Abstract

Purpose. To review the biology of renal cell carcinoma (RCC) and the clinical results of vascular endothelial growth factor (VEGF) blockade in metastatic RCC.

Methods. A review of relevant published literature regarding VEGF, von Hippel-Lindau (VHL) gene inactivation, and VEGF overexpression in RCC was performed. Further, a review of the mechanism, toxicity, and clinical development of VEGF-targeted therapy in metastatic RCC was undertaken.

Results. VHL tumor suppressor gene inactivation is observed in the majority of clear cell RCC cases, leading to VEGF overexpression. Therapy with agents directed against the VEGF protein or the VEGF receptor have demonstrated initial clinical activity in metastatic RCC.

Conclusions. Therapeutic targeting of VEGF in RCC has strong biologic rationale. Substantial clinical activity has been reported in initial clinical trials with VEGF-targeting agents. Further investigation is needed to optimally use these agents for maximal clinical benefit.

Introduction

Metastatic renal cell carcinoma (RCC) is a disease in which only a limited subset of patients experiences clinically meaningful benefit from standard interleukin-2 and/or interferon (IFN)-α therapy [1]. A growing understanding of the underlying biology of RCC has identified vascular endothelial growth factor (VEGF) as a logical therapeutic target. Therapy directed against the biologic activity of VEGF is now undergoing clinical development in RCC.

VEGF

VEGF (also known as vascular permeability factor [VPF] and VEGF-A) is a dimeric glycoprotein and a member of the platelet-derived growth factor (PDGF) superfamily of...
growth factors. It has been found to have critical importance in both normal and tumor-associated angiogenesis through increased microvascular permeability to plasma proteins [2], induction of endothelial cell division and migration [3, 4], promotion of endothelial cell survival through protection from apoptosis [5], and reversal of endothelial cell senescence [6]. The VEGF gene is differentially spliced to encode four major isoforms (VEGF$_{121}$, VEGF$_{165}$, VEGF$_{189}$, and VEGF$_{206}$) [7]. VEGF$_{165}$ is the predominant isoform, with physical characteristics that result in optimal bioavailability and potency.

VEGF exerts its biologic effect through interaction with receptors present on the cell surface. These transmembrane tyrosine kinase receptors include VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), selectively expressed on vascular endothelial cells, VEGFR-3 (Flt-4), expressed on lymphatic and vascular endothelium, and the neuropilin receptor (NRP-1), expressed on vascular endothelium and neurons [8]. Upon binding of VEGF to the extracellular domain of its receptor, dimerization and autophosphorylation of the intracellular receptor tyrosine kinases occurs and a cascade of downstream proteins is activated. VEGFR-2 appears to be the main receptor responsible for mediating the proangiogenic effects of VEGF.

VEGF expression is regulated by a number of factors. Pertinent to RCC, VEGF expression results from inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene, which is observed in the majority of RCC cases (vide infra), thus identifying VEGF as a critical component of RCC tumor angiogenesis and a particularly relevant therapeutic target in RCC.

**Biology of VEGF Expression in Renal Cell Carcinoma**

**VHL Gene Expression in RCC**

Based on the observation that VHL syndrome patients often develop clear cell RCC tumors, Latif et al. analyzed VHL syndrome kindreds and mapped the VHL gene to chromosome 3p25-26 [9]. According to a two-hit model requiring inactivation of both gene alleles for tumor development [10], VHL was defined as a tumor suppressor gene in VHL syndrome patients [11]. In sporadic (noninherited) RCC, VHL gene allele deletion (loss of heterozygosity) has been demonstrated in 84%–98% of sporadic renal tumors [12–16]. Examination of RCC tumors has revealed mutation in the remaining VHL allele in 34%–57% of clear cell RCC tumors (Table 1) [12, 13–15, 17, 18]. VHL gene inactivation in RCC may also occur through gene silencing by methylation (Table 1) [14, 15, 19, 20]. Taken together, the above data suggest that biallelic VHL gene inactivation occurs in the majority of clear cell RCC tumors. Nonclear cell tumors do not demonstrate significant VHL gene inactivation.

