Sorafenib in Renal Cell Carcinoma

Keith T. Flaherty

Abstract  Sorafenib is an orally available inhibitor of vascular endothelial growth factor receptors, platelet-derived growth factor receptor-β, and RAF kinases. A dose of 400 mg twice daily administered continuously was selected for phase 2 testing, although 600 mg twice daily formally met criteria for a maximum tolerated dose. It is well tolerated compared with cytokine therapy. Antitumor activity was shown clearly in the context of a randomized discontinuation phase 2 trial. In this setting, even disease stabilization was established as a treatment-related phenomenon. A phase 3 trial with sorafenib confirmed a benefit of therapy across the vast majority of patients treated with sorafenib as opposed to placebo. Limited investigations into the mechanism of action of sorafenib in renal cell carcinoma support vascular endothelial growth factor receptor antagonism as the primary mediator of effect. The toxicity profile of sorafenib allows for its use in combination regimens. The focus of efforts to improve on the efficacy of sorafenib is on use with IFN, bevacizumab, or temsirolimus. Preliminary evidence with this approach is promising and will be the subject of the next generation of randomized trials in renal cell carcinoma.

Recently approved by the Food and Drug Administration, sorafenib (Bayer Pharmaceuticals, West Haven, CT) is an agent with established single-agent efficacy in metastatic renal cell carcinoma (RCC). It is among 17 inhibitors of vascular endothelial growth factor (VEGF) receptor (VEGFR) 2 (VEGFR2 or KDR) in clinical testing. However, the spectrum of kinase inhibition offered by sorafenib differentiates it from other agents and deserves consideration (Table 1). The unique clinical development path of sorafenib has uncovered some properties of the agent that may contribute to further advances in RCC treatment. In this review, we consider the important preclinical, clinical, and translational data that support the efficacy of sorafenib and the avenues for further clinical investigation.

Sorafenib was initially identified as a potent inhibitor of c-RAF. When hypertension was observed in the context of phase 2 trials, sorafenib was hypothesized to be a VEGFR inhibitor. Biochemical assays established that it is a potent inhibitor of VEGFR2 and VEGFR3. Both of these transmembrane receptors are implicated in tumor pathophysiology (Fig. 1). VEGFR2, previously referred to as KDR, is one of two high-affinity receptors for VEGF-A (VEGF165; ref. 1). Transgenic mice lacking VEGFR2 do not survive embryogenesis and have impaired blood vessel formation (2). Selective disruption of the VEGF-A/VEGFR2 interaction is associated with tumor growth inhibition in human tumor xenografts in mice (3). The importance of VEGFR1 in VEGF-A-mediated angiogenesis is less clear because abrogation of the kinase activity of VEGFR1 does not affect vessel formation in transgenic mice (4). VEGFR3 has been identified as a high-affinity receptor for VEGF-C and VEGF-D, which mediate lymphangiogenesis (5). Tumor metastasis via lymphatics can be inhibited by interference with the VEGF-C VEGFR3 interaction (6). Thus, activity against VEGFR2 and VEGFR3 in a disease that is VEGF driven, such as RCC, is an attractive feature of sorafenib.

Platelet-derived growth factor (PDGF) receptor-β has been the focus of several recent preclinical investigations and seems to be a valid target for angiogenesis inhibition. In addition to VEGF, hypoxia also induces secretion of PDGF (7). PDGF and PDGF receptor expressions are up-regulated in microvessel endothelial cells compared with mature vessels (8). Signaling through the PDGF receptor pathway in pericytes is essential for their recruitment (9, 10). In turn, the recruitment of pericytes is essential to the maturation and stabilization of immature blood vessels (11). Microvessels that are endowed with pericytes are no longer dependent on VEGF for their survival (12). Similar to the role of VEGF as a survival factor for immature endothelial tubes, under hypoxic conditions, pericytes are dependent on PDGF for survival (13). Inhibition of PDGF, in the absence of VEGF inhibition, inhibits blood vessel formation and tumor growth in human tumor xenografts (14). In an animal model of pancreatic islet cell tumors, the dual inhibition of VEGF and PDGF seems to be markedly more effective at blocking angiogenesis than blockade of either alone (15).

