Review

Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling

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Abstract

Although patients with advanced refractory solid tumors have poor prognosis, the clinical development of targeted protein kinase inhibitors offers hope for the future treatment of many cancers. *In vivo* and *in vitro* studies have shown that the oral multikinase inhibitor, sorafenib, inhibits tumor growth and disrupts tumor microvasculature through antiproliferative, antiangiogenic, and/or proapoptotic effects. Sorafenib has shown antitumor activity in phase II/III trials involving patients with advanced renal cell carcinoma and hepatocellular carcinoma. The multiple molecular targets of sorafenib (the serine/threonine kinase Raf and receptor tyrosine kinases) may explain its broad preclinical and clinical activity. This review highlights the antitumor activity of sorafenib across a variety of tumor types, including renal cell, hepatocellular, breast, and colorectal carcinomas in the preclinical setting. In particular, preclinical evidence that supports the different mechanisms of action of sorafenib is discussed. [Mol Cancer Ther 2008;7(10):3129–40]

Introduction

Over the past three decades, there has been no significant improvement in survival for patients with advanced refractory solid tumors. Five-year survival rates are as low as 4% to 6% for some patients, particularly those with pancreatic, kidney, or liver cancers (1–4). However, the advent of imatinib mesylate (Gleevec; ref. 5) and the ongoing clinical development of over 30 targeted protein kinase inhibitors designed to inhibit tumor growth and progression offer promise for the future (6, 7).

Sorafenib (Nexavar) is an oral multikinase inhibitor approved by the U.S. Food and Drug Administration for the treatment of patients with advanced renal cell carcinoma (RCC) and those with unresectable hepatocellular carcinoma (HCC). It is also approved by the European Medicines Agency for the treatment of patients with HCC and patients with advanced RCC with whom prior IFN-α or interleukin-2-based therapy had failed or those considered to be unsuitable for such therapy. Recommended daily dosing is 400 mg p.o. bid. Sorafenib is undergoing phase II/III clinical evaluation in a wide variety of other solid tumors, including melanoma and non-small cell lung cancer (NSCLC; refs. 8–12).

The significant increase in overall survival reported recently for sorafenib-treated patients with advanced HCC in a phase III, placebo-controlled trial represents a breakthrough in the management of this complex disease, which until now was the only solid tumor without systemic treatment options—a clear unmet medical need (13). Hence, the molecular mechanism of action of this drug in otherwise classically refractory solid tumors, such as RCC and HCC, warrants further investigation.

Although originally identified as a Raf kinase inhibitor, sorafenib also inhibits several receptor tyrosine kinases involved in tumor progression and tumor angiogenesis (14–27). In this review, we discuss the mechanism of action of sorafenib across a variety of tumor types, including RCC, HCC, and breast and colorectal carcinomas. Moreover, we discuss preclinical evidence supporting the antiproliferative, antiangiogenic, and proapoptotic mechanisms of action of sorafenib on the tumor and tumor endothelia and the contribution of known molecular targets of sorafenib to these effects.

Targets for Sorafenib

Sorafenib has multiple known protein kinase targets (Fig. 1) as identified in biochemical and cellular assays *in vitro* (27, 28). In an initial screening, sorafenib was identified as a potent inhibitor of Raf serine/threonine kinase isoforms

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in vitro (27, 28). Sorafenib has since been shown to have potent inhibitory effects on other Raf isoforms in biochemical assays, with an order of potency of Raf-1 > wild-type B-Raf > oncogenic B-Raf V600E (Table 1; refs. 27, 28). However, sorafenib does not inhibit MEK-1 or extracellular signal-regulated kinase (ERK)-1 kinase activity *in vitro* (27, 28). Sorafenib has been shown to inhibit ERK signaling, as measured by the reduction in ERK phosphorylation, in several cell lines from both hematopoietic malignancies and solid tumors. Sorafenib is capable of inhibiting ERK signaling in tumor cell lines with wild-type K-Ras and B-Raf and no known oncogenic activation of the ERK pathway as well as in cell lines containing oncogenic K-Ras or B-Raf. The antiproliferative activity of sorafenib varies widely depending on the oncogenic signaling pathways driving proliferation. For tumor cell lines with a single activating oncogenic tyrosine kinase mutation [such as MV4-11 and EOL-1 leukemic cell lines that contain a Flt-3 gene, mutant T670I cKIT that renders patients with gastrointestinal stromal tumor refractory to imatinib (29, 30), or oncogenic RET variants (15, 31) in metastatic thyroid cancer; ref. 32], the antiproliferative activity of sorafenib is in the low nanomolar concentration range (14, 33). For tumor cell lines without an activating receptor tyrosine kinase mutation and with multiple signaling pathways driving cell growth, the antiproliferative activity of sorafenib is in the low micromolar concentration range. Sorafenib has shown dose-dependent inhibition of the proliferation of several human tumor cell lines containing oncogenic K-Ras or B-Raf mutations, such as human MDA-MB-231 breast tumor cells containing oncogenic G463V B-Raf and K-Ras (codon 13; refs. 27, 34). Sorafenib also abolished the growth of human Mia-PaCa-2 pancreatic tumor cells (34) and significantly reduced the growth of human HCT-116 colon tumor cells (34). Both of these tumor cell lines contain the constitutively active V12 K-Ras oncogene. Sorafenib has also been shown recently to sequester Raf-1 and B-Raf in a stable inactive complex in treated tumor cell lines expressing wild-type B-Raf but not V600E B-Raf mutant (35). This alteration of Raf-1 protein complexes by sorafenib may result in perturbation of other Raf-1 complexes with MST-1 and ASK-1, which are involved in tumor cell survival signaling mechanisms (36, 37).

In addition to targeting Raf serine/threonine kinases, sorafenib also potently inhibits the proangiogenic vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, VEGFR-3, and platelet-derived growth factor receptor-β (PDGFR-β) tyrosine kinases in biochemical assays *in vitro*. In cellular assays, sorafenib inhibits the VEGF-mediated autophosphorylation of VEGFR-2 (human endothelial cells and NIH 3T3 fibroblasts expressing VEGFR-2), VEGFR-3, and PDGF-mediated autophosphorylation of PDGFR-β in HAoSMCs (27).