**Biologic Consequences of VHL Gene Inactivation in RCC**

The VHL gene encodes a 213 amino acid protein (pVHL). In conditions of normoxia and normal VHL gene function, pVHL is the substrate recognition component of a ubiquitin ligase complex that targets a protein transcription factor, hypoxia-inducible factor (HIF), for proteolysis [21–23]. In conditions of hypoxia or defective pVHL function, the interaction between pVHL and HIF is dysfunctional; HIF is not subject to proteolysis and is thus constitutively activated. HIF translocates into the nucleus and leads to transcription of hypoxia-inducible genes [24, 25]. Several hypoxia-inducible genes are induced by this process, including VEGF [24, 25] and PDGF [26]. Examination of RCC tumors for VEGF (mRNA transcripts and/or VEGF protein) has demonstrated VEGF overexpression in the vast majority of samples [27–33].

Taken together, the above data provide compelling evidence for VHL inactivation in the majority of clear cell RCC tumors, leading to VEGF overexpression that drives tumor angiogenesis. Thus, inhibition of VEGF has been pursued as a therapeutic target in RCC.

**Table 1. VHL gene inactivation in renal cell carcinoma: selected series**

<table>
<thead>
<tr>
<th>Study</th>
<th>VHL gene mutation in clear cell RCC</th>
<th>VHL gene mutation in nonclear cell RCC</th>
<th>VHL gene methylation in clear cell RCC</th>
<th>VHL gene methylation in nonclear cell RCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gnarra et al. [12]</td>
<td>57% (63/110)</td>
<td>NR</td>
<td>19% (5/26)</td>
<td>0% (0/5)</td>
</tr>
<tr>
<td>Shuin et al. [13]</td>
<td>56% (22/39)</td>
<td>0% (0/8)</td>
<td>15% (7/45)</td>
<td>0% (0/16)</td>
</tr>
<tr>
<td>Gallou et al. [17]</td>
<td>56% (73/130)</td>
<td>0% (0/21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schraml et al. [18]</td>
<td>34% (38/113)</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NR = not reported; RCC = renal cell carcinoma; VHL = von Hippel-Lindau.
CLINICAL DEVELOPMENT OF VEGF-TARGETED THERAPY IN RCC

Strategies to inhibit VEGF in RCC, including binding of the VEGF protein and blockade of the VEGF receptor, have recently undergone clinical testing in metastatic RCC. Recent approaches with reported phase II results are described here.

Anti-VEGF Antibody

A recombinant human monoclonal antibody against VEGF, bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA, http://www.gene.com), binds and neutralizes all biologically active isoforms of VEGF [34]. This humanized antibody was demonstrated to inhibit bovine capillary endothelial cell proliferation in response to VEGF and to have antitumor effects in sarcoma and breast cancer cell lines [34].

The clinical utility of bevacizumab in metastatic RCC was investigated in a randomized phase II trial in which 116 patients with treatment-refractory, metastatic clear cell RCC were randomized to receive placebo, low-dose (3 mg/kg) bevacizumab, or high-dose (10 mg/kg) bevacizumab i.v. every 2 weeks [35]. All groups were well balanced with regard to established prognostic factors [36]. There were four partial responses, all in the high-dose bevacizumab arm (4/39; 10% objective response rate). An intent-to-treat analysis demonstrated a significantly longer time to progression (TTP) in the high-dose bevacizumab arm than in the placebo arm (4.8 months versus 2.5 months; p < .001 by log rank test). There were no life-threatening toxicities or deaths attributable to bevacizumab. In the high-dose bevacizumab arm, hypertension of any grade occurred in 36% of patients, and grade 3 hypertension, defined as hypertension not controlled by one standard medication, was observed in 21% of patients. Asymptomatic proteinuria without renal insufficiency was observed in 64% of patients in the high-dose bevacizumab arm. All toxicities were reversible with cessation of therapy. Grade 1 or 2 hemoptysis was observed in two patients receiving bevacizumab and two patients receiving placebo. No thromboembolic events were reported in any arm.

