Phase I Trial of Sorafenib in Combination with IFN α-2a in Patients with Unresectable and/or Metastatic Renal Cell Carcinoma or Malignant Melanoma

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Abstract

Purpose: To determine the safety, maximum tolerated dose, pharmacokinetics, and efficacy, and to evaluate biomarkers, of the multikinase inhibitor sorafenib plus IFN α-2a in advanced renal cell carcinoma (RCC) or melanoma.

Experimental Design: Patients received 28-day cycles of continuous, oral sorafenib twice daily and subcutaneous IFN thrice weekly: sorafenib 200 mg twice daily plus IFN 6 million IU (MIU) thrice weekly (cohort 1); and sorafenib 400 mg twice daily plus IFN 6 MIU thrice weekly (cohort 2); or plus IFN 9 MIU thrice weekly (cohort 3). Tumor response was assessed by Response Evaluation Criteria in Solid Tumors and dynamic contrast-enhanced ultrasonography.

Results: Thirteen patients received at least one dose of sorafenib plus IFN (12 RCC; one melanoma). The maximum tolerated dose was not reached [only one dose-limiting toxicity (grade 3 asthenia)]. Most frequently reported drug-related adverse events were grade 2 or less in severity, including fatigue, diarrhea, nausea, alopecia, and hand-foot skin reaction. One (7.7%) RCC patient achieved partial response and eight (61.5%) had stable disease (including the melanoma patient). Good responders assessed by dynamic contrast-enhanced ultrasonography had increased progression-free survival and overall survival, relative to poor responders. IFN had no effect on the pharmacokinetics of sorafenib. There were no significant changes in absolute values of lymphocytes, levels of proangiogenic cytokines, or inhibition of phosphorylated extracellular signal-regulated kinase in T cells or natural killer cells, with combination therapy.

Conclusions: This sorafenib combination was well tolerated, with preliminary antitumor activity in advanced RCC and melanoma patients. There were no drug-drug interactions and the recommended dose for future studies is sorafenib 400 mg twice daily plus IFN 9 MIU.

Renal cell carcinoma (RCC) and melanoma have poor prognoses when diagnosed in advanced stages (1), and are associated with a median survival of ≤12 months (2, 3). RCC accounts for 80% to 95% of kidney tumors, and ∼30% of RCC patients have metastatic disease at diagnosis (1, 4, 5).

The Raf/extracellular signal-regulated kinase (ERK) kinase (MEK)/ERK signaling pathway, which regulates cell proliferation and survival (6), is implicated in the onset and development of RCC (7) and melanoma (6). Overactivation of Raf-1 is detected in 55% of RCC biopsies in the absence of oncogenic ras/raf mutations (7). Clear cell carcinomas, which account for 75% to 85% of renal tumors, are characterized by loss of the von Hippel-Lindau tumor-suppressor gene (8), leading to overexpression of proangiogenic vascular endothelial growth factor (VEGF) and platelet-derived growth factor-β (9, 10). Activating b-raf mutations occur in >60% of melanomas, of which ∼90% are V600E (11, 12). The V600E b-raf mutant is constitutively active and implicated in tumor development (12). Inhibiting Raf/MEK/ERK signaling induces apoptosis in melanoma cells, but not in normal melanocytes, suggesting that oncogenic b-raf may promote cell survival (6).

Although recombinant IFN α-2a is a standard treatment for RCC and melanoma, complete responses in RCC are rare (<5%) and mean response rate is only 15% (13), whereas response rates of 10% to 20% were reported in phase II studies in metastatic melanoma (2, 14). RCC and melanoma are particularly resistant to radiotherapy and chemotherapy, and there is a need to develop effective, and better tolerated, targeted therapies for these diseases (15).

Sorafenib (Nexavar) is an oral multikinase inhibitor that targets Raf kinase and receptor tyrosine kinases, including VEGF receptor-2 and platelet-derived growth factor receptor-β (16). Sorafenib inhibited tumor growth in animal cancer models by targeting the tumor or tumor endothelia to inhibit proliferation...
and angiogenesis (12, 16–18). In phase II/III trials, sorafenib significantly prolonged progression-free survival versus placebo and showed good tolerability in advanced RCC patients (19, 20). Based on these phase II/III RCC findings, sorafenib was approved recently for the treatment of advanced RCC. Sorafenib may be acting through inhibition of angiogenesis in RCC, but the precise mechanism of action by which sorafenib exerts its clinical effects, and the etiologic role of the Raf/MEK/ERK pathway, are undergoing further investigation in this tumor type.