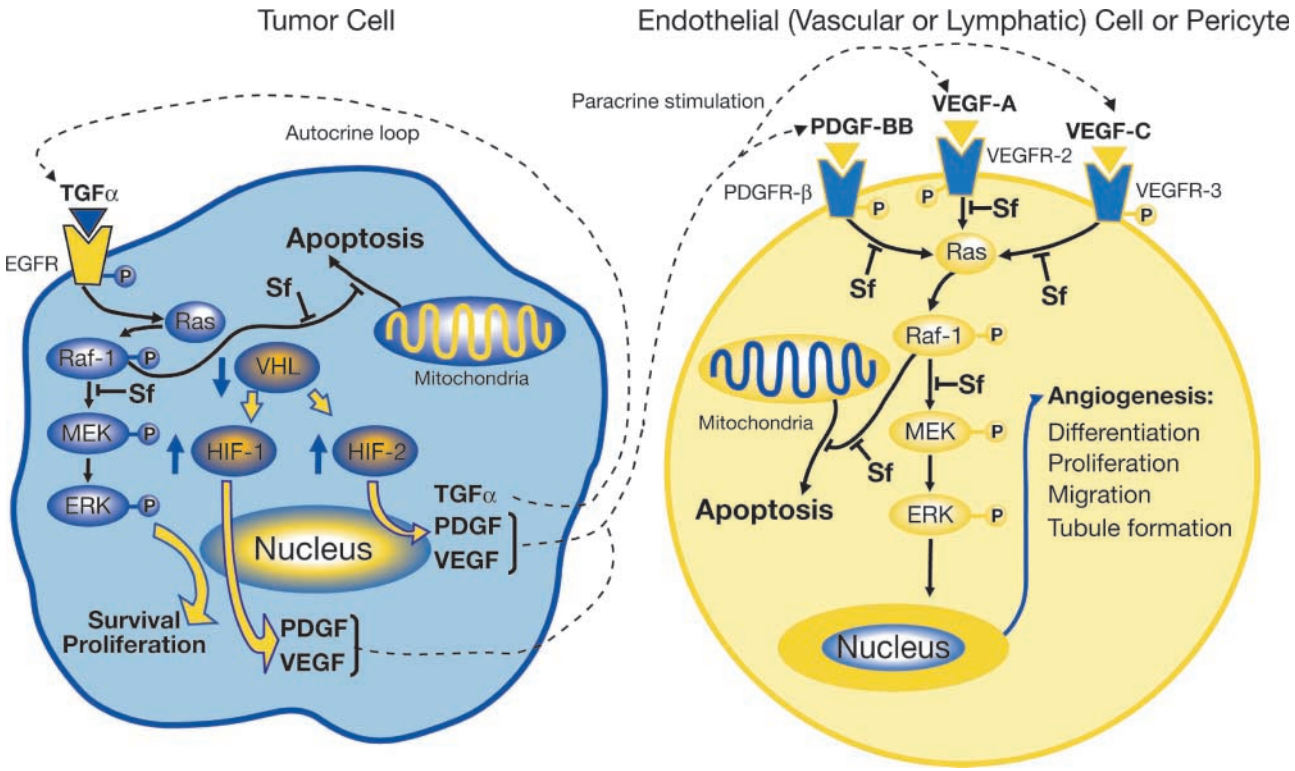

Figure 1. Dysregulated signaling through Raf-1 in tumor cells, endothelial cells, and/or pericytes could result in tumor growth and/or angiogenesis by an autocrine mechanism in RCC.

Table 1. *In vitro* cellular profile of sorafenib

Cellular assay	Mutational status	Histologic type	Reference
Inhibition of pERK signaling			
A375	*b-raf* V600E	Melanoma	83
ATC	*b-raf* V600E	Thyroid	47
Bx PC	WT-Ras, WT-B-Raf	Pancreatic	27
Colo829	*b-raf* V600E	Melanoma	83
HepG2	*k-ras*	HCC	35
LOX	*b-raf* V600E	Melanoma	27
MDA-MB-231	*b-raf* (G463V)/*k-ras*	Breast	27
PLC/PRF/5	*k-ras*	HCC	46
U937	Unknown	Leukemia	84
UACC 903	*b-raf* V600E	Melanoma	74
WM-266-4	*b-raf* V600D	Melanoma	83
Inhibition of tumor cell proliferation			
ATC	*b-raf* V600E	Thyroid	47
EOL-1	Flt-1 3 ITD	Leukemia	14
HepG2	*k-ras*	HCC	27
HepG2	*k-ras*	HCC	46
MDA-MB-231	*b-raf* (G463V)/*k-ras*	Breast	27
MV4;11	Flt-1 3 ITD	Leukemia	14
PLC/PRF/5	*k-ras*	HCC	46
RS4-11	Flt-1 WT	Leukemia	14
UACC 903	*b-raf* V600E	Melanoma	75
SK-MEL 2	*n-ras*	Melanoma	78
SK-MEL 28	*b-raf* V600E	Melanoma	78
A2058	*b-raf* V600E	Melanoma	78
Inhibition of receptor tyrosine kinase autophosphorylation			
c-Kit T670I	Kit	—	29, 30, 33
Flt-3 ITD	Flt-3	—	14, 33
PDGF-β dependent	PDGFR-β wt	—	27
Mutant PDGFR-β	ETV6-PDGFRβ	—	33
VEGF-dependent	VEGFR-2	—	27
RET	PTC3, C634R, M918T, V804L, V804M	—	15, 31
Down-regulation of Mcl-1 in tumor cells			
786-O	VHL-/-	RCC	85
A549	*k-ras*	NSCLC	38
ACHN	Unknown	RCC	38
HT-29	*b-raf* V600E	Colon	38
Jurkat	Unknown	Leukemia	84
MDA-MB-231	*b-raf* (G463V)/*k-ras*	Breast	38
U937	Unknown	Leukemia	85
Induction of apoptosis in tumor cells			
A549	*k-ras*	NSCLC	38
EOL-1	Flt-3 ITD	Leukemia	14
HepG2	*k-ras*/unknown	HCC	46
KMCH	Unknown	Cholangiocarcinoma	38
MDA-MB-231	*b-raf* (G463V)/*k-ras*	Breast	38
MV4;11	Flt-3 ITD	Leukemia	14
PLC/PRF/5	*k-ras*	HCC	46
U937	Unknown	Leukemia	84

