time/INR, clinical ascites, and encephalopathy. While none of these parameters is tumor specific, this scoring systems is used for hepatocellular carcinoma by default. Other more tumor and risk factor specific scoring systems include the CLIP (Cancer of the Liver Italian Program) for hepatitis C–related hepatocellular carcinoma and CUPI (Chinese University Prognostic Index) for hepatitis B–related hepatocellular carcinoma.

HCC (hepatocellular carcinoma): HCC is a type of adenocarcinoma. This is the most common form of liver cancer.

Microarray: A miniature array of regularly spaced DNA or oligonucleotide sequences printed on a solid support at high density that is used in a hybridization assay. The sequences may be cDNAs or oligonucleotide sequences that are synthesized in situ to make a DNA chip.

pERK (phosphorylated extracellular signal regulated kinase): ERK is a downstream enzyme of the MAP kinase pathway that is directly activated by Raf to pERK. In case of Raf inhibition like with sorafenib, the level of pERK serves as a surrogate of this inhibition and could be correlated to response to therapy.

Raf kinase: Receptor activation factor (RAF) kinase, or MAPKK kinase, or MAPKKK is an essential component of the MAP Kinase pathway which is a key signaling mechanism that regulates many cellular functions such as cell growth, transformation, and apoptosis. Raf can be mutated or overexpressed in certain types of cancer. Raf kinase is a target of inhibition by sorafenib. The regulation of Raf is complex and involves the integration of other signaling pathways as well as intramolecular interactions, phosphorylation, dephosphorylation, and protein-protein interactions.

Sorafenib: A substance belonging to the family of drugs called raf kinase inhibitors and anti-VEGF that is being studied in the treatment of cancer.

VEGFR (vascular endothelial growth factor receptor): VEGFRs are transmembrane tyrosine kinase receptors to which the VEGF ligand binds. VEGFR-1 (also called Flt-1) and VEGFR-2 (also called KDR/Flk-1 [murine homologue]) are expressed on endothelial cells, while VEGFR-3 (also called Flt-4) is expressed on cells of the lymphatic and vascular endothelium. VEGFR-2 is thought to be principally responsible for angiogenesis and for the proliferation of endothelial cells. Typically, most VEGFRs have seven extracellular immunoglobulin-like domains responsible for VEGF binding, and an intracellular tyrosine kinase domain.