In two human melanoma xenograft models, sorafenib inhibited tumor growth by blocking tumor cell VEGF production, resulting in antiangiogenic, antiproliferative, and apoptotic effects (12). Although sorafenib has not shown antitumor activity as a single agent in melanoma, phase I/II trials with sorafenib in combination with dacarbazine (21) and carboplatin/paclitaxel (22) showed promising efficacy and acceptable tolerability in patients with this disease. It is unclear exactly why these combinations are particularly effective; however, combining agents with different mechanisms of action may help overcome tumor drug resistance mechanisms and enhance antitumor activity (23). Although it remains to be determined in appropriately controlled trials in patients with melanoma whether combination therapies with sorafenib show superior efficacy to chemotherapy alone, sorafenib has shown almost double the clinical benefit of historical controls of carboplatin/paclitaxel alone in second-line melanoma patients (85% versus 45%; refs. 24, 25). Whether this effect is mediated through inhibition of the Raf kinase pathway, rather than inhibition of angiogenesis, is also presently unclear.

It is widely accepted that tumors depend on angiogenesis for growth, invasion, and metastasis. Highly vascularized tumors are generally larger than tumors with no arterial vascularization (26). There is some evidence to suggest that targeted therapies can induce changes in tumor structure (e.g., by decreasing tumor vascularity or inducing necrosis), consistent with a therapeutic response, before a change in tumor size or volume is observed (27, 28). Furthermore, changes in tumor vascularization can be predictive of subsequent changes in tumor volume (29, 30). Standard response end points based on unidimensional and bidimensional measurements, such as Response Evaluation Criteria in Solid Tumors or WHO criteria, originally designed to evaluate cytotoxic drugs, do not accurately reflect changes in tumor volume and, therefore, often fail to accurately register responses to targeted agents, which are typically cytostatic (31). Imaging techniques that provide morphologic and functional perfusion data, such as Doppler ultrasonography with contrast-agent injection (32), may be combined with standard criteria to better assess the efficacy of targeted agents. This technique may prove useful as a surrogate marker of response, as it can accurately determine the size of abdominal tumors and the proportion of contrast agent taken up by the tumor—a measure of tumor vascularity. An early reduction in tumor vascularity and/or tumor volume was shown to correlate with response, and was predictive of progression-free survival and overall survival (33).

This phase I study was undertaken to determine the safety, maximum tolerated dose (MTD), and pharmacokinetics of sorafenib plus IFN in advanced RCC or melanoma patients. IFN was selected as it is used widely alone or in combination to treat a range of cancers. Dynamic contrast-enhanced ultrasonography (DCE-US) was used to measure the effects of sorafenib/IFN on the tumor and tumor vasculature.

Materials and Methods

Patient selection

Patients with histologically or cytologically confirmed unresectable and/or metastatic RCC or malignant melanoma, refractory to standard treatment, were eligible for inclusion. Additional inclusion criteria included age ≥18 years; Eastern Cooperative Oncology Group performance status of 0 or 1; life expectancy ≥12 weeks; and adequate bone marrow, liver, and renal function. Exclusion criteria included cardiac disease; congestive heart failure or uncontrolled hypertension; cerebral disease; serious infections; cirrhosis; brain tumors; seizure disorder; history of organ allograft; anticancer chemotherapy or hormonal therapy 4 weeks before initiating IFN (i.e., day −14); radiotherapy within 3 weeks of study entry; major surgery within 4 weeks of study entry; or prior exposure to Ras, MEK, or VEGF pathway inhibitors.

The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonisation Good Clinical Practice Guideline (Note for Guidance on Good Clinical Practice), and the German drug law (AMG). All patients gave written informed consent in accordance with institutional and federal guidelines before study participation.