Sorafenib has also been shown to induce apoptosis in several tumor cell lines. Although the mechanism through which sorafenib induces apoptosis is not fully elucidated and may vary between cell lines, a commonly observed theme is the inhibition of phosphorylation of the initiation factor eIF4E and loss of the antiapoptotic protein myeloid cell leukemia-1 (Mcl-1). The initiation factor eIF4E regulates the translation of a large number of mRNAs, including the Bcl-2 family member Mcl-1. Constitutive overexpression of Mcl-1 in cells significantly inhibits sorafenib-induced apoptosis, whereas Mcl-1 down-regulation by RNA interference enhances sorafenib-induced apoptosis (38). Down-regulation of Mcl-1 by sorafenib is associated with the release of cytochrome *c* from mitochondria into the cytosol, caspase activation, and apoptotic cell death.

Although a correlation has been shown in several tumor cell lines between the induction of apoptosis by sorafenib and the inhibition of eIF4E phosphorylation and decrease of Mcl-1 protein level (39), there remains a temporal and potency disconnect between the inhibition of eIF4E phosphorylation, which occurs with hours at nanomolar concentrations, the loss of Mcl-1, which occurs within hours at micromolar concentrations, and the induction of apoptosis, which occurs after at least 24 h of exposure to the compound. This temporal disconnect may be due in large part to the redundancy in antiapoptotic pathways activated in tumor cells. Although the precise mechanism of sorafenib-medicated apoptosis is not fully understood, the compound clearly sensitizes tumor cells to apoptosis induced by other agents *in vitro*. The proapoptotic activity of sorafenib is significantly enhanced when combined with chemotherapy and signal transduction inhibitors, such as the mammalian target of rapamycin inhibitor (38, 40, 41). The full clinical activity of sorafenib may therefore be manifest in combination with chemotherapy and/or signal transduction inhibitors targeting other pathways important in tumor cell growth and survival (13, 42–45).

Due to the multiple targets inhibited by sorafenib, effects in different tumor types are likely to be mediated through a variety of mechanisms. Although it may be difficult or even impossible to determine the precise contribution of individual targets to each tumor type, evaluation of preclinical data may help elucidate the contributions of given mechanisms of action of sorafenib in different tumor types. Oral sorafenib inhibited tumor growth in a wide variety of preclinical cancer models, including human breast, colon, ovarian, thyroid, and pancreatic carcinomas, melanoma, and RCC, HCC, and NSCLC (27, 34, 46, 47). Tumor growth was inhibited in preclinical cancer models at plasma drug exposures within the range of those observed in patients receiving the standard dose of 400 mg bid. The mean $AUC_{(0\text{-}12\ h)}$ in patients receiving sorafenib at 400 mg bid continuously for 7 days is 121.7 μmol/L h (10), which is within the range of the observed for mouse plasma $AUC_{(0\text{-}24\ h)}$ at the efficacious doses of 10 mg/kg (62 μmol/L h) and 30 mg/kg (210 μmol/L h; refs. 39, 46, 48–50). At the 400 mg bid dose, the C_{max} at steady state is between 6 and 15 μmol/L, with a $t_{1/2}$ of 22 to 27 h (51), and it is highly protein-bound to human plasma (99.4%: ref. 52). No major adverse effects were reported in any of the models tested, even with wide concentration ranges up to 100 mg/kg in a HCC model (46).

Renal Cell Carcinoma

RCC is characterized by the loss of von Hippel-Lindau tumor suppressor protein, resulting in dysregulation of growth factor signaling, including VEGF, PDGF-β, and transforming growth factor-α. These factors play key roles in angiogenesis and lymphangiogenesis as well as in dysregulation of Raf pathways that regulate tumor growth and survival (53–58). Daily treatment with sorafenib produced dose-dependent growth inhibition of human RCC 786-O and Renca tumor xenografts (Fig. 2; refs. 48, 49). In the 786-O xenograft model, a dose of 15 mg/kg produced 28% tumor growth inhibition, whereas treatment with 30, 60, or 90 mg/kg dose resulted in 80% tumor growth inhibition; tumor stabilization occurred at doses of 60 or 90 mg/kg (49). Similarly, in the Renca tumor model, a dose of 15 mg/kg produced 53% tumor growth inhibition, whereas treatment with a 60 or 90 mg/kg dose produced 82% inhibition and resulted in tumor stabilization during treatment (48, 49). No detectable decrease in pERK was observed in the 786-O or Renca tumors after sorafenib treatment.

The effect of sorafenib on angiogenesis was assessed by measuring the level of CD31 endothelial marker in the tumor (48, 49). A significant reduction in 786-O tumor vasculature was evident within 3 days of sorafenib treatment at the 15, 30, or 60 mg/kg dose level. Mean microvessel area (MVA), as measured by the level of CD31 staining, was decreased by 70% at the 15 mg/kg dose and by 90% at the 30 or 60 mg/kg dose. Similar results were obtained in the Renca murine model, in which significant inhibition of MVA was observed at all doses tested (48, 49). The reduction in tumor vasculature and increase of tumor hypoxia correlated with an induction of apoptosis and necrosis in a dose- and time-dependent manner. A 3-day treatment at a dose of 30 or 60 mg/kg resulted in an increase of terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling-positive area by 17.7% and 28.9%, respectively, and a prolonged treatment of 5 days resulted in 31.3% and 40.8% increase, respectively.

The models described above indicate that sorafenib acts on RCC through inhibition of VEGFR and consequent antiangiogenic effects. Given that VEGFR plays a key role in the development of RCC (56), this mechanism may explain the benefits of sorafenib seen in clinical studies. Consistent with the preclinical data in RCC xenograft models, correlative science studies in RCC patients using dynamic contrast-enhanced Doppler ultrasound with perfusion software showed that good responders had a significant decrease (60%) in contrast uptake after 3 weeks of treatment with sorafenib 400 mg bid. In this study, good response by dynamic contrast-enhanced Doppler ultrasound was shown to correlate with a statistically significant difference in progression-free survival in this cohort of RCC patients compared with poor response (59). Similar findings in a recent dynamic contrast-enhanced magnetic resonance imaging study in RCC patients enrolled in an open-label pilot study (60) showed a significant decrease in K_{trans} or vascular permeability after 12 weeks of sorafenib 400 mg bid. This decrease in vascular permeability was associated with improved outcome. In addition, the investigators of this study found that baseline K_{trans} was a predictive marker of favorable response to therapy. Sorafenib has shown an advantage compared with placebo in phase II/III clinical trials in patients with treatment-refractory metastatic RCC, prolonging progression-free survival by 2- to 4-fold (61, 62).