Several lines of evidence suggest that inhibition of RAF signaling inhibits angiogenesis. In cells that overexpress the VEGF receptor, flk-1/KDR, VEGF signaling through the RAF–mitogen-activated protein kinase/extracellular signal-regulated kinase kinase–mitogen-activated protein kinase pathway is necessary for proliferation (16). In endothelial cells, VEGF-induced
activation of mitogen-activated protein kinase is inhibited by expression of a truncated form of RAF that binds to Ras but cannot phosphorylate mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (17). These data support the necessity of RAF activity in the VEGF-induced activation of endothelial cells. Hood et al. (18) have developed a nanoparticle delivery system that specifically targets tumor endothelium. Coupling of an integrin 3 ligand to the surface of nanoparticles results in selective trafficking of particles to angiogenic blood vessels in tumor-bearing mice. Delivery of a mutant RAF gene, incapable of binding ATP, to tumor endothelium results in inhibition of angiogenesis and tumor-associated endothelial cell apoptosis. This is associated with apoptosis of tumor cells and prolonged regression of primary and metastatic tumors.

In vivo, it is difficult to determine the relative contribution of each sorafenib target to the antitumor activity. Nonetheless, the agent clearly affects the maintenance of tumor vasculature and angiogenesis (19). It should be noted that in vitro, sorafenib inhibits the proliferation of RCC cell lines, an experimental system that is independent of angiogenesis. Although it still difficult to discern which of the targets of sorafenib might be mediating this effect, direct tumor cytotoxicity may be a component of the clinical efficacy observed in RCC. Consideration of other cancers, such as melanoma, where BRAF harbors activating mutations and is notable for the large randomized discontinuation phase 2 trial, in which activity in RCC was first observed (21). This trial is significant for the large number of prior therapies, most patients had clear cell RCC, and the median number of prior therapies was 2. Of the 202 RCC patients enrolled, 11% of patients had 38% regression of progressions of <25%, and 32% had progression of <25%. This last group of

### Table 1. Spectrum of sorafenib in isolated kinase assays

<table>
<thead>
<tr>
<th>Kinase assays</th>
<th>IC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-RAF</td>
<td>2 nmol/L</td>
</tr>
<tr>
<td>mVEGFR2, VEGFR3</td>
<td>6-10 nmol/L</td>
</tr>
<tr>
<td>WT BRAF, V599E BRAF</td>
<td>20-40 nmol/L</td>
</tr>
<tr>
<td>p38, PDGFR-β</td>
<td>28-38 nmol/L</td>
</tr>
<tr>
<td>FLT-3, c-KIT</td>
<td>40-80 nmol/L</td>
</tr>
<tr>
<td>EGFR, PKC, MEK, ERK</td>
<td>Inactive at 10 nmol/L</td>
</tr>
</tbody>
</table>

Abbreviations: PDGFR-β, PDGF receptor-β; EGFR, epidermal growth factor receptor; PKC, protein kinase C; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; ERK, extracellular signal-regulated kinase.

dose, and then after 2 months of therapy with 400 mg. Nonetheless, 400 mg twice daily was selected for further development. Pharmacokinetic data support the selection of this dose because exposure did not increase significantly between cohorts treated with 400, 600, and 800 mg.

It is possible to link some of the common sorafenib-associated toxicities with known molecular targets by comparing the toxicities seen with other targeted therapies with overlapping spectrum of activity. Hypertension is class effect of essentially all VEGF-targeted therapies and is discussed in detail below. The macular-papular rash associated with sorafenib is not commonly observed with bevacizumab (a VEGF antibody), sunitinib, and other small-molecule inhibitors of VEGFR and PDGF receptor. This leads one to speculate that a unique target of sorafenib, such as RAF, might mediate this effect. Given that it has been the only RAF kinase inhibitor to be evaluated clinically, it is difficult to verify this point. Other targets, such as p38, are relatively unique to sorafenib and could be implicated in any of the toxicities that are unique to this agent. Sorafenib is one of the few toxicities that emerges relatively late in the course of treatment, when other toxicities, such as rash, have dissipated. This time course makes one wonder if this is less likely a signal transduction effect on intestinal epithelial cells and perhaps more related to chemical irritation from the carriers in the formulation.

The phase 3 trial comparing sorafenib to placebo in cytokine-refractory RCC provides the best summary of sorafenib-related toxic effects at 400 mg twice daily (Fig. 2). In that study, grade 3 events were infrequent. Considering all grades of toxic effects, the three toxic effects that were clearly treatment related were rash, diarrhea, and hand-foot syndrome. Pruritus is exclusively present in the setting of rash.

