Vascular Endothelial Growth Factor–Targeted Therapy in Renal Cell Carcinoma: Current Status and Future Directions

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Abstract Renal cell carcinoma is a highly vascular tumor associated with expression of vascular endothelial growth factor (VEGF). Recently, VEGF-targeted therapies have been identified as a promising therapeutic approach. Three agents targeting the VEGF pathway have shown clinical activity as monotherapy in metastatic renal cell carcinoma: the anti-VEGF monoclonal antibody, bevacizumab, and small-molecule VEGF receptor tyrosine kinase inhibitors, sorafenib and sunitinib. This article explores these agents in terms of their mechanisms of action, clinical efficacy, and toxicity profiles. This article also reviews future development strategies, including combination regimens and drug sequencing, trial design considerations, and patient selection opportunities.

Historically, treatment options for metastatic renal cell carcinoma (RCC) have been limited because of inherent tumor resistance to chemotherapy and radiotherapy. As immune mechanisms are thought to be important in regulating tumor growth and progression in metastatic RCC, immunotherapeutic agents, such as interleukin-2 and IFN-α (IFNA), have been evaluated. Only a limited subset of patients, however, benefit from these cytokines with modest objective response rates (ORR) in an unselected cohort (1, 2). For this reason, new therapies for metastatic RCC patients have long been sought.

Several treatment strategies have been investigated in metastatic RCC. One approach is to block the signals initiated by angiogenic growth factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (3). VEGF is the major factor responsible for tumor angiogenesis, and there is a particularly strong rationale for blocking VEGF in RCC. Approximately 60% of clear cell RCC tumors have an inactivated *von-Hippel Lindau* (*VHL*) tumor suppressor gene, either through somatic mutation (~50% of RCC tumors) or promoter methylation (~10% of tumors; ref. 4). A normal VHL protein indirectly represses transcription of hypoxia-inducible genes, such as *VEGF*, by targeting the α subunit of hypoxia-inducible factor-α for proteolytic degradation (5–7). Under hypoxic conditions or when the *VHL* gene is inactivated, hypoxia-inducible factor-α is not degraded, resulting in induction of VEGF transcription, overexpression of VEGF protein, and angiogenesis (5, 8, 9). Indeed, near universal up-regulation of hypoxia-inducible factor–mediated genes in RCC, such as *PDGF* and *carbonic anhydrase 9*, in addition to VEGF supports the hypothesis that mechanisms of VEGF pathway activation in addition to *VHL* gene silencing are relevant in RCC. RNA and protein microarray studies show that nearly all RCC tumors express high levels of VEGF and that this overexpression results in RCC tumor vascularity, which correlates with tumor progression and poor prognosis (10). RCC tumors become dependent on VEGF, and blocking this factor can result in regression of both the tumor and its vasculature. Finally, in addition to its angiogenic properties, there is also evidence that VEGF may suppress antitumor immune responses by inhibiting recruitment and activation of dendritic cell function, supporting the possibility that VEGF blockade may enhance an existing immune response against the tumor (11). Against this background, targeting VEGF has emerged as a promising treatment strategy in patients with metastatic RCC.

Two main strategies have been evaluated for targeting VEGF signaling: ligand blockade through a monoclonal antibody, soluble receptor/ligand trap or an aptamer, and blocking signaling by targeting the receptor with either a monoclonal antibody or a small-molecule tyrosine kinase inhibitor. The three VEGF-targeting agents with the most advanced clinical development in RCC are the monoclonal antibody, bevacizumab (Avastin, Genentech, Inc., South San Francisco, CA), and the small-molecule VEGF receptor (VEGFR) tyrosine kinase inhibitors, sorafenib (BAY 43-9006, Nexavar, Bayer Pharmaceuticals, West Haven, CT and Onyx Pharmaceuticals, Emeryville, CA) and sunitinib (SU11248, Sutent, Pfizer, Inc., New York, NY).

The mechanisms of action, monotherapy data, and toxicity profiles will be examined in this review to characterize bevacizumab, sorafenib, and sunitinib. In addition, this article reviews strategies to maximize the clinical potential of these agents in metastatic RCC, including combination and sequential therapy, trial design, and patient selection.

Mechanisms of Action of Bevacizumab, Sorafenib, and Sunitinib

Bevacizumab binds VEGF, preventing interaction with its receptors and activation of downstream signaling pathways (Fig. 1). This ultimately leads to vascular regression, leaving the

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tumor dormant. Preclinical studies with A4.6.1, the murine precursor of bevacizumab, showed significant reductions (25-95%) in the rate of tumor progression compared with control mice in a variety of human xenograft tumor models, including RCC models (reviewed in ref. 12). In addition, preclinical studies have shown that bevacizumab has activity against metastases (reviewed in ref. 12) and that A4.6.1 prevented lung metastases from Wilms' tumors implanted into kidneys of nude mice (13).