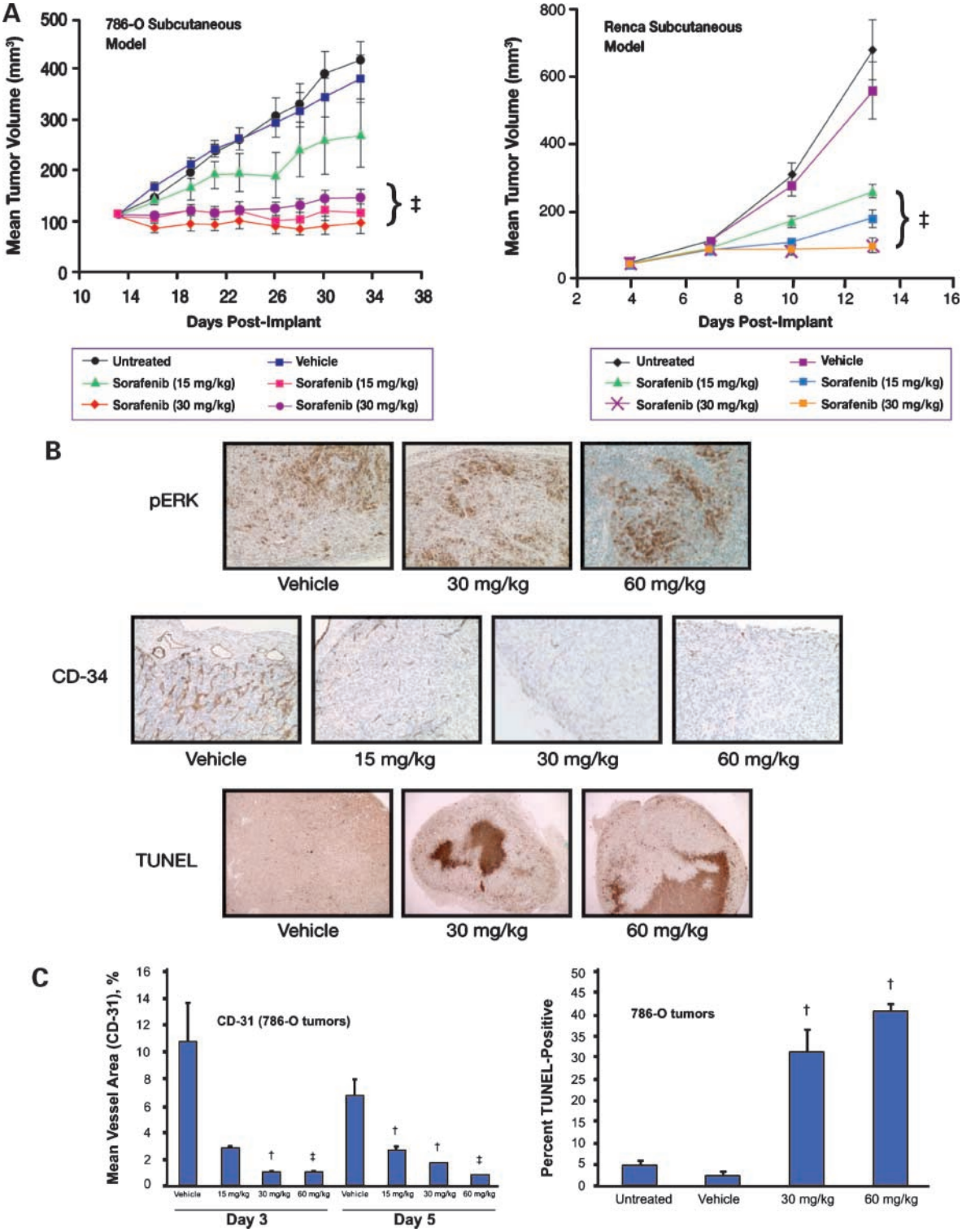

Figure 2. Sorafenib inhibits the growth of s.c. implanted human 786-O and murine Renca RCC tumors (adapted from refs. 42, 66). **A,** female athymic NCr *nu/nu* mice were implanted s.c. with 786-O tumor fragments or Renca cells. Sorafenib or vehicle control was administered orally, once a day, for 21 d (786-O) or 9 days (Renca) at the indicated dose. $n = 10$ per group. ‡, $P < 0.001$. **B,** sorafenib reduced CD34 but did not alter pERK level in 786-O tumors. Treatment began when tumors reached a volume of 200 to 400 mm (3). Sorafenib and vehicle control were administered orally, once a day, for 5 d at the indicated dose. Tumors were collected and then immunostained with anti-CD34 or anti-pERK antibody. **C,** level of CD34 and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling in the 786-O tumors was evaluated on images captured using bright-field microscopy. Average of more than 10 random tumor sections taken from three different tumor samples.

A very important clinical issue is cross-resistance to VEGFR tyrosine kinase inhibitors, such as sunitinib, or the VEGF-A monoclonal antibody, bevacizumab—both of which are used in the treatment paradigm for patients with metastatic RCC. In a recent preliminary report (63), 37 patients assessed after failure with either sunitinib or bevacizumab were then switched to oral sorafenib. Thirty-eight percent exhibited some degree of tumor shrinkage or disease stabilization, with an average progression-free survival of 3.8 months. This indicates that it may be possible to derive additional clinical benefit after failure of either primary or secondary treatment with an antivascular targeted agent. The optimal sequence of these agents requires further investigation.

