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 activity 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.

Targets
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 PDGFR receptor expressions are up-regulated in microvessel endothelial cells compared with mature vessels (8). Signaling through the PDGFR 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 might serve a more definitive test of sorafenib RAF-inhibitory properties, is beyond the scope of this review.

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 regression or progression of <25%. This last group of

Clinical Data

Phase 1 and toxicity. Sorafenib was evaluated in four phase 1 trials, investigating distinct schedules of administration: three with interrupted dosing and one with continuous administration (20). At doses of 200 mg twice daily and higher, inhibition of mitogen-stimulated RAF activity was observed in peripheral mononuclear cells. Dose-limiting toxicity was observed in 1 of 6 patients treated at 200 mg twice daily, none of the 15 treated at 400 mg twice daily, 4 of 14 at 600 mg twice daily, and 3 of 6 at 800 mg twice daily. The most common moderate or severe toxic effects were rash, hand-foot syndrome, diarrhea, and fatigue. Although it is reasonable to infer that the 600 mg dose level was intolerable based on a 29% dose-limiting toxicity rate, most patients experience a decline in toxic effects after the first 4 to 6 weeks of treatment. Therefore, it is possible that long-term therapy at 600 mg twice daily is feasible, if not as a starting

![Fig. 1. Sorafenib targets in tumor cells, endothelial cells, and pericytes.](image-url)
vascular permeability and has been validated in preclinical studies. This technique takes advantage of the influence of VEGF on vascular permeability, which is detectable using contrast-enhanced magnetic resonance imaging as a method of assessing tumor vascular permeability. Vascular permeability changes are more readily detected than normal vessel permeability. 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 used baseline dynamic contrast-enhanced magnetic resonance imaging and a follow-up assessment after several weeks of therapy. Reduction in tumor permeability (Kv) was significantly lower after sorafenib treatment (median, 60.3% decline; 95% confidence interval, 46.1-74.6%). As hypothesized, reduction in tumor permeability was 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 Kv is detecting this type of effect and that changes in tumor size are somewhat independent. Unexpectedly, elevated Kv at baseline was significantly correlated with time-to-progression (P = 0.02). The Kaplan-Meier PFS for patients with baseline Kv > 3 mL/min/m and baseline Kv < 3 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 Kv 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 of 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 Kv. 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-specific 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 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-specific 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 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-specific 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 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-specific 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

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. A more thorough evaluation of the blood pressure as a potential marker of VEGF inhibition is warranted.

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?
Fig. 6. Mechanism of sorafenib-induced hypertension in patients with metastatic RCC.

Dr. Flaherty: One potential explanation is that the dependence of the rim on tumor vasculature to deliver nutrients and oxygen is different than the core. So, tumor cells in the rim probably aren’t dependent on tumor vasculature entirely for delivery of blood and nutrients. The other potential explanation is that the fraction of VEGF-dependent neovessels is differentially present as you move through zones of the tumor.

Dr. Rosen: Might the neovessels at the tumor rim and those at the tumor center behave differently—as they may not be at the same stage of maturation—even though they are all driven by the same angiogenic signals from the tumor?

Dr. George: These are not homogeneous masses. The bigger they get, the more heterogeneous.

Dr. Figlin: How does that explain how these drugs work in this disease?

Dr. George: There may be different compartments to the vasculature: compartments that are immature, VEGF-dependent compartments, and perhaps compartments that are more complex with both VEGF-dependent components as well as components dependent on other growth factors, such as platelet-derived growth factor. These inhibitors are probably only working on a subset of the tumor vasculature. To what extent we can add to them and optimize the antiangiogenic effect remains to be seen.

Dr. Sukhatme: Is it true that the hypertension seen with this drug is actually less than what is seen with, for example, bevacizumab or in any of the early drugs on VEGF trial?

Dr. Flaherty: We’ve never compared bevacizumab and this drug in a rigorous way. I do not believe so, but I’ve never seen any data that quantify the percentage of, for example, people who have blood pressure elevations over systolic of 150 mm Hg or some other significant threshold.

Dr. Atkins: Was there any correlation between rash and hand-foot syndrome and clinical benefit? Of the targets that this drug is hitting, what factors do you think are responsible for those side effects?

Dr. Flaherty: The rash didn’t correlate with progression-free survival. The rash may be a RAF-driven phenomenon because it is different from the epidermal growth factor receptor rash.

Dr. Sokasman: But the hand-foot syndrome is seen across the board with all the multitargeted tyrosine kinase inhibitors.

Dr. Flaherty: Yes, that’s seen with the other drugs as well.

Dr. Atkins: My hypothesis for hand-foot syndrome and mucositis is that those areas of the body are constantly undergoing subclinical trauma. With these agents, you do not have the normal tissue repair, and over time, this lack of normal tissue repair causes either ulcerations at pressure points in the mouth or on the hands and feet.

Dr. Flaherty: Exactly. Everyone believed 5 years ago that angiogenesis happened only in wound healing and after a myoccardial infarction in adults, but that is not the case. You have angiogenesis that is part of minor levels of trauma that occur every day.

Dr. McDermott: You said that you do not believe rebound is happening with this drug. However, we have all seen data, from you and others, that when you block the VEGF receptor with tyrosine kinase inhibitors serum VEGF levels increase. Wouldn’t this lead to rebound growth of RCC when VEGF receptor inhibitors are stopped abruptly?

Dr. Flaherty: With SU11248, the VEGF levels are clearly up on therapy, but after the 2-week break, they’re down again to baseline. This is a small window of overdrive. Is that really biologically important in terms of the population of patients? I know there have been anecdotes of patients having minor tumor progression during that 2-week break. With sorafenib, we do not use intermittent dosing. That is why it is harder to make those comments. It might be there, but to a degree that is not clinically meaningful.

References


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