Broadly speaking for all solid tumors, bevacizumab is thought to act through three potential mechanisms: (*a*) regression of existing microvasculature; (*b*) normalization of mature vasculature; and (*c*) inhibition of the production of new vasculature. The early effects of bevacizumab therapy include the regression of existing microvasculature (14) resulting in consistent improvements in response rates by approximately 10% to 15% over chemotherapy alone (15, 16). Additionally, a normalization process, in which the tumor vasculature is pruned of dilated, tortuous, and permeable blood vessels, results in a more efficient vasculature, reduced permeability, and reduced interstitial pressure (17, 18), which can aid the delivery of chemotherapy to the tumor (15, 16). The continued use of bevacizumab can prevent the growth of new vessels (19), thus improving the duration of response, rate of disease stabilization, and delaying disease progression (15, 16, 20). The precise mechanism of effect of bevacizumab in RCC has not been fully elucidated. Presumably, direct effects on microvasculature, on which RCC is heavily dependent, leads to tumor regression and slowing of tumor growth. Improved delivery of additional systemic therapy has not been to date relevant in RCC given lack of chemotherapy effectiveness, although this potential mechanism may be relevant to future combination trials.

Sorafenib is an oral multikinase inhibitor that inhibits VEGFRs 1 to 3, PDGF receptor (PDGFR)-β, and the serine threonine kinase Raf-1, which acts through the canonical Raf/mitogen-activated protein kinase/extracellular signal-regulated kinase signaling pathway and plays a role in cellular proliferation and tumorigenesis (Fig. 1; refs. 21, 22). In preclinical studies, sorafenib significantly inhibited neovascularization in human colon and breast cancer xenograft models, showing significant tumor growth inhibition in both models (22). A recent study showed that sorafenib inhibits the growth of renal adenocarcinoma through inhibition of tumor angiogenesis (23). The contribution of the Raf kinase inhibitory properties of sorafenib to the antitumor effect in RCC is not well understood. Activating Ras or Raf mutations are rare in RCC (24, 25), yet mitogen-activated protein kinase/extracellular signal-regulated kinase kinase/extracellular signal-regulated kinase pathway activation is observed. Further investigation is needed to understand whether manipulation of this pathway in RCC is of therapeutic benefit.

Sunitinib is an oral inhibitor with potent *in vitro* and cellular activity against several related protein tyrosine kinase receptors, including PDGFR-β, stem cell factor receptor (KIT), and Fms-like tyrosine kinase-3, as well as VEGFRs 1 to 3 (Fig. 1; refs. 26, 27). Preclinical studies in various tumor cell lines have shown that the antiangiogenic effects of sunitinib are mediated through VEGFR and PDGFR-β, and it also has a direct antitumor effect through KIT and Flt3 in diseases where these pathways are biologically relevant (26, 28). Based on

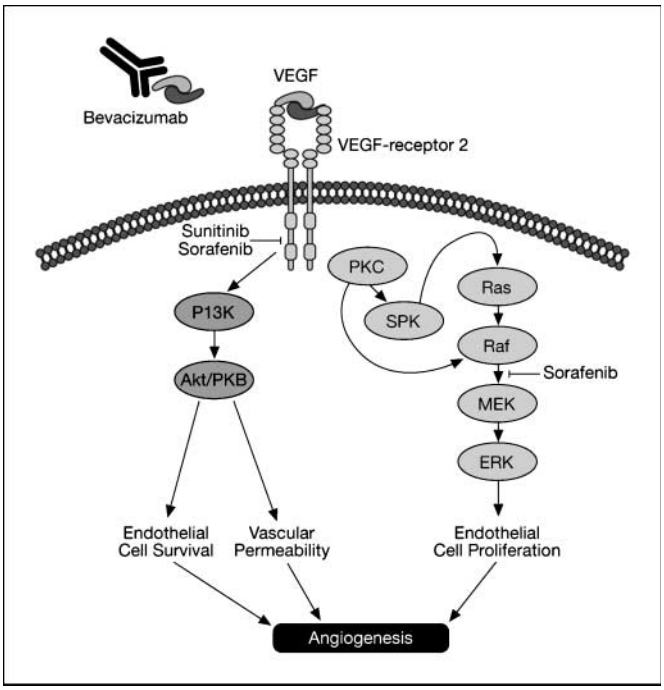

Fig. 1. Mechanism of action of inhibitors of the VEGF/VEGFR signaling pathway. Bevacizumab binds VEGF, preventing interaction with its receptors and activation of downstream signaling pathways. Sunitinib is an oral tyrosine kinase inhibitor with potent activity against several related protein tyrosine kinase receptors, including VEGFRs 1 to 3. Sorafenib is also an oral multikinase inhibitor that, in addition to inhibiting VEGFRs 1 to 3, also inhibits the serine threonine kinase Raf-1 involved in the Raf/mitogen-activated protein kinase/extracellular signal-regulated kinase (*ERK*) kinase (*MEK*)/extracellular signal-regulated kinase signaling pathway activated after VEGF binding.

preclinical and clinical evidence, the mechanism of action of sunitinib in RCC is thought to be through inhibition of the VEGF pathway in endothelial cells and PDGFR-β expressed on the supporting pericytes (26). Preclinical models have shown a more potent angiogenic effect through inhibition of both VEGFR on endothelial cells and PDGF on pericytes than inactivation of endothelial cells alone (29). There is, however, inconclusive evidence supporting direct effects of inhibiting either VEGFR or PDGFR signaling on RCC cells.