Hepatocellular Carcinoma

In the PLC/PRF/5 HCC xenograft model, 10 mg/kg sorafenib inhibited tumor growth by 49% and produced complete tumor growth inhibition at a dose of 30 mg/kg (Fig. 3; ref. 39). A dose of 100 mg/kg produced partial tumor regressions in 50% of the mice. In this tumor model, sorafenib induced apoptosis that resulted in tumor shrinkage, reaching the level of objective regression after only 10 days of dosing (39). Sorafenib increased tumor cell apoptosis (assessed by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling), inhibited Raf signaling (pERK immunohistochemistry analysis), and decreased MVA (CD34 immunohistochemical analysis). Thus, both vascular targeting and inhibition of tumor Raf signaling, resulting in tumor cell apoptosis, may contribute to the tumor regression observed in the PLC/PRF/5 tumor model.

Recent observations of pERK as a biomarker in a phase II HCC clinical trial also support a role for Raf inhibition in the mechanism of action of sorafenib in HCC. In a recent phase II trial of patients with advanced, inoperable HCC, in which continuous administration of 400 mg bid sorafenib showed antitumor activity, tumor biopsies from 33 patients were evaluated immunohistochemically for pERK at baseline. The observed significant correlation of increased baseline pERK staining intensity with prolonged time to progression ($P < 0.001$) suggests that high baseline pERK levels may predict response to sorafenib (64).

The results described above suggest that, in HCC, sorafenib may act by targeting both vascularization and tumor cell survival. These mechanisms may provide the underlying basis for results seen in a phase III randomized, placebo-controlled trial in which sorafenib improved overall survival in patients with advanced HCC (stage C of the Barcelona Clinic Liver Cancer classification; ref. 65) and in those who progressed after locoregional therapies (13) and are consistent with current knowledge of the pathophysiology of HCC. Signaling through the Raf/MEK/ERK cascade, as well as angiogenesis, are reported to have important roles in the development of HCC (66). A recent report showed the ubiquitous activation of the Ras/mitogen-activated protein kinase (MAPK) pathway in HCC, mainly due to loss of function of tumor suppressor genes such as NOREB1 and RASSF1A, and of the Janus kinase/signal transducer and activator of transcription pathway (66). Unlike several other tumor types, B-Raf-activating mutations are relatively rare in HCC. However, Raf-1 kinase is overexpressed in a high number of HCC tumors, and the Raf/MEK/ERK pathway can be activated by major etiologic factors such as hepatitis B or C virus infection and mitogenic growth factors. Regarding activating mutations of Ras, the association between exposure to vinyl chloride and K-Ras mutations is well known, with rates of mutation as high as 42% (67). However, K-Ras and H-Ras mutations induced by chronic hepatitis infection or alcohol intake are rare in patients with HCC (68). Finally, HCC is notoriously hypervascular as evidenced by both its diagnostic perfusion pattern on imaging studies and its propensity for vascular invasion. Several reports have shown a significant overexpression of VEGFR mRNA and protein in human HCC samples (69), and in experimental HCC models, VEGFR blockade diminishes tumor growth (70).

Breast Cancer

The MDA-MB-231 breast cancer model was sensitive to sorafenib treatment, with a 30 mg/kg dose producing a 42% reduction in the mean size of these tumors after only 9 days of treatment (27). Sorafenib-treated tumors showed significant tumor necrosis after 5 days of treatment as visualized by hematoxylin staining (27). Daily oral administration of a 30 or 60 mg/kg dose of sorafenib strongly decreased MVA and microvessel density in the sorafenib-treated tumors, showing significant inhibition of angiogenesis in this tumor model. Immunohistochemistry analysis of the tumor sections showed a substantial decrease in the level of pERK and Ki-67 proliferation marker (27). Although in the majority of preclinical models sorafenib seems to act predominantly to prevent the growth of the tumors, in the MDA-MB-231 model sorafenib showed evidence of tumor regression after only 9 days of oral dosing (27). Aberrant cell proliferation is likely driven by expression of mutant K-Ras or B-Raf in certain cell lines. MDA-MB-231 cells contain activating mutations in both K-Ras and B-Raf proto-oncogenes. The presence of these activating mutations might confer a selective proliferative advantage to cells associated with greater dependence on signaling through the Raf/MEK/ERK pathway for survival. Indeed, in this tumor model, sorafenib induced cell death as early as 5 days after initiation of drug treatment as evidenced by extensive tumor cell necrosis. These results in the MDA-MB-231 model indicate that sorafenib may act in breast cancer through inhibition of the MAPK pathway and inhibition of angiogenesis. In this model, sorafenib-induced tumor shrinkage inhibited proliferation and angiogenesis, leading to tumor shrinkage (27).

Colon Cancer

Sorafenib induced complete tumor stasis when administered orally at doses of 30 or 60 mg/kg in two early-stage

human colon xenograft models (HT-29 and Colo-205, both expressing V600E B-Raf mutant). The growth of HCT-116 tumor xenografts has also been shown to be inhibited by 64% after 14 days of dosing with sorafenib at 30 mg/kg (27, 34). Furthermore, sorafenib doses as low as 3 mg/kg significantly slowed the growth of advanced-stage HCT-116 tumor xenografts (weighing ≥1 g at the start of treatment; ref. 34). Interestingly, the growth of HCT-116