Given the promising data demonstrating an effect of bevacizumab on TTP in RCC, an Intergroup phase III trial investigating the addition of bevacizumab to initial systemic therapy in RCC is under way [37]. Patients with metastatic clear cell RCC without prior systemic therapy are being randomized to receive either low-dose IFN-α2b (Intron® A; Schering-Plough Corporation, Kenilworth, NJ, http://www.sch-plough.com), 9 MU three times weekly, or the same dose and schedule of IFN-α2b in combination with bevacizumab at a dose of 10 mg/kg i.v. every 2 weeks. Patients are stratified by nephrectomy status and established prognostic factors to ensure balanced randomization [36, 38, 39]. The primary end point of the trial is overall survival, and the study is designed to detect an improvement in median survival from 13 months for IFN-α alone [36] to 17 months for the combination, representing a hazard ratio of 1.3. Seven hundred patients will be enrolled over 3 years with a two-sided significance level of 0.05 and a power of 89%. A similarly designed phase III trial is under way in Europe using IFN-α2a (Roferon-A®-A; Hoffmann-La Roche, Grenzach-Wyhlen, Germany. http://www.roche.com) instead of IFN-α2b.

Bevacizumab has been further investigated in combination with an antiepidermal growth factor receptor (EGFR) strategy. Transforming growth factor (TGF)-α is a VHL-regulated growth factor for RCC, with a biologic effect through interaction with the EGFR [40–42]. Single-agent studies, however, with small molecules or antibodies directed against the EGFR have demonstrated a limited antitumor effect [43]. Nonetheless, preclinical investigation in human RCC xenograft models of bevacizumab and erlotinib (Tarceva®; OSI Pharmaceuticals, Inc., Melville, NY, http://www.osip.com), a small molecule EGFR inhibitor, has demonstrated a potential benefit of combination therapy on tumor growth inhibition [44], perhaps because EGFR resistance is mediated through VEGF [45]. A clinical trial in metastatic RCC with bevacizumab (10 mg/kg i.v. every 2 weeks) in combination with erlotinib (150 mg orally daily) reported a 25% partial response rate [46]. A recently completed randomized phase II trial of bevacizumab with or without erlotinib in untreated, metastatic RCC patients may provide further insight into potential additive or synergistic clinical effects of this combination therapy.

Small-Molecule VEGF Receptor Inhibitors

An alternative approach to VEGF inhibition involves small-molecule tyrosine kinase inhibitors. These agents inhibit not only the VEGFR, but also other receptors in the split kinase domain superfamily of receptor tyrosine kinases, including the PDGFR. PDGFR is expressed in pericytes, which serve as structural supporting cells for endothelial cells, and thus class effects of these drugs on PDGFR may have therapeutic relevance.

SU11248

SU11248 (Pfizer, Inc., La Jolla, CA, http://www.pfizer.com) is an orally bioavailable oxindole small-molecule tyrosine kinase inhibitor of VEGFR-2 and PDGFR-B. In vitro assays have demonstrated inhibition of VEGF-induced proliferation of endothelial cells and PDGF-induced proliferation of mouse fibroblast cells [47]. Investigation in mouse xenograft models demonstrated growth inhibition...
of various implanted solid tumors and eradication of larger, established tumors.

SU11248 was investigated in a single-arm, multi-institutional phase II trial in advanced RCC patients failing initial cytokine treatment (n = 63) [48]. Patients were treated with 50 mg of SU11248 orally daily on a 4-weeks-on/2-weeks-off cycle. Fifteen patients (24%) obtained partial responses per Response Evaluation Criteria In Solid Tumors (RECIST) criteria. An additional five patients (8%) demonstrated partial responses but await confirmation of response status. Of the 15 patients who achieved partial responses, one has progressed at 5 months and 14 remain progression free, with a median duration of response of 6+ months.