Monotherapy Trials

Phase II and III data with bevacizumab, sorafenib, and sunitinib show that each of these anti-VEGF agents is effective as monotherapy in cytokine-refractory, metastatic RCC (Table 1; refs. 30–35). The first clinical trial to show that anti-VEGF therapy was effective in metastatic RCC was a placebo-controlled randomized double-blind phase II trial of bevacizumab monotherapy 3 or 10 mg/kg every 2 weeks in 116 cytokine-refractory patients (30, 31). This trial was closed early after interim analysis showed that time-to-progression was significantly longer in patients treated with bevacizumab 10 mg/kg (4.8 months) than in those patients treated with placebo (2.5 months; hazard ratio = 2.55; $P < 0.001$; Fig. 2A). This trial used strict progression criteria, resulting in several patients meeting progression criteria with a lower tumor burden than baseline. The value of bevacizumab monotherapy in untreated RCC was defined recently from a bevacizumab monotherapy arm of a randomized phase II trial of bevacizu-

mab ± erlotinib. The ORR was 13% and median progression-free survival (PFS) with bevacizumab monotherapy was a substantial 8.5 months (36). There was no improved clinical outcome with the addition of erlotinib to bevacizumab.

In an initial sorafenib trial, metastatic RCC patients (n = 202) were treated with 12 weeks of sorafenib 400 mg bid, and then patients with tumor burden change within 25% of baseline were randomized to placebo or to continuation of sorafenib. A PFS advantage of 24 versus 6 weeks (P = 0.0087) was shown in the randomized cohort of 65 patients at 12 weeks postrandomization (33). A subsequent 905 patient, placebo-controlled, randomized trial of sorafenib 400 mg bid in cytokine-refractory, metastatic RCC was conducted. The trial investigators reported a PFS advantage in the sorafenib arm of 5.5 versus 2.8 months (P < 0.000001; Fig. 2B; ref. 32). A 2% Response Evaluation Criteria in Solid Tumors (RECIST)–defined ORR was seen in the sorafenib arm, but 74% of patients overall had some degree of tumor burden shrinkage, thus accounting for the PFS benefit. Sorafenib was approved by the Food and Drug Administration as monotherapy for advanced RCC in December 2005. A randomized phase II trial of sorafenib versus IFNA in untreated metastatic RCC is ongoing to define the activity of sorafenib in this setting (37).

Sunitinib showed an ORR of ~40% in patients with cytokine-refractory, metastatic RCC in two sequential phase II trials with a combined median PFS of 8.2 months (Fig. 2C; ref. 34, 35). A recent phase III randomized trial of first-line sunitinib versus IFNA in 750 patients with metastatic clear cell RCC showed statistically significant improvements in ORR and PFS with sunitinib compared with IFNA. Median PFS as assessed by an independent review was 11 months in the sunitinib arm versus 5 months in the IFNA arm, and ORR was 31% versus 6%, respectively (P < 0.000001; ref. 38). Sunitinib was approved by the Food and Drug Administration as monotherapy for advanced RCC in January 2006. Data from the phase III trials of sorafenib and sunitinib are not at present mature enough to permit assessment of overall survival (OS). Table 2 summarizes completed, ongoing, and planned trials of bevacizumab, sorafenib, and sunitinib in metastatic RCC.

It is noteworthy in the monotherapy trials of bevacizumab, sorafenib, and sunitinib that, although RECIST-defined ORRs varied from 2% to 40%, a relatively uniform 70% to 75% of patients experienced shrinkage of total tumor burden (defined as the sum of RECIST-defined target lesions). Thus, a significant percentage of patients experience tumor burden reduction from 1% to 29%, not meeting the 30% RECIST criteria for an objective response, but showing an antitumor effect of the drug. It is unknown if the similar degree of tumor shrinkage reflects the similarity among antitumor mechanism(s) and if different ORRs imply significant differences in antitumor mechanism of a given drug. Other considerations for response rate differences could be dosing and scheduling of each agent, which could affect on the attainment of therapeutic drug levels. Importantly, PFS was increased with each of these agents over control, supporting the notion that tumor shrinkage not meeting RECIST criteria for objective response can lead to PFS benefits for the overall cohort (Fig. 2). There are no firm data to correlate degree of tumor shrinkage or ORRs with PFS or OS for these agents in metastatic RCC. Alternative or additional thresholds for response in addition to the RECIST-defined 30% tumor burden shrinkage requirement require investigation. Similarly, the 20% tumor burden increase threshold of RECIST criteria may not be adequate for all patients receiving these agents. Tumor appearance change independent of size (e.g., darkened center consistent with tumor necrosis) is not presently accounted for by RECIST criteria but may reflect a beneficial drug effect. Patients with substantial reductions in tumor burden followed by slow tumor growth may still have an antitumor effect of the drug despite a 20% tumor burden increase from the smallest amount of tumor burden. Randomized, prospective trials will be required to decipher the value of continuing an agent in this setting. Additionally, alternative imaging modalities, such as dynamic contrast-enhanced magnetic resonance imaging, which assesses vascular permeability and blood flow, are being investigated to complement tumor size reduction in RCC patients on VEGF-targeted therapy and also to potentially permit early selection of patients for response through correlation of baseline and/or early time point imaging results with clinical outcome.