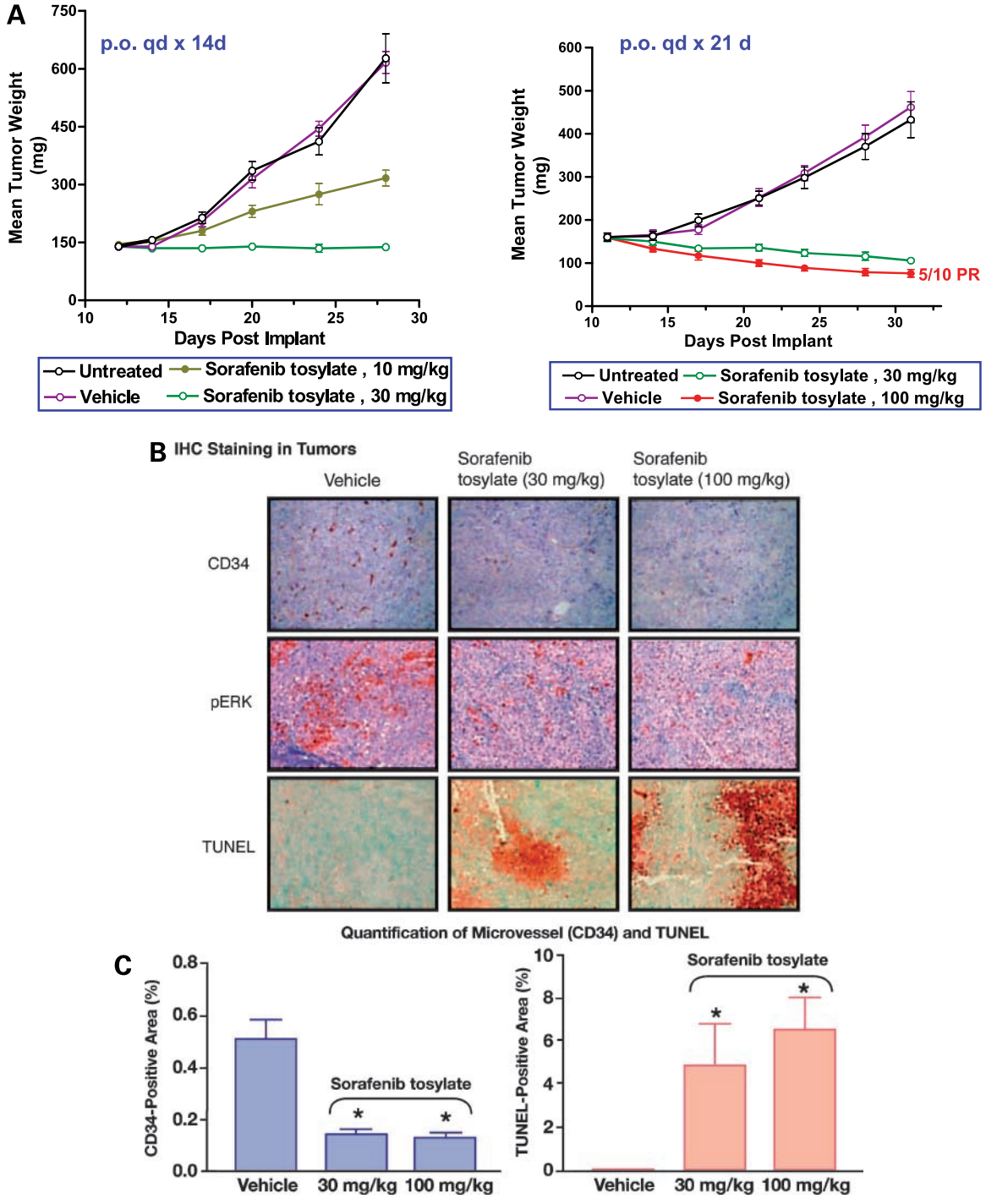

Figure 3. Sorafenib strongly inhibits the growth of PLC/PRF/5 HCC tumors in a xenograft mouse model (adapted from ref. 35). **A,** *in vivo* efficacy of sorafenib dosed orally, once a day, for 14 or 21 d. **B,** sorafenib decreases pERK and CD34 level and induces cell death in PLC/PRF/5 HCC tumors in mice. Tumors were collected and then immunostained with anti-pERK or anti-CD-34 antibody. **C,** sorafenib significantly inhibits MVA (CD34) in PLC/PRF/5 HCC tumors in mice. MVA and microvessel density were plotted.

tumor xenografts was still significantly reduced up to 14 days after cessation of sorafenib treatment (34). In contrast to the MDA-MB-231 breast tumor model, in which sorafenib induced tumor regression, sorafenib appeared to have a cytostatic effect in colon xenograft models (Fig. 4), as tumor size before treatment did not change significantly with increased duration of treatment (34). Human colon DLD-1 (K-Ras mutation) and Colo-205 (V600E B-Raf), which only harbor a single oncogene, are less sensitive to the Raf-inhibitory effects of sorafenib (pERK, IC_{50} = 2,000 and 4,000 nmol/L, respectively; ref. 27).

In the HT-29 colon tumors, tumor growth inhibition correlated with a decrease in ERK phosphorylation (27). Sorafenib treatment was also associated with significant (50-80%) inhibition of HT-29 tumor neovascularization, indicating that the effects of sorafenib on HT-29 tumor growth may be mediated by inhibition of both MAPK signaling pathway and tumor angiogenesis. In contrast, in the Colo-205 colon tumor model, sorafenib treatment did not affect ERK phosphorylation, but CD31 staining was significantly reduced, suggesting that, in this model, tumor growth inhibition was likely due to decreased tumor angiogenesis, not modulation of the MAPK signaling pathway. The differing results in HT-29 and Colo-205 tumor models indicate that although sorafenib targets both tumor cell proliferation and tumor angiogenesis, the importance of these two mechanisms may differ between different colon carcinomas.

Non-Small Cell Lung Cancer

Sorafenib has been shown to strongly inhibit tumor growth in two NSCLC tumor xenografts models, H460 and A549, both of which harbor a mutant K-Ras oncogene. In the A549 xenograft model, sorafenib induced complete tumor stasis (27). Sorafenib induces apoptosis in A549 or NCI-H460 NSCLC cells by down-regulating Mcl-1, but no such effect has been observed with the MEK inhibitor U0126, suggesting that sorafenib acts independently of signaling through MEK and ERK in these NSCLC lines (38). There is evidence to suggest that Raf-1 is involved in mediating the post-translational up-regulation of Mcl-1 to prevent apoptosis in tumor cells and that inhibition of Raf-1 down-regulates Mcl-1 protein levels in tumor cells (71). Therefore, it is conceivable that sorafenib may inhibit the growth of human A549 or NCI-H460 NSCLC cells and xenografts by blocking Raf-1 to promote Mcl-1 degradation and apoptosis by a MEK/ERK-independent mechanism. However, pERK levels are not affected by sorafenib concentrations as high as 10 μmol/L in human A549 or NCI-H460 NSCLC tumor cells despite the fact that these tumor lines both contain oncogenic K-Ras mutations (27). Although this suggests that the growth-inhibitory effects of sorafenib are not mediated through MEK and ERK, this observation does not necessarily preclude a direct (MEK/ERK-independent) effect of Raf. Several studies have suggested that wild-type Raf-1 inhibits apoptotic pathways to promote cell survival by interacting directly with apoptosis-regulatory proteins