Study design

Phase I, single-center, open-label, noncontrolled, dose-escalation study. IFN alone [either 6 or 9 million IU (MIU)] was administered during a 2-week run-in period to allow recovery from acute IFN-related toxicities (e.g., flu-like symptoms). Patients then received 28-day cycles of continuous, oral sorafenib twice daily and s.c. injections of IFN thrice weekly on days 1, 3, and 5, until progressive disease, unacceptable toxicity, or death.

Patients were entered into three cohorts: sorafenib 200 mg twice daily and IFN 6 MIU thrice weekly (cohort 1); sorafenib 400 mg twice daily and IFN 6 MIU thrice weekly (cohort 2); or IFN 9 MIU thrice weekly (cohort 3). Dose escalation was done in groups of three to six patients (Fig. 1), based on the occurrence of dose-limiting toxicities (DLT). DLTs were defined by the National Cancer Institute-Common Terminology Criteria for Adverse Events version 3.0 as follows: grade 4 neutropenia (absolute neutrophil count <500/mm3) for 7 days; grade 4 thrombocytopenia of any duration with sepsis or a fever ≥38.5°C; platelet count <25,000/mm3; grade 3/4 nonhematologic toxicity (excluding grade 3 nausea and vomiting and transient fever). The MTD was the dose at which at least two of three or six patients developed a DLT during the first treatment cycle.

Study outcomes

The primary objectives were to determine the MTD of sorafenib plus IFN, identify the recommended dose for future studies, and evaluate safety. Secondary objectives evaluated tumor response, overall duration of response, progression-free survival, overall survival, pharmacokinetics, and biomarkers.

Safety. Patients who received at least one dose of sorafenib/IFN combination were evaluable for safety. Adverse events were graded according to the National Cancer Institute-Common Terminology Criteria for Adverse Events version 3.0.

Efficacy. Patients who completed at least two treatment cycles were evaluable for tumor response. Baseline radiologic examinations were done within 28 days before administration of sorafenib/IFN. Tumor response was evaluated every two cycles (8 weeks) according to Response Evaluation Criteria in Solid Tumors. Partial response was defined as ≥30% decrease in the sum of the longest diameter of target lesions; progressive disease was ≥20% increase; and stable disease was a change in tumor measurement between these two variables.
Evaluation of efficacy using DCE-US

Morphologic studies. Three diameters of each lesion (one or two per patient) were measured with electronic calipers, and tumor volume was calculated as depth \times length \times width/2 (mm$^3$). Only patients with lesions accessible to ultrasonography could be analyzed in this part of the study.

Dynamic studies. A total of 29 DCE-US examinations were done before treatment and after 2 and 4 weeks of sorafenib using an Aplio sonograph (Toshiba Medical, Puteaux, France) with a 4.4 MHz C37 convex array. The percentage uptake of Sonovue (after i.v. bolus injection of 4.8 mL at 8 μL/mL) contrast agent (Bracco S.P.A., Milan, Italy), which consists of sulfur hexafluoride–filled microbubbles (comprising encapsulated stable perfluorocarbon gas), throughout each lesion was measured qualitatively by Vascular Recognition Imaging software, and validated by two radiologists. Advanced Dynamic Flow allowed simultaneous, but independent, real-time visualization of the both the tumor and contrast agent. The dynamic sequence was recorded on a digital tape. Two emission processes were used at a low mechanical index to avoid destroying contrast agent microbubbles, which enabled microvessel flow direction (red/blue) and perfusion (green) to be detected. Qualitative and quantitative evaluations were done. For qualitative analysis, the percentage of contrast uptake throughout the lesion was evaluated by the radiologist conducting the examination and validated by two independent radiologists. For the quantitative analysis, quantification of mean contrast uptake by digital analysis of images was done using the following procedures: the tumor was outlined using Adobe Photoshop, which automatically discriminates colors, distinguishing two zones in two different tonalities; the image was analyzed with the Matrox Inspector software, which quantifies image pixels, allowing discrimination of the tonalities and evaluation of the percentage of perfused tissue.

Morphologic and dynamic variables were combined to evaluate tumor response: A good response was defined as a ≥20% decrease in contrast uptake coupled with stability or a decrease in tumor volume or a ≥30% decrease of volume if no modification of vascularization was visualized.