Long-term therapy

The early clinical results with VEGF-targeting agents in RCC are noteworthy for robust objective response and/or tumor shrinkage rates, but this is balanced against the paucity of complete responses and the resulting need for long-term

Table 1. Phase II/III data from key trials of bevacizumab, sorafenib, and sunitinib in metastatic RCC

	Study population	No. patients	PFS	ORR (%)
Bevacizumab vs placebo (30)	2nd Line	116	4.8 vs 2.5 mo	10 vs 0*
Bevacizumab ± erlotinib (36)	1st Line	104	8.5 vs 9.9 mo	13 vs 14†
Sorafenib vs placebo (32)	2nd Line	905	5.5 vs 2.8 mo	2 vs 0†‡
Sorafenib (33)	2nd Line or later	202	24 wk	4†
Sunitinib (34)	2nd Line	63	8.7 mo	40†§
Sunitinib (35)	2nd Line	106	8.1 mo	44†§
Sunitinib vs IFNA (38)	1st Line	750	11 vs 5 mo	31 vs 6‡

*Partial response: ≥50% of the sum of products of the maximum perpendicular diameters of measured lesions lasting for a minimum of 1 mo with no progression of any lesion or appearance of new lesions.
†RECIST criteria.
‡Third-party independent review.
§Investigator assessed.

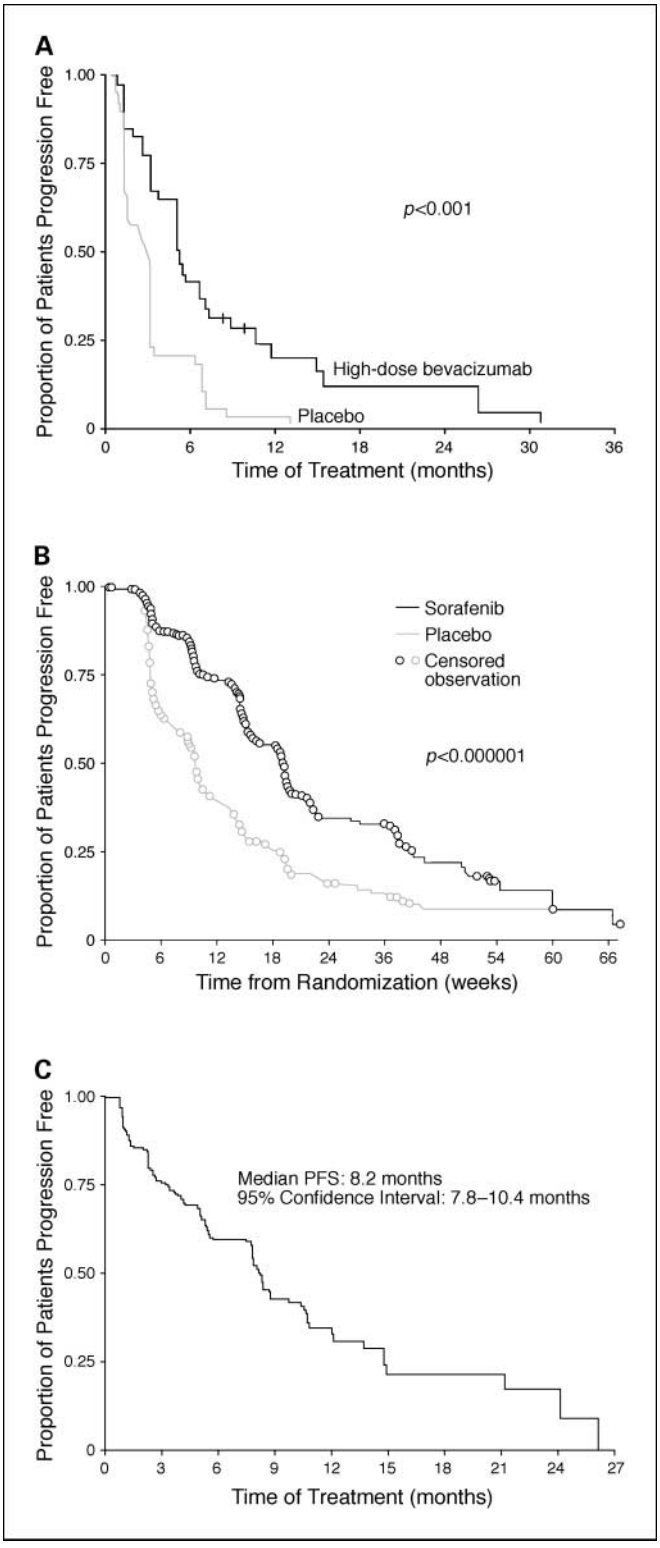

Fig. 2. PFS data from bevacizumab (*A*; ref. 30), sorafenib (*B*; ref. 32), and sunitinib (*C*; ref. 35) phase II/III monotherapy trials. *A* and *B,* significant improvements in PFS rates with high-dose 10 mg/kg bevacizumab monotherapy versus placebo (*A*) and with 400 mg bid sorafenib monotherapy versus placebo (*B*) in randomized phase II/III trials of cytokine-refractory RCC patients. *C,* combined median PFS in a pooled population of patients with cytokine-refractory metastatic RCC ($n = 168$) from two sequential phase II trials of sunitinib monotherapy. *A,* Copyright © 2003 Massachusetts Medical Society. All rights reserved (30). *B,* reproduced with kind permission of Bernard Escudier. *C,* reproduced with kind permission from the American Medical Association (35).