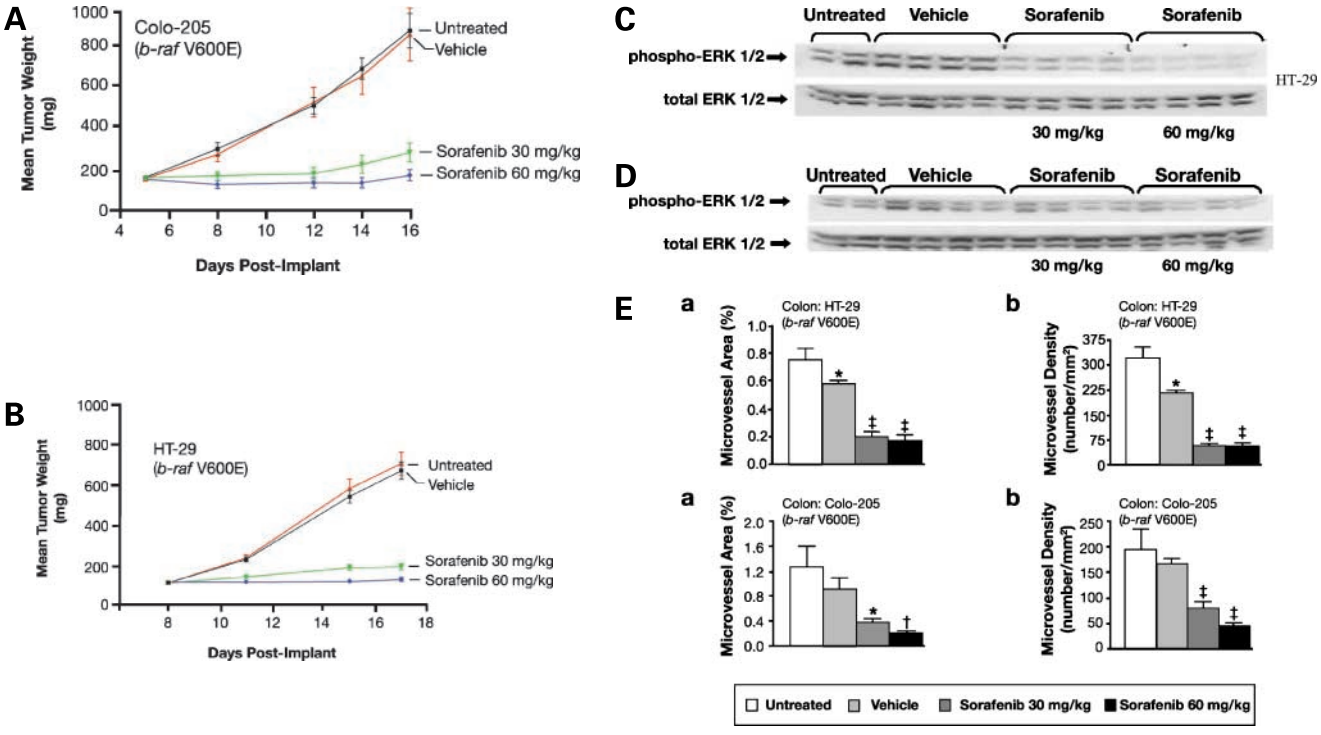

Figure 4. Effect of sorafenib on growth of (**A**) Colo-205 and (**B**) HT-29 human colon carcinoma xenografts [adapted from Wilhelm et al. (27)]. *In vivo* efficacy of sorafenib dosed orally, once a day, for 9 d. **C,** Western blot analysis using anti-pERK and anti-ERK antibodies. **D,** treatment with sorafenib inhibited tumor growth without substantially reducing MAPK activation in Colo-205 xenografts. **E,** tumors were collected and then immunostained with anti-CD31 antibody. MVA and microvessel density were plotted. Mice with tumors measuring 100 to 200 mg received 5 d of sorafenib treatment.

to alter their function without the need for activation of downstream MEK and ERK (72–74).

Melanoma

Sorafenib has been shown to inhibit the growth of human UACC 903 and 1205 Lu melanoma xenografts, which harbor the oncogenic B-Raf V600E mutation (75). Sorafenib (50 mg/kg i.p. every 48 h) significantly inhibited B-Raf V600E signaling within melanoma tumors as evidenced by a 3-fold decrease in pERK compared with vehicle-treated control mice. This antiproliferative effect of sorafenib was confirmed by a significant reduction in the incorporation of bromodeoxyuridine in tumor cells from treated animals (75). The primary effect of sorafenib in this model was the prevention of further vascular development of advanced-stage tumors by markedly inhibiting the secretion of VEGF, thereby leading to increased apoptosis within the UACC 903 tumor xenografts (75). These antiangiogenic and antiproliferative effects of sorafenib halt tumor growth but do not lead to regression of preexisting (advanced-stage) tumors (75). Inhibition of the expression of the B-Raf V600E oncogene in this model, using small interfering RNA, also blocks VEGF secretion by melanoma tumors (75). This results in antiangiogenic effects on the tumor vasculature, antiproliferative effects on tumor cells, and an overall cytostatic effect, similar to those observed with sorafenib. Similarly, sorafenib inhibits tumoral VEGF production, vascular development, and tumor growth in another B-Raf V600E-positive melanoma xenograft model (75). In contrast, inhibition of Raf-1 gene expression by small interfering RNA in these B-Raf V600E melanoma xenograft models had no effect on VEGF production, vascular development, tumor proliferation, or tumor growth (75). Also, despite decreasing pERK levels, sorafenib had no effect on the growth of human C8161 melanoma xenografts, which lack the B-Raf V600E oncogene (75). Therefore, although oncogenic B-Raf is important in driving the development of malignant melanoma, other signaling pathways, such as c-MET or c-Kit, can also drive tumorigenesis in this tumor type (76–79).