Plasma pharmacokinetics

Pharmacokinetics of sorafenib were determined on day 15 of cycle 1, and plasma samples were obtained up to 8 h postdose. Maximum concentration ($C_{\text{max}}$), area under the plasma concentration versus time curve (AUC$_{0-8}$), and time to maximum concentration (t$_{\text{max}}$) were calculated using noncompartmental methods. Plasma concentrations of sorafenib were quantified using a validated liquid chromatography/tandem mass spectrometry assay with a lower limit of quantification of

![Fig. 1. Dose-escalation schematic.](image)

Table 1. Patient demographics

<table>
<thead>
<tr>
<th>Gender</th>
<th>Cohort 1 (n = 4)</th>
<th>Cohort 2, (n = 3)</th>
<th>Cohort 3 (n = 6)</th>
<th>Total, (N = 13)</th>
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<tbody>
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<td>Male</td>
<td>3 (75)</td>
<td>2 (67)</td>
<td>3 (50)</td>
<td>8 (62)</td>
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<td>1 (25)</td>
<td>1 (33)</td>
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<td>60</td>
<td>59</td>
<td>60</td>
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<td>Range</td>
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<td>24-76</td>
<td>44-72</td>
<td>24-76</td>
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<td>2 (67)</td>
<td>4 (67)</td>
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<td>0 (0)</td>
<td>1 (33)</td>
<td>2 (33)</td>
<td>3 (23)</td>
</tr>
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<td>Site of primary tumor lesion</td>
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<td>3 (100)</td>
<td>6 (100)</td>
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<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (8)</td>
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<tr>
<td>Prior anticancer therapy*</td>
<td>Systemic therapy</td>
<td>4 (100)</td>
<td>2 (67)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Surgery†</td>
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<td>3 (100)</td>
<td>6 (100)</td>
<td>13 (100)</td>
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<td>1 (33)</td>
<td>3 (50)</td>
<td>4 (31)</td>
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<td>No. prior therapies</td>
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<td>0 (0)</td>
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<td>2</td>
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<td>3 (50)</td>
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<td>≥3</td>
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<td>0 (0)</td>
<td>3 (50)</td>
<td>6 (46)</td>
</tr>
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</table>

NOTE: All values expressed as n (%). Abbreviation: ECOG, Eastern Cooperative Oncology Group.
*Both diagnostic and therapeutic procedures.
†Twelve patients had prior nephrectomy (four, cohort 1; two, cohort 2; six, cohort 3), including the melanoma patient in cohort 1 who had prior nephrectomy for metastasis in the kidney.
Biosciences, 20

Gated to phycoerythrine (PhosFlow reagent, Becton Dickinson

Labeled with anti-pERK1/2 (T202/Y204) monoclonal antibody conju-

gated

Chased from Becton Dickinson Biosciences, Le Pont de Claix, France).

Phosphorylated ERK (pERK) is an activated form of the MAPK (mitogen-activated protein kinase) signaling pathway and is a marker of cell activation. The Raf/MEK/ERK pathway is involved in various cellular processes, including proliferation and survival. Stimulation of the pathway can be assessed by measuring the phosphorylation status of ERK using antibodies specific for the phosphorylated forms of ERK (pERK1/2). In PBLs, the activation of the Raf/MEK/ERK pathway was assessed as the fold change in pERK1/2 expression compared to unstimulated cells.

Peripheral blood samples for measuring immunologic variables were taken from patients receiving sorafenib/IFN on days 1 and 15 of treatment cycle 1; day 1 of cycles 2, 4, and 7; and at study end, using EDTA as the anticoagulant. All biomarker data from immunologic analyses are presented as mean ± SD.

Absolute values (cells/mm³) of peripheral blood lymphocytes (PBL) and subpopulations were determined by flow cytometry using four-color immunostaining with anti-CD45-FITC (all lymphocytes), anti–CD3-phycoerythrin-cyanine5 and anti–CD56-phycoerythrin-cyanine5 [natural killer (NK) lymphocytes] monoclonal antibodies and relevant controls (all purchased from Becton Dickinson Biosciences, Le Pont de Clax, France).