Toxicities in the phase II trial, most commonly grade 1 or 2, included fatigue/asthenia (78%), nausea (56%), diarrhea (51%), and stomatitis (44%). Grade 3/4 toxicities included lymphopenia (30%), elevated lipase (21%) and amylase (8%) without clinical signs of pancreatitis, elevated phosphorus (13%), and fatigue/asthenia (8%). Two patients were taken off study for asymptomatic decreases in left ventricular ejection fraction of >20% compared with baseline. A confirmatory single-arm, phase II trial in 100 cytokine-refractory, metastatic RCC patients and a randomized phase III trial versus IFN-α monotherapy in untreated metastatic RCC patients are ongoing.

**PTK787/ZK222584**

PTK787/ZK222584 (PTK787; Novartis Pharmaceuticals Corporation, Hanover, NJ, http://www.pharma.us.novartis.com) is an oral, selective inhibitor of VEGFR-1, VEGFR-2, and PDGFR-B tyrosine kinases [49]. Preclinical data demonstrated that PTK787 inhibits VEGF-induced endothelial cell proliferation, migration, and survival [49]. PTK787 demonstrated inhibition of both VEGF- and PDGF-induced vascularization in a growth factor implant model and reduced vascularization induced by tumors (A431 epithelial) implanted s.c. into nude mice. In an immune-competent murine renal carcinoma model (RENC), PTK787 inhibited the growth of primary tumors, and PTK787 reduced the number of metastases in treated animals compared with vehicle control animals with a concomitant decrease in tumor blood vessel density [49, 50].

A phase I/II trial of PTK787 in 45 patients with metastatic RCC has been reported [51]. Therapy was well tolerated, and the maximum-tolerated dose was not reached at 1,500 mg/day. Clinical activity, assessed using 41 evaluable RCC patients, included partial responses in two patients (5%) and minor responses (25%–50% tumor shrinkage) in six patients (15%), as defined by the sum of the bidimensional measurement of tumors [52].

**BAY 43-9006**

BAY 43-9006 (Bayer Pharmaceuticals, West Haven, CT, http://www.bayerpharma-na.com and Onyx Pharmaceuticals, Richmond, CA, http://onyx.pharma.com) is an orally bioavailable bi-aryl urea Raf kinase inhibitor, with demonstrated inhibition in Ras-dependent human tumor xenograft models [53]. Activated Ras promotes cell proliferation through the Raf/MEK/ERK pathway by binding to and activating Raf kinase. BAY 43-9006 has also demonstrated direct inhibition of VEGFR-2, VEGFR-3, and PDGFR-B [54]. Xenograft models treated with daily BAY 43-9006 demonstrated significant inhibition of tumor angiogenesis, as measured by anti-CD31 immunostaining.

A phase II randomized discontinuation study with BAY 43-9006 has been reported in refractory solid tumors, including 112 patients with metastatic RCC [55]. All patients received oral BAY 43-9006, 400 mg twice a day, and patients with stable disease after 12 weeks of treatment were randomized to either continue the drug or receive placebo. Patients with a ≥25% tumor shrinkage, by the sum of the bidimensional measurement, at 12 weeks (defined as responders) continued open-label BAY 43-9006. Of the 65 RCC patients who had reached the initial 12-week assessment, 25 (38%) achieved a response and 18 (28%) achieved stable disease (defined as tumor burden within 25% of baseline). A randomized, placebo-controlled phase III trial in cytokine-refractory RCC patients is ongoing.

Table 2 summarizes the clinical data on anti-VEGF agents in RCC. Comparison of anti-VEGF agents is not currently possible due to separate studies employing different patient selection, methodology, and outcome criteria. Nonetheless, significant antitumor activity has been observed (both objective responses and tumor regression not meeting criteria for response recorded as stable disease), placing VEGF blockade strategies at the forefront of RCC clinical investigation.