therapy to maintain benefit in responding patients. Thus, the feasibility of chronic therapy is of clinical relevance for this class of agents. Preclinical studies show that sustained VEGF blockade can result in long-term tumor growth inhibition and prolonged survival coincident with a decrease in microvascular density (39). In the phase II monotherapy trial of bevacizumab, four patients underwent long-term bevacizumab therapy without tumor progression for 3 or more years (31). Side effects were limited and consisted mainly of transient proteinuria that returned to baseline after a brief drug holiday. Patients also showed additional tumor shrinkage on resumption of bevacizumab after off periods. These limited data suggest that RCC tumors may continue to rely on VEGF signaling, and in some cases, will continue to respond to persistent and/or repeated VEGF blockade. There are fewer published data on long-term therapy with sorafenib and sunitinib, although it has been reported that sunitinib was administered for more than 2 years in responding patients (34, 35). An additional consideration as noted above is whether administration of these agents should be continued beyond disease progression, especially if a patient initially responded to therapy and the pace of disease progression is slower than before treatment. Several trials have allowed treatment beyond RECIST-defined disease progression if there was clinical benefit in the opinion of the treating physician. Variable application of this strategy, however, makes firm conclusions difficult. Furthermore, issues of interpatient variability in the metabolism of sorafenib and interrupted dosing (4 weeks on/2 weeks off) of sunitinib introduce the possibility that dose/schedule optimization may be needed for individual patients who progress on these therapies. Additional prospective investigation into alternative dosing/scheduling strategies, continuation of therapy beyond progression, and feasibility/toxicity of long-term therapy is needed.

Managing adverse events

Understanding adverse events associated with bevacizumab, sorafenib, and sunitinib therapy will guide how these agents are used in clinical practice. Initial studies with bevacizumab showed that it was generally well tolerated with the most common toxicities being hypertension and proteinuria. Proteinuria was asymptomatic and not associated with renal dysfunction, even in patients undergoing long-term therapy, and hypertension was managed with standard antihypertensive agents (31). Other less common toxicities were noted in large studies in colorectal cancer and included arterial thromboembolic events, such as myocardial infarction (1.5%) and cerebrovascular accident (0.5%), and gastrointestinal perforations (2.0%; ref. 15). Sorafenib was also generally well tolerated. The most commonly reported grade 3/4 toxicities were hand-foot syndrome, fatigue, and hypertension. The incidence of grade 3/4 toxicities seemed to be lower in the phase III trial when compared with the phase II trial (32, 33), but this may be because patients with several adverse risk factors were excluded from the phase III trial. The most common adverse events with sunitinib were constitutional, gastrointestinal, and dermatologic and hypertension (34, 35). Serious gastrointestinal complications, including gastrointestinal perforation, have occurred rarely in patients with intra-abdominal malignancies treated with sunitinib. Vascular toxicities, such as hypertension and bleeding, are biologically

Table 2. Selected completed and ongoing trials of bevacizumab, sorafenib, and sunitinib in RCC