Activation of the Raf/MEK/ERK pathway in PBLs was assessed as previously described (34). Briefly, batches of 10⁶ PBLs were stimulated with 40 μmol/L phorbol myristate acetate (Sigma-Aldrich, Saint-Quentin Fallavier, France) or left unstimulated for 10 min at 37°C, then fixed in 2% formaldehyde PBS. After washing in PBS containing 4% FCS, cell suspensions were then resuspended in 2% formaldehyde PBS. Immunostaining with anti-CD45-FITC, anti–CD56-phycoerythrin-cyanine5 and anti–CD3-phycoerythrin-cyanine5 monoclonal antibodies (all purchased from Becton Dickinson Biosciences) for 20 min at room temperature, then resuspended in 2% formaldehyde PBS. Negative controls were done in the same conditions using isotype-matched immunoglobulins. After acquisition of 10⁶ CD45⁺ cells on a four-color FACSCalibur flow cytometer (Becton Dickinson Biosciences), analysis of pERK1/2 intensity was done using the CellQuest pro software after gating either CD45⁺CD3⁺CD56⁻ [T lymphocytes] or CD45⁺CD3⁻CD56⁺ (NK lymphocytes) cells. Raf/MEK/ERK pathway activation was expressed as stimulation index by calculating the ratio of mean fluorescence intensity of pERK1/2 after stimulation with phorbol myristate acetate versus unstimulated intensity levels. Therefore, the stimulation index reflects the number of cells with activated pERK among all T or NK lymphocytes.

Plasma levels of proangiogenic biomarkers and cytokines (VEGF, basic fibroblast growth factor, tumor necrosis factor α, interleukin (IL)-6, and IL-10) were also measured. Cytokines were measured using ELISA kits for VEGF and basic fibroblast growth factor (R&D Systems, Minneapolis, MN), IL-6, IL-10, and tumor necrosis factor α (Immuno-tech, Marseille, France) with sensitivity thresholds of 5, 3, 3, 6, and 5 pg/mL, respectively. Standard curves were derived for each assay from duplicate samples and experimental values were computed using linear regression analysis.

Table 2. Drug-related adverse events in ≥10% of patients

<table>
<thead>
<tr>
<th>Biomarker analyses</th>
</tr>
</thead>
<tbody>
<tr>
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Statistical analyses

The statistical analyses for baseline demographics, pharmacokinetics, and efficacy are based on all data available up to the cutoff date of the study.
July 4, 2005. The statistical evaluation of demographics was done by using the Statistical Analysis System software package (version 8.2). The following statistics were calculated for each of the pharmacokinetic sampling points: arithmetic mean, SD and coefficient of variation, geometric mean, geometric SD (retransformed SD of the logarithms) and coefficient of variation; minimum, median, maximum value; and the number of measurements. Statistical analyses of PBLs and biomarkers data were done using paired Student’s t tests comparing each data set for each time point to baseline values.

**Results**

**Patient characteristics**

Fifteen patients were enrolled in this study, but two patients (n = 1 each RCC and melanoma) enrolled in cohort 3 were subsequently removed from the study during the screening phase, due to elevated transaminases and anemia. Of 13 patients who received at least one dose of sorafenib/IFN combination, 12 had RCC and one had malignant melanoma (Table 1). The majority of patients had failed prior systemic treatment (92%) and had at least two prior therapies (92%).

All 13 patients were evaluable for safety measurements; 12 were evaluable for MTD, tumor response measurements, and biomarkers; and 11 were evaluable for pharmacokinetics.

Five patients discontinued treatment: two due to an adverse event (n = 1 fatigue in cohort 1 during cycle 1, because of personal choice, despite being only grade 2 in severity; n = 1 in cohort 2 after cycle 3 due to grade 2 fatigue and diarrhea, without disease progression) and three due to disease progression (n = 1 in cohort 2 and n = 2 in cohort 3 after cycle 2). Subsequently, two additional patients with disease progression discontinued while receiving sorafenib alone. As of July 1, 2006, six patients are ongoing (n = 2, n = 1, and n = 3 in cohorts 1-3, respectively).