**FUTURE DIRECTIONS**

The exciting preliminary clinical response data with VEGF inhibition in RCC has provided an opportunity for treatment advances in this historically resistant malignancy. As the clinical activity of existing agents is further defined in ongoing trials, several questions on optimizing their utility remain. Modest but real antitumor effects of cytokines, most evident in the durable complete response subset, require that anti-VEGF agents be investigated in combination and comparative studies. Further enrichment of patients susceptible to VEGF blockade, beyond restriction to clear cell histology, is needed. The mechanism of resistance to anti-VEGF agents in RCC and the utility of alternative anti-VEGF
approaches in this setting also require clinical testing. Investigation into blood- and tissue-based correlates of response and resistance to these agents should be undertaken as clinical development proceeds. Combination anti-VEGF therapy with agents targeting other aspects of the VEGF pathway or with therapy directed against other targets of VHL inactivation (PDGF, EGFR, TGF-α) deserves investigation.

**CONCLUSION**

VHL inactivation is a frequent event in clear cell RCC leading to overexpression of VEGF, driving the malignant biology of RCC. Therapeutic inhibition of VEGF via antibody or receptor blockade results in antitumor activity. Further, definitive studies are ongoing and will determine the optimal timing, sequence, and clinical utility of these agents in RCC.

**Table 2. Summary of clinical results with VEGF targeting agents in metastatic RCC**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Trial design</th>
<th>Clinical activity</th>
<th>Common toxicity</th>
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<tbody>
<tr>
<td><strong>VEGF-binding antibody</strong></td>
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<tr>
<td>Bevacizumab</td>
<td>Randomized, placebo-controlled trial; 100% pretreated patients</td>
<td>10% response rate (WHO criteria(^a)); delay in TTP versus placebo (2.5 months versus 4.9 months)</td>
<td>hypertension, proteinuria</td>
</tr>
<tr>
<td>Bevacizumab + erlotinib</td>
<td>Single-arm phase II; 32% pretreated</td>
<td>21% response rate (RECIST criteria(^b))</td>
<td>Not reported</td>
</tr>
<tr>
<td><strong>VEGF receptor inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SU011248</td>
<td>Single-arm phase II; 100% pretreated</td>
<td>33% response rate (RECIST criteria)</td>
<td>fatigue/asthenia, nausea, diarrhea, stomatitis</td>
</tr>
<tr>
<td>PTK787/ZK</td>
<td>Phase I/II; 53% pretreated</td>
<td>5% response rate (WHO criteria)</td>
<td>nausea, fatigue, vomiting</td>
</tr>
<tr>
<td>BAY 43-9006</td>
<td>Randomized discontinuation design; 86% pretreated</td>
<td>15% response rate (WHO criteria)</td>
<td>hand-foot syndrome, rash, fatigue, diarrhea, hypertension</td>
</tr>
</tbody>
</table>

Abbreviations: RECIST = Response Evaluation Criteria In Solid Tumors; TTP = time to progression; VEGF = vascular endothelial growth factor; WHO = World Health Organization.

\(^a\)The WHO defines objective response as a 50% or greater reduction in the sum of the bidimensional measurement of tumors.

\(^b\)RECIST defines objective response as a 30% or greater reduction in the sum of the unidimensional measurement of tumors.

**REFERENCES**


This article was retracted on October 20, 2011


Retraction

It has been brought to the Editors’ attention that the article by Brian Rini, M.D., entitled “VEGF-Targeted Therapy in Metastatic Renal Cell Carcinoma” (The Oncologist 2005;10:191–197) contained both previously (Rini and Small, 2005) and subsequently (Rini, Sosman, and Motzer, 2005; Rathmell, Wright, and Rini, 2005; Choueiri, Bukowski, and Rini, 2006