Agent	Study design	Dose of antiangiogenic agent	Status	Investigator, sponsor (reference)
Bevacizumab	Randomized, placebo-controlled, phase II trial of bevacizumab	3 or 10 mg/kg i.v. q2w	Completed	Yang, NCI, Bethesda, MD (30)
	Single-arm, phase II trial of bevacizumab + erlotinib	10 mg/kg i.v. q2w	Completed	Hainsworth, Sarah Cannon Research Institute, Nashville, TN (56, 57)
	Randomized, double-blind, phase II trial of bevacizumab ± erlotinib (RACE)	10 mg/kg i.v. q2w	Completed	Bukowski, Genentech (36)
	Randomized, phase III trial of bevacizumab + IFNA-2b vs IFNA-2b	10 mg/kg i.v. q2w	Ongoing	Rini, CALGB (48)
	Randomized, placebo-controlled, phase III trial of bevacizumab + IFNA-2a vs IFNA-2a	10 mg/kg i.v. q2w	Ongoing	Escudier, Roche
	Single-arm, phase II trial of bevacizumab + erlotinib (neoadjuvant)	10 mg/kg i.v. q2w	Ongoing	Jonasch, M. D. Anderson Cancer Center, Houston, TX
	Phase I/II trial of bevacizumab + sorafenib (all solid tumors)	MTD: bevacizumab = 5-10 mg/kg q2w; sorafenib = 200 mg bid	Ongoing	Kohn, NCI, Bethesda, MD (63)
	Phase I/II trial of bevacizumab + sorafenib	Dose escalation design	Ongoing	Sosman, Vanderbilt-Ingram Cancer Center Nashville, TN/NCI (51)
	Phase I trial of bevacizumab + sunitinib (solid tumors)	Dose escalation starting at 25 mg sunitinib + 5 mg/kg bevacizumab	Ongoing	Rini, Cleveland Clinic Taussig Cancer Center, Cleveland, OH/NCI
	Phase I trial of bevacizumab + sunitinib (RCC only)	Dose escalation of sunitinib + 10 mg/kg bevacizumab	Ongoing	Motzer, MSKCC, New York, NY Genentech
	Single-arm, phase II trial of bevacizumab + high-dose interleukin-2	10 mg/kg i.v. q2w	Ongoing	Ernstoff, Cytokine Working Group
	Single-arm, phase II trial of bevacizumab + low-dose interleukin-2	10 mg/kg i.v. q2w	Ongoing	Garcia, Cleveland Clinic Taussig Cancer Center, Cleveland, OH
	Phase II trial of bevacizumab and RAD001 (everolimus)	10 mg/kg i.v. q2w	Ongoing	Hainsworth, Sarah Cannon Research Institute, Nashville, TN
Sorafenib	Randomized, placebo-controlled, phase III trial of sorafenib	400 mg oral bid	Completed	Escudier, Gustave-Roussy Institute, France/Bayer (32)
	Randomized discontinuation phase II trial of sorafenib	400 mg oral bid	Completed	Ratain, University of Chicago, Chicago, IL (33)
	Randomized, phase II trial of sorafenib vs IFNA	400 mg oral bid to 600 mg bid (following progression)	Ongoing	Escudier, Bayer (37)
	Randomized, phase II trial of sorafenib ± low-dose IFNA	400 mg oral bid	Ongoing	Jonasch, M. D. Anderson Cancer Center, Houston, TX
	Single-arm, phase II trial of sorafenib + IFNA	400 mg oral bid	Ongoing	Gollob, Duke Cancer Comprehensive Center, Durham, NC/NCI (49)
	Single-arm, phase II trial of sorafenib + IFNA	400 mg oral bid	Ongoing	Ryan, SWOG (50)
	Single-arm, phase II trial of sorafenib in RCC patients refractory to sunitinib or bevacizumab	400 mg oral bid to 800 mg bid (following progression)	Ongoing	Rini, Cleveland Clinic Taussig Cancer Center, Cleveland, OH
Sunitinib	Two, single-arm, phase II trials of sunitinib	50 mg oral daily; 4 wk on/2 wk off	Completed	Motzer, Pfizer (34, 35)
	Randomized, phase III trial of sunitinib vs IFNA	50 mg oral daily; 4 wk on/2 wk off	Completed	Motzer, Pfizer (38)
	Single-agent, phase II trial of sunitinib (bevacizumab-refractory metastatic RCC)	50 mg oral daily; 4 wk on/2 wk off	Completed	Rini, Cleveland Clinic Taussig Cancer Center, Cleveland, OH, Pfizer (59)
	Single-arm, phase II trial of sunitinib (continuous daily regimen)	37.5 mg oral daily	Ongoing	Pfizer (64)
	Randomized phase III trial of two sunitinib dosing schedules (EFFECT trial)	50 mg oral daily; 4 wk on/2 wk off vs 37.5 mg oral daily	Ongoing	Pfizer
	Single-arm, phase II trial of sunitinib in patients with unresectable primary RCC tumors with or without metastases	50 mg oral daily; 4 wk on/2 wk off	Ongoing	Rini/Campbell; Cleveland Clinic Taussig Cancer Center, Cleveland, OH
	Sunitinib precytoreductive or postcytoreductive nephrectomy in metastatic RCC	50 mg oral daily; 4 wk on/2 wk off	Ongoing	Jonasch, M. D. Anderson Cancer Center, Houston, TX

Abbreviations: NCI, National Cancer Institute; RACE, rapid amplification of cDNA ends; CALGB, Cancer and Leukemia Group B; MTD, maximum tolerated dose; MSKCC, Memorial Sloan-Kettering Cancer Center; SWOG, Southwest Oncology Group.

plausible based on the known effects of VEGF on blood pressure and existing vasculature, yet the precise mechanism of each of these toxicities is at present poorly understood. One report of 20 patients treated with sorafenib failed to find a relationship between changes in blood pressure and several measured variables, including VEGF, renin, and aldosterone (40). In terms of serious cardiovascular events, three patients with metastatic RCC in the phase II sunitinib trials experienced grade 3 myocardial ischemia and one patient experienced myocardial infarction while on treatment, which was fatal. As use of these agents increases in prevalence and duration, the toxicity in an unselected RCC population will be further defined. Rare, but potentially serious toxicities may emerge, and toxicity associated with long-term use, such as cardiac toxicity, will require careful vigilance. Perioperative issues of wound healing will also have a greater importance. The toxicity of these agents requires a deeper mechanistic understanding so that prevention and intervention strategies can be formulated.

Maximizing the Clinical Benefit of Bevacizumab, Sorafenib, and Sunitinib

Approaches that may maximize the clinical benefit of these therapies include evaluating appropriate combination regimens, implementation of novel trial designs with relevant clinical end points, and identifying predictive markers of response.