**Safety**

Because only one DLT (grade 3 asthenia) was observed in one patient of six in cohort 3, the MTD was not reached. As the DLT was recorded during the 2-week run-in phase, in which IFN alone was administered, the patient subsequently received full doses of both sorafenib and IFN.

The most common drug-related adverse events (all grades) throughout the entire treatment period were constitutional (fatigue and weight loss), gastrointestinal (diarrhea, anorexia, and nausea), and dermatologic (alopecia, hand-foot skin reaction, and pruritus; Table 2). The incidence of drug-related toxicities was similar between the three cohorts. The number of patients with hand-foot skin reaction and alopecia did not seem to be increased at the highest combination doses (cohort 3) compared with those on lower doses (i.e., cohorts 1 and 2; Table 2). Grade 3 drug-related adverse events were infrequent: fatigue was observed in three (23%) patients (one patient in cohort 2 and two in cohort 3). No grade 4 drug-related adverse events were observed. No deaths were reported during the treatment period of this study, up to the data cutoff of July 4, 2005.

**Efficacy**

**Response evaluation criteria in solid tumors.** Partial response was achieved in one RCC patient (7.7%) in cohort 1 (Table 3). Ten patients had a change in tumor measurement that seemed to meet stable disease criteria (Fig. 2); however, an overall best response of stable disease was observed and confirmed in eight (61.5%) of these patients (two in cohort 1, two in cohort 2, and four in cohort 3), including the melanoma patient. Including the patient with partial response, a total of 8 of the 12 patients evaluable for response had evidence of tumor shrinkage at some point during the study (Fig. 2). Time-to-event analysis is limited.

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**Fig. 2.** Maximum change from baseline in sum of longest diameter of target lesions in patients evaluable for tumor response (n = 12). Bid, twice daily.

**Fig. 3.** Hepatic metastasis from a RCC in a good responder (62-yr-old woman) receiving sorafenib plus IFN. Dynamic contrast-enhanced ultrasonography pretreatment (A), after 2 wks of treatment (B), and after 4 wks of treatment (C). The contrast uptake was estimated to be 80%, 60%, and 40%, respectively.
because no deaths occurred, and disease progression was observed in only 3 of the 12 patients during the study.  

**DCE-US.** As of July 2006, six of nine patients evaluated with DCE-US were alive. One (17%) of these patients had a decrease of tumor volume of ≥30%, but without a change in tumor vascularity, whereas five (83%) had decreases of tumor vascularity of ≥20% (Fig. 3). None of the three patients who died exhibited a change in tumor vascularity with combination treatment. Mean progression-free survival was 319 days in good responders (n = 6) and 90 days in poor responders (n = 3). Mean overall survival was 319 days in good responders (n = 6) and 173 days in poor responders (n = 3).

**Pharmacokinetics**

Concomitant administration of IFN (6 or 9 MIU) had no effect on the pharmacokinetics of sorafenib (Fig. 4; Table 4). Increasing the dose of IFN from 6 to 9 MIU (cohort 2 versus cohort 3, respectively) had no effect on the AUC₀-₈ or Cₘ₅ₐₓ of sorafenib (Table 4). Doubling the dose of sorafenib from 200 to 400 mg twice daily (cohort 1 versus cohorts 2 and 3, respectively) increased the plasma exposure (AUC₀-₈ and Cₘ₅ₐₓ) of sorafenib in a dose-proportional manner (Table 4).

**Immunologic variables and biomarkers**

Although there was a trend toward a reduction in absolute values of lymphocytes over the treatment period, there was no significant change from baseline in lymphocyte subsets (T cells, B cells, and NK cells; Fig. 5A). Similarly, no significant dose-dependent inhibition of ERK phosphorylation in T cells or NK cells was shown with combination therapy (Fig. 5B). Activation of Raf/MEK/ERK signaling (increase in pERK) was evidenced after phorbol myristate acetate stimulation in T lymphocytes from 10 patients, and a similar range of stimulation index was observed at baseline (3.0-6.8) and after a 4-month period of sorafenib (2.5-7.8).