### Phase 2 and 3 trials

The clinical development of sorafenib is notable for the large randomized discontinuation phase 2 trial, in which activity in RCC was first observed (21). This trial used modified WHO response criteria to avoid randomizing patients with clinically significant reductions in tumor volume. Eligibility was not restricted based on histologic subtype or number of prior therapies. Most patients had clear cell RCC, and the median number of prior therapies was 2. Of the 202 RCC patients enrolled, 11% of patients had 50% regression by WHO criteria, meeting criteria for a conventional objective response. According to the modified WHO criteria used in the protocol, 36% had tumor regressions of at least 25% and 32% had progression or progression of <25%. This last group of
vascular permeability and has been validated in preclinical models. Gadolinium diffuses across tumor vasculature more readily than normal vessels. In the setting of VEGF inhibition, gadolinium diffusion into tumors is reduced. This can be quantified by comparing the area under the time-enhancement curves before and during treatment. Among 17 patients with varied RCC histologic subtypes, we did baseline dynamic contrast-enhanced magnetic resonance imaging and a follow-up assessment after several weeks of therapy. $K_{tr}$ was significantly lower after sorafenib treatment (median, 60.3% decline; 95% confidence interval, 46.1-74.6%). As hypothesized, reduction in $K_{tr}$ significantly correlated with prolonged time-to-progression ($P = 0.01$) but not with the amount of tumor regression by computed tomography ($P = 0.22$). Of note, each of these variables was treated as continuous. Given that some tumors develop macroscopic evidence of necrosis, it is possible that $K_{tr}$ is detecting this type of effect and that changes in tumor size are somewhat independent. Unexpectedly, elevated $K_{tr}$ at baseline was significantly correlated with time-to-progression ($P = 0.02$). The Kaplan-Meier PFS for patients with baseline $K_{tr} > 3 \text{mL/mL/min}$ and baseline $K_{tr} < 3 \text{mL/mL/min}$ is shown in Fig. 4. This cutpoint was chosen based on the equal distribution of patients above and below this value. Previous studies in patients with other tumor types have correlated elevated $K_{tr}$ with high microvessel density and serum VEGF levels, both adverse prognostic signs in RCC. Thus, in the context of sorafenib therapy, this marker of otherwise poor prognosis seems to identify those most likely to receive clinical benefit. Of course, this small pilot study will require validation in a larger trial. This will happen in the context in the E2804 (to be discussed later).

Although dynamic contrast-enhanced magnetic resonance imaging can assess metastatic tumors in a variety of metastatic sites, relatively large tumors are needed (>3 cm in some cases) to obtain robust measurements of $K_{tr}$. In addition, patients must at least undergo imaging studies beyond those required for clinical staging, and interpreting the results requires a significant amount of expertise. Therefore, identification of a serum marker of pharmacodynamic effect would be preferable. We have observed that serum VEGF levels increase during the initial weeks of therapy with sorafenib. This is not a tumor-related phenomenon but rather a systemic response to VEGF blockade, as it is seen even among people without cancer. Among a cohort of 20 patients treated with sorafenib, those who had a doubling or more in serum VEGF had a more

![Graph showing frequency of symptomatic toxicities in Phase III renal cell carcinoma trial](image)

**Fig. 2.** Frequency of symptomatic toxicities in Phase III renal cell carcinoma trial (black bar, any CTC grade; grey bar, grade 3).

patients ($n = 65$) were randomized to continue sorafenib or cross over to placebo. The comparison of progression-free survival (PFS) in this cohort was the primary end point of the study. The significant difference in PFS (median, 24 versus 6 weeks; $P = 0.0087$) confirmed that disease stabilization was attributable to sorafenib. Taken together with the patients with tumor regression (median PFS, 40 weeks), this trial established clinical activity in most patients.

The phase 3 trial of sorafenib in RCC used a more conventional one-to-one randomization at the time of study entry to sorafenib versus placebo. Nine hundred three patients with clear cell RCC refractory to one prior therapy were enrolled. Although this study was designed to compare overall survival, the first interim analysis for PFS was revealed to the investigators, and the trial was terminated prematurely with placebo-treated patients crossed over to sorafenib (22). The median PFS (5.5 versus 2.8 months; $P < 0.00001$) for sorafenib was nearly identical to that observed in the phase 2 trial (Fig. 3). The investigator-assessed objective response rate was 10% by Response Evaluation Criteria in Solid Tumors criteria. Clearly, the objective response rate does not adequately summarize the effect of sorafenib on the natural history of metastatic RCC. This is, in part, due to the related observation that many metastatic RCC lesions develop radiographically evident necrosis while on sorafenib therapy. This seems to be a class phenomenon for the VEGF-targeted agents in this disease. An interim analysis for overall survival revealed a nonsignificant trend toward improved survival (hazard ratio, 0.72; $P = 0.018$). $P < 0.0005$ was required to yield a definitive benefit. Although these patients continue to be followed up for overall survival, 48% of the patients initially assigned to placebo crossed over to sorafenib soon after the PFS analysis. This will undoubtedly limit the statistical power to detect a survival advantage for sorafenib.