Combination therapy

Preclinical rationale. The rationale for targeting VEGF in RCC is strong, particularly because of the frequent disruption of *VHL* and the resulting hypoxia-inducible factor–mediated up-regulation of VEGF. Other factors, however, are also up-regulated by this mechanism, including PDGF-B, transforming growth factor-α, and erythropoietin. PDGF-A and PDGF-B are secreted as homodimer or heterodimer and bind to the dimeric PDGF-β receptor. These receptors are expressed at high levels both in the pericytes supporting the tumor vasculature and in the renal epithelial cancer cells (41). The related PDGF-D has also been found to be up-regulated in renal and ovarian tumors (42). RCC therefore has the potential for both autocrine and paracrine signaling through PDGFs in either the tumor cells or the tumor vasculature. Conceivably, enhanced antitumor activity could be achieved by simultaneous targeting of both VEGF and PDGF signaling. Various preclinical studies suggest that IFN is also an antiangiogenic agent (43), and combining two agents with antiangiogenic properties may result in additive or synergistic antitumor activity. Preclinical studies have also shown that targeting three angiogenesis factors, VEGF, VEGFR-2, and Tie2, a receptor tyrosine kinase expressed principally on vascular endothelium, with tumor immunotherapy can lead to synergistic antitumor activity (44). Pharmacodynamic analyses of early phase I studies of sorafenib suggested that T-cell activation was inhibited (45, 46). In addition, a recent report substantiates the ability of sunitinib to reduce immunosuppressive T regulatory cells and favorably alter the inherently immunosuppressive T helper 2 bias in RCC (47). Taken together, these data support potential immunomodulatory effects of the targeted agents in RCC, opening the door for several combination therapy strategies. Further characterization of the effects of these agents on cellular immune variables is needed to allow for the most rational strategies. Based on these considerations, anti-VEGF therapy is being combined with other antiangiogenics and with immunotherapy in the clinic.

Clinical trials. Clinical trials have been initiated to examine the feasibility of combining anti-VEGF agents with several other agents. Addition of bevacizumab to IFNA is being evaluated compared with IFN monotherapy in two randomized, placebo-controlled phase III trials as first-line therapy for patients with metastatic RCC (48). These trials will provide additional data about the efficacy of bevacizumab in the front-line setting and combination data with IFN but will not fully define the additive or synergistic effects of this combination due to lack of bevacizumab monotherapy control arm. Additional trials are investigating sorafenib in combination with IFN. Preliminary data from the 24 evaluable patients in a single-arm, phase II trial of sorafenib plus IFNA-2b in patients with metastatic RCC showed one complete response and nine partial responses for an overall response rate of 38% (49). In another identically designed study of the combination as first-line therapy in patients with advanced RCC, the preliminary overall response rate for 53 evaluable patients was 19% (50). Grade 3+ toxicities included fatigue, rash, leukopenia, neutropenia, and hypophosphatemia. The value of this combination over either agent as monotherapy will require further study. A phase I trial of sunitinib in combination with IFN and a phase I trial of sunitinib in combination with an antibody against the immunosuppressive molecule CTL antigen 4 are also ongoing.

The feasibility of combining VEGF-targeting approaches is also being studied. In a phase I trial, 24 patients with metastatic RCC received 3, 5, or 10 mg/kg bevacizumab every 2 weeks plus 200 mg qd, 200 mg bid, or 400 mg bid sorafenib. The combination showed preliminary evidence of antitumor activity, but the full doses of each agents were not reached due to dose-limiting toxicity related primarily to hand-foot syndrome, functional stomatitis, anorexia, and fatigue (51). Further testing of this combination remains ongoing. Similar findings were observed in another phase 1 dose-escalation study of bevacizumab plus sorafenib in patients with advanced solid tumors (52). Phase I trials of bevacizumab in combination with sunitinib have also begun accrual.

Another approach examined anti-VEGF agents combined with targeted agents, such as HER1/epidermal growth factor receptor tyrosine kinase inhibitors. Studies suggest that HER1/epidermal growth factor receptor is overexpressed in human RCC (53) and is associated with rapid tumor cell proliferation in metastatic RCC (54). A single-arm phase II trial in 63 patients of bevacizumab in combination with erlotinib hydrochloride (Tarceva), an oral, small-molecule HER1/epidermal growth factor receptor tyrosine kinase inhibitor, was conducted after preclinical studies showed the combination administered concomitantly or in sequence was highly effective in three different human RCC xenografts (55). Despite initial promising results (56, 57), a recent, randomized, multicenter, double-blind, phase II trial showed that erlotinib does not add to the clinical efficacy of bevacizumab (36). The later results underscore the inherent bias of single-arm phase II studies and the need for appropriately controlled comparisons for adequate evaluation.

Combination of anti-VEGF strategies with agents targeting other relevant proteins are also planned. The most prominent

agent is temsirolimus (CCI-779, Wyeth Pharmaceuticals, Collegeville, PA), a mammalian target of rapamycin inhibitor, which showed a significantly increased PFS (3.7 versus 1.9 months; $P = 0.0001$) and OS (10.9 versus 7.3 months; $P = 0.0069$) compared with IFN in a phase III randomized study in 626 poor-risk advanced RCC patients (58). One potential mechanism of antitumor activity of temsirolimus in RCC is decreased transcription of hypoxia-inducible factor and therefore decreased VEGF production. Additive effects with other anti-VEGF approaches based on this or other antitumor mechanisms of temsirolimus are possible and will be tested in clinical trials. It is vital for any combination strategy to produce both statistically and clinically meaningful benefits that outweigh those of each agent as monotherapy and also that of sequential monotherapy with each agent to balance the expected increased toxicity, patient inconvenience, and cost of combination therapy.