A trend was observed toward increased plasma levels of the proangiogenic cytokines, VEGF, and IL-6, up to cycle 2 (~8 weeks) of combination treatment. However, VEGF levels declined to baseline levels during the remaining 20 weeks of treatment (Fig. 5C and D). There was no significant change from baseline at any time point for these cytokines with combination treatment, due to the high degree of interindividual variability. Similarly, plasma basic fibroblast growth factor, tumor necrosis factor α, and IL-10 did not change significantly with combination treatment.

### Discussion

The combination of sorafenib plus IFN was generally well tolerated in this phase I trial, with only two patients discontinuing due to adverse events. The MTD for this combination was not reached, as only one patient of six in cohort 3 reported a DLT. No clear increase was observed in the frequency or severity of drug-related adverse events with increasing combination doses, including dermatologic toxicities typically associated with sorafenib monotherapy (i.e., hand-foot skin reaction and alopecia), which were actually lower at the higher dosage. Diarrhea, a common toxicity with single-agent sorafenib (35), was the most frequently observed gastrointestinal toxicity, whereas fatigue, which occurs in ~70% of patients receiving IFN (36), was the most frequently observed toxicity in this trial. This combination was not associated with an increased incidence of toxicities above the level expected with either agent alone.

The combination also showed preliminary efficacy, as one RCC patient achieved partial response and eight (62%) patients had disease stabilization (seven RCC, one melanoma). The incidence of disease stabilization was more frequent among patients on the higher combination dosages.

Targeted agents are generally disease stabilizing rather than cytotoxic (37). They may often induce tumor necrosis that increases tumor volume rather than inducing a marked decrease in tumor size. Consequently, objective responses may be missed or wrongly classified as progressive disease by standard response criteria. Assessment of tumor neovascularization and changes in tumor volume with imaging techniques aims to identify early responses and provide a more reliable measure of efficacy for targeted therapies and antiangiogenic treatments (28, 38). Preliminary evidence from the present study suggests that a relationship may exist between a good response, as assessed by morphologic and dynamic variables, and improved outcome (prolonged progression-free survival and overall survival). A good response comprised a ≥20%

### Table 4. Pharmacokinetic variables of sorafenib at steady-state after multiple oral doses of 200 or 400 mg twice daily with doses of 6 or 9 MIU IFN [geometric means / geometric SD (range); n = 11]

<table>
<thead>
<tr>
<th>Cohort</th>
<th>AUC₀-₈ (mg h/L)</th>
<th>Cₘ₅ₐₓ (mg/L)</th>
<th>tₘ₅ₐₓ (h)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean/SD</td>
<td>Range</td>
<td>Mean/SD</td>
</tr>
<tr>
<td>Cohort 1 (n = 3)</td>
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<td>(11.8-20.4)</td>
<td>2.61/1.54</td>
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<tr>
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<td>27.1/1.16</td>
<td>24.4-30.2</td>
<td>4.93/1.15</td>
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<tr>
<td>Cohort 3 (n = 6)</td>
<td>26.2/1.50</td>
<td>16.4-44.0</td>
<td>4.18/1.58</td>
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</tbody>
</table>
A decrease in tumor vascularity (i.e., decreased contrast uptake) with a stable or decreased tumor volume, or a ≥30% decrease in tumor volume with no effect on the tumor vasculature. These DCE-US data would need to be evaluated further in a larger number of patients to correlate dynamic changes in tumor volume and/or tumor vasculature by DCE-US with improved outcome. A recent prospective study of sorafenib in RCC patients confirmed that DCE-US is a simple, accurate, and noninvasive measure of tumor vascularity (33). The combination of a decrease in tumor vascularity (i.e.,