Sorafenib also inhibits the proliferation of the human melanoma cell lines SK-MEL 28 and A2058 (both of which express the B-Raf V600E mutant) and SK-MEL 2 (which expresses oncogenic N-Ras) at low micromolar concentrations *in vitro* (78). In addition to its antiproliferative effects on tumor cells, sorafenib has also been shown to inhibit the proliferation of tumor endothelial cells (79). In the K1735 murine melanoma model, 7 days of treatment with sorafenib at 30 mg/kg significantly impairs endothelial cell cycling as evidenced by a reduction in Ki-67 immunostaining (79). Therefore, sorafenib may act primarily by impairing angiogenesis and thereby disrupting the tumor vasculature in this murine melanoma model.

The results of a recently reported preclinical study suggests that, in melanoma cells, sorafenib may induce apoptosis by a mechanism different to that described for other tumor types (80). In B-Raf V600E-positive human SK-MEL 5 and A2058 melanoma cells, sorafenib down-regulates the antiapoptotic proteins Bcl-2 and Bcl-XL and induces apoptosis in a caspase-independent manner, largely by stimulating the nuclear translocation of apoptosis-inducing factor (80). Therefore, sorafenib may induce apoptosis by affecting more than one pathway.

Conclusions

The oral multikinase inhibitor sorafenib targets the Raf serine/threonine kinases (Raf-1, wild-type B-Raf, and oncogenic B-Raf V600E) and receptor tyrosine kinases (VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-β, Flt-3, and c-Kit) implicated in tumorigenesis and tumor progression. Sorafenib inhibits tumor growth in preclinical models of human melanoma, renal, colon, pancreatic, hepatocellular, thyroid, and ovarian carcinomas and NSCLC. Furthermore, sorafenib produced partial tumor regressions in mice bearing PLC/PRF/5 HCC and induced substantial tumor regression in a breast cancer model harboring B-Raf and K-Ras oncogenic mutations.

Preclinical studies suggest that sorafenib acts on tumors and tumor vasculature by inhibiting cellular proliferation and angiogenesis and/or by inducing apoptosis. In most tumor types, sorafenib inhibited signaling through Raf as evidenced by reduced pERK levels. Sorafenib induces apoptosis primarily by down-regulation of the antiapoptotic protein Mcl-1 possibly by a MEK/ERK-independent mechanism. Sorafenib also inhibited tumor angiogenesis in xenograft models, including a renal cancer model. It remains to be determined which molecular targets are responsible for anticancer effects across various tumor models.

In the clinic, sorafenib showed significant antitumor activity primarily due to a disease-stabilizing effect observed in phase III clinical trials in advanced RCC and HCC (two neoplasms resistant to classic chemotherapy). In advanced HCC, sorafenib significantly increased median overall survival (10.7 months for sorafenib versus 7.9 months for placebo) and median time to progression (5.5 months for sorafenib versus 2.8 months for placebo), indicating that sorafenib provides survival advantages by delaying the disease progression. This effect is consistent with data showing that sorafenib generally induces tumor cytostasis. The abrogation of two critical pathways in advanced HCC (Raf/MEK/MAPK and VEGF signaling) is assumed to be responsible for this effect, although genomic studies are under way to elucidate the molecular signatures of responders and biomarkers of response. A phase II HCC trial has shown that high baseline intratumoral pERK levels correlate with response to sorafenib. However, post-treatment evaluations of pERK are required to validate this putative biomarker and confirm that sorafenib acts clinically by decreasing Raf/MEK/ERK signaling *in vivo*.

The initial clinical success of sorafenib has been as monotherapy in RCC and HCC. Early-stage clinical trials show that sorafenib in combination with chemotherapy is well tolerated and improves the disease control rate across a wide range of tumor types and chemotherapeutic regimens (42). Results from late-stage trials in combination

with chemotherapy have been mixed. In a randomized, double-blind, placebo-controlled phase II study of patients with HCC, sorafenib in combination with doxorubicin doubled the median overall survival from 6.5 months in the doxorubicin-treated group to 13.7 months in the combination group (43). This study confirms the absence of activity of doxorubicin as a single systemic agent in HCC. Regarding the effect in combination, further phase III studies should clarify whether this is the result of a true synergistic effect or if it represents a benefit of sorafenib in a selected subgroup of patients. In fact, the median survival of 13 to 14 months is similar to that obtained in the subgroup of sorafenib-treated patients within the phase III trial who were stage Barcelona Clinic Liver Cancer-B or had HCV-related HCC (13). In other studies, no improvement in overall survival was found with sorafenib in combination with either dicarbazine or paclitaxel plus carboplatin in patients with melanoma despite having activity in preclinical models and despite patient biopsy samples showing a variable decrease in pre- and post-nuclear and cytosolic pERK staining (81) and a statistically significant increase in terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling-positive cells (81) following treatment (44, 81). Similar negative results were seen with paclitaxel and carboplatin in patients with advanced NSCLC (44, 81, 82). Although these results were disappointing, studies are ongoing with paclitaxel and cisplatin in patients with melanoma in the first-line setting where better activity may be seen in chemotherapy-naive patients and with a different chemotherapy regimen (capecitabine) in patients with NSCLC. Additional well-controlled, randomized studies will be needed to identify the optimal chemotherapeutic regimen and schedule for use in combination with sorafenib. Studies with signal transduction inhibitors such as bevacizumab and erlotinib are still in the early stages of clinical exploration. Further investigation of promising biomarkers for predicting response and prognosis is clearly warranted.

In conclusion, sorafenib is a multikinase inhibitor that acts by inhibiting tumor growth and disrupting tumor microvasculature through antiproliferative, antiangiogenic, and proapoptotic effects. Studies in xenograft models showed that sorafenib acts through several mechanisms to inhibit tumor angiogenesis, induce tumor cell apoptosis, and inhibit the MAPK signaling cascade. The multiple molecular targets of sorafenib, which include Raf-1, wild-type B-Raf, B-Raf V600E, VEGFR-1, VEGFR-2, VEGFR-3, PDGFR-β, Flt-3, and c-Kit, may explain its broad preclinical activity across tumor types and its clinical activity in RCC and HCC.

Disclosure of Potential Conflicts of Interest

S.M. Wilhelm, L. Adnane, and M. Lynch: employees of Bayer HealthCare Pharmaceuticals. No other authors disclosed potential conflicts of interest.

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