Sequenced therapy

Questions about the efficacy and toxicity of sequenced approaches have emerged as resistance to these agents develops. Resistance to or failure of antiangiogenic agents is currently defined by the evidence of progressive disease per RECIST criteria despite therapy. Clinically, it is recognized that not all 'resistance' is identical. That is, a subset of patients fails to achieve any tumor shrinkage to therapy and progresses rapidly. Other patients experience minor degrees of tumor shrinkage followed by progression and still others experience dramatic and prolonged antitumor effects followed by a slow progression and may reach RECIST-defined progressive disease with a lower total tumor burden than pretherapy. These patient subgroups are likely clinically and biologically different and should be thought of as distinct in the approach to treatment of their therapy-refractory disease. Sunitinib has been investigated in 61 metastatic RCC patients resistant to prior bevacizumab-based therapy and showed an ORR of 23% and overall tumor shrinkage rate of 74%, supporting the clinical hypothesis that VEGF and related receptor signaling are still therapeutically relevant after VEGF ligand blockade (59). There is also anecdotal evidence of activity of sunitinib or sorafenib on failure of the other agent (60). There is as yet no insight into mechanism of resistance to these agents and whether the most appropriate maneuver on disease progression is dose/schedule modification, incorporation of an additional agent, or targeting another pathway with a different agent. Several trials are ongoing or planned in patients with metastatic RCC who are refractory either to bevacizumab, sorafenib, or sunitinib. These data will provide insight into the degree of clinical cross-resistance to these therapies and perhaps begin to identify an optimal sequence of agents.

Clinical end points

As discussed, anti-VEGF agents administered as monotherapy in RCC may contribute to inhibiting tumor growth in some patients rather than promoting frank tumor regression, and it is of note that a response-independent survival benefit has been observed with bevacizumab in other settings (20). The traditional approach of measuring RECIST-defined response rate in single-arm phase II trials, therefore, may not be the most appropriate design for evaluating these agents. For example, the low response rates did not entirely capture the clinical benefit achieved with regard to the delayed time-to-progression in patients receiving bevacizumab or sorafenib compared with placebo (30, 31). Thus, for some agents, a randomized comparison with a PFS end point may be needed to identify a signal of activity for further investigation. It is also important to remember that OS is still the gold standard on which to judge new treatments. This standard, however, is currently complicated in RCC with the simultaneous emergence of several active agents noted above and the availability of active agents on disease progression. A randomized phase II trial with a PFS end point is an appropriate initial evaluation to generate a signal for further development.

Patient selection

By preselecting patients most likely to respond to anti-VEGF therapy, such agents could be optimized in the clinical setting. Clear cell histology has been an inclusion criterion in nearly all trials, based on the biology of *VHL* inactivation and subsequent VEGF overexpression, which is confined to this histology. There have, however, been anecdotal reports of activity of anti-VEGF agents in patients with non–clear cell histology. A completed compassionate use trial of sorafenib was not restricted to patients with clear cell histology. This trial and the community use of both sorafenib and sunitinib in non–clear cell RCC due to the lack of other effective agents will provide some insight into the potential activity in this setting. Any activity of active agents in non–clear cell RCC underscores the complex biology of RCC that can lead to VEGF dependence and the fact that it is not solely *VHL* inactivation (which is exclusive to clear cell RCC) that drives VEGF expression.

Predictive biomarkers of response are also being sought. VEGF expression is an obvious candidate, and studies have found a correlation between VEGF levels and poor prognosis in many tumor types (10), but results to date in metastatic RCC have been inconclusive. Plasma VEGF levels were measured in the bevacizumab trial (31), although no significant correlation was found between pretreatment levels and clinical response or time-to-progression in either of the bevacizumab groups. Soluble VEGFR-2 levels correlated modestly with exposure to sunitinib therapy in metastatic RCC patients (61). Additionally, in these trials of sunitinib, VEGF levels were increased in patients with partial responses compared with those experiencing stable disease or rapid progression. It can be hypothesized that a greater degree of tumor regression was associated with more effective VEGFR inhibition (as reflected in lower soluble VEGFR-2 and higher VEGF protein levels), but this hypothesis requires further investigation. The prognostic significance of VEGF and VEGFR levels therefore remains to be fully elucidated. Regarding *VHL* status and response to VEGF-targeted agents, an initial report in metastatic RCC patients treated with VEGF-targeting agents did not find a correlation between VHL inactivation and response (62). However, the subset of patients with *VHL* methylation or a mutation predicted to truncate or shift the *VHL* reading frame (and thus presumably disrupt VHL protein function) had a longer median time-to-progression versus patients with no methylation, truncation, or shift in the *VHL* reading frame (13.3 versus 7.4 months, respectively; $P = 0.06$). Additional molecular profiling studies, including assessment of *VHL* activation status and RNA/protein expression studies, are ongoing to determine whether certain tumor expression profiles are predictive of response or resistance to therapy.

Conclusion

The VEGF pathway is pertinent to pathogenesis of renal carcinoma. Agents targeting VEGF have shown substantial clinical activity as monotherapy in metastatic RCC. Additional data about the efficacy versus toxicity profile of each of these agents are required for a more comprehensive understanding of their clinical usefulness. Data from phase III clinical trials are also beginning to emerge, with data supporting PFS over conventional therapy. Strategies for dosing, combination regimens, and sequencing these agents are also being considered. It is clear that the clinical development of these agents is far outpacing the ability to test them in preclinical models. As such, issues, such as optimal combination and sequence, cross-resistance, and patient selection, may indeed be initially investigated in the clinical setting. Additional investigations of the molecular effects of these agents (e.g., on immune function) and the molecular basis for response are needed to advance the substantial therapeutic benefit that this class of agent represents for RCC.

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