Fig. 5. Immunologic analyses were done at baseline, at various time points throughout the study (on days 1 and 15 of treatment cycle 1, day 1 of cycles 2, 4, and 7), and at study end. A, immunophenotyping of total (CD45+), T (CD3+), B (CD19+), and NK lymphocytes (CD56+). Columns, mean of absolute values (cells/mm²); bars, SD. B, activation of the Raf/MEK/ERK pathway (pERK) in T lymphocytes and NK lymphocytes. Columns, mean of stimulation index; bars, SD. C and D, measurement of circulating cytokines (n = 12). Columns, mean of cytokine concentration (pg/mL); bars, SD. bFGF, basic fibroblast growth factor; TNFα, tumor necrosis factor α.
≥10% decrease in contrast-agent uptake) with either unchanged or decreased tumor volume was predictive of progression-free survival and overall survival in this prospective study (33). There was no evidence of drug-drug interactions, as concomitant IFN had no significant effect on the pharmacokinetics of sorafenib. Plasma exposure to sorafenib increased proportionately with increasing dose from 200 to 400 mg twice daily and was not associated with increased toxicity. The pharmacokinetics of sorafenib in the present combination study are similar to those reported in monotherapy trials (35) and combination trials, including gemcitabine (39), oxaliplatin (40), dacarbazine (21), and carboplatin/paclitaxel (22), in which there were also no evident drug-drug interactions. Sorafenib has shown promising antitumor activity and good tolerability in combination studies in melanoma patients (21, 22). Combining sorafenib with drugs that have different mechanisms of action may help to maximize its therapeutic potential and overcome tumor resistance, which commonly limits the effectiveness of monotherapies (23).

Increased signaling through the Raf/MEK/ERK pathway, due to dysregulated receptor tyrosine kinase activation or Ras mutations, is common in human cancers. The biological effects of molecular targeted anticancer agents on normal tissues can be measured as a surrogate marker of activity (34). Therefore, levels of pERK in circulating PBLs may provide a surrogate marker for the effects of Raf/MEK/ERK inhibitors in cancer patients (34). In contrast to a phase I trial of sorafenib monotherapy (35), no significant inhibition of phorbol myristate acetate–induced ERK phosphorylation was observed in T or NK lymphocytes with sorafenib/IFN in the present study. Phorbol myristate acetate–induced ERK phosphorylation was completely inhibited by concomitant addition of sorafenib in vitro PBL assays (data not shown), suggesting that IFN may be antagonizing the effects of sorafenib on pERK in PBLs in vivo. Further studies are required to confirm whether inhibition of ERK phosphorylation was selectively occurring in tumor cells. In addition, the exact molecular mechanisms by which sorafenib exerts its effects in a range of tumor types, including RCC and melanoma, have yet to be confirmed in appropriately designed clinical trials.

Overexpression of VEGF occurs in ~70% of renal tumors and is associated with poor prognosis (41). Serum levels of VEGF and basic fibroblast growth factor can be elevated in RCC patients (42). The angiogenesis inhibitor SU5416 combined with IFN significantly reduced plasma VEGF levels in RCC patients (43). In contrast, levels were elevated by sorafenib treatment (44). In the present study, a nonsignificant trend toward increasing plasma VEGF and IL-6 was observed during the first 8 weeks, which was subsequently followed by a decline in levels of these cytokines. The precise mechanism of how sorafenib affects plasma VEGF levels is currently unknown. However, it is conceivable that vascular disruption by sorafenib could explain the initial elevation of VEGF levels over the first 8 weeks, as it could lead to increased levels of intratumoral hypoxia. Studies have shown that a significant correlation exists between serum VEGF levels and the number of platelets (45). Although it is also conceivable that IFN-induced thrombocytopenia could have resulted in reduced production or release of VEGF from platelets, thereby explaining the decline in VEGF after 8 weeks, this is unlikely because very few patients had platelet-related toxicities in the present study. Further studies are necessary to determine how sorafenib affects plasma VEGF levels. The observed variability might be reduced by evaluating a larger number of patients.

Data from two phase II trials involving sorafenib and IFN in advanced RCC patients were presented at American Society of Clinical Oncology this year (46, 47). Sorafenib plus IFN showed considerable efficacy, and this combination was well tolerated in untreated patients in the first- and second-line setting (47).

In conclusion, sorafenib plus IFN was generally well tolerated, with promising efficacy and no evidence of drug-drug interactions in metastatic RCC patients and a patient with malignant melanoma in this phase I trial. The recommended dose for future studies is sorafenib 400 mg twice daily plus IFN 9 MIU. Further investigations of sorafenib combined with immunotherapies are ongoing.

References

Phase I Trial of Sorafenib in Combination with IFN α-2a in Patients with Unresectable and/or Metastatic Renal Cell Carcinoma or Malignant Melanoma


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