**Correlative studies.** The antiangiogenic activity of sorafenib in vivo has been investigated in the context of the large phase 2 trial of sorafenib. We conducted a pilot study using dynamic contrast-enhanced magnetic resonance imaging as a method of detecting changes in tumor vascular permeability ($K_{tr}$; ref. 23). This technique takes advantage of the influence of VEGF on vascular permeability and has been validated in preclinical

![Graph showing progression-free survival Kaplan-Meier analysis in Phase III renal cell carcinoma trial](image)

**Fig. 3.** Progression-free survival Kaplan-Meier analysis in Phase III renal cell carcinoma trial.
favorable outcome than those with little or no change in VEGF (Fig. 5). This threshold was selected based on nearly even numbers of patients above and below this value. There are several potential explanations for this relationship. Greater increases in VEGF levels in the setting of VEGFR blockade may simply relate to higher drug exposure. In addition, the development of new areas of tumor necrosis within tumors, which is manifested on computed tomography scans, may be evidence of greater degrees of tumor hypoxia, triggering more VEGF production.

An even more convenient biomarker of sorafenib effect might be treatment-related hypertension. This is a class effect observed not only with VEGFR inhibitors but also with the VEGF monoclonal antibody. A thorough analysis of the relation between changes in blood pressure and outcome has not been reported. However, we conducted an investigation of the mechanism of sorafenib-induced hypertension in patients with metastatic RCC (24). We observed a consistent elevation in blood pressure for the entire cohort (Fig. 6). We found no significant relationship between previously described mediators of blood pressure and magnitude of increase. Although we could not measure it directly, we suspect that sorafenib is inhibiting nitric oxide production in endothelial cells, which has been shown previously to be VEGF mediated (25). This is supported by the rapid normalization of blood pressure following interruption of sorafenib. A more thorough evaluation of the blood pressure as a potential marker of VEGF inhibition is warranted.

**Sorafenib in combination regimens.** Angiogenesis is mediated by other factors in addition to VEGF and PDGF. In particular, basic fibroblast growth factor, epidermal growth factor, transforming growth factors α and β, and interleukin 8 have all been implicated in tumor angiogenesis (26). For that reason, it is hypothesized that targeting multiple angiogenic growth factors will yield even greater benefits in RCC. The armamentarium of targeted agents against these factors is limited. Sorafenib is sufficiently tolerable to be combined with other agents. One combination of particular interest is simultaneous blockade of VEGFRs with depletion of secreted VEGF. The rationale behind this approach is founded on the observation that serum VEGF levels increase following administration of VEGFR inhibitors, including sorafenib. Although we have observed that increases in VEGF correlate with clinical benefit, it remains possible that increased VEGF production eventually mediates resistance to therapy. Bevacizumab (Genentech, South San Francisco, CA) has been shown previously to deplete serum VEGF to undetectable levels at doses of 3 mg/kg and higher (27). The combination of sorafenib and bevacizumab is in the midst of phase 1 evaluation.

In addition to bevacizumab, temsirolimus (CC1-779, Wyeth Pharmaceuticals, Collegeville, PA) is another agent that seems to affect angiogenesis at a point that is different from sorafenib. Mammalian target of rapamycin, the target of temsirolimus, regulates the expression of hypoxia-inducible factor 1-α (28), which is up-regulated by the loss of the von Hippel Lindau gene in RCC. By down-regulating hypoxia-inducible factor 1-α in the tumor cell, temsirolimus may complement the effects of sorafenib at the level of the endothelial cell. The combination of sorafenib and temsirolimus is being evaluated in a phase 1 trial.

As the number of targeted agents with relevance to RCC increases, the number of potential combination regimens multiplies. In an effort to evaluate the currently available agents as doublets in parallel, we are initiating a randomized phase 2 trial in the cooperative groups (E2804). In that study, sorafenib will be combined with bevacizumab and temsirolimus.

**Conclusions**

Sorafenib has established efficacy in RCC and is well tolerated. The currently available evidence suggests that VEGFR antagonism is being achieved. The spectrum of kinases inhibited by sorafenib goes far beyond VEGFRs and is unique compared with other agents in this class. The contribution of these various targets to the activity of sorafenib in RCC is not known. Based on its toxicity profile and target spectrum, sorafenib is well suited to inclusion in combination regimens. This will be the focus of investigations in the next generation of clinical trials.

**Open Discussion**

Dr. Figlin: Is it possible that the vascular endothelial growth factor (VEGF) receptor antagonists are working best on the hypoxic environment of VEGF-dependent tumors and are not working well on the VHL-dependent tumor in the outer rim?
References
Sorafenib in Renal Cell Carcinoma

Keith T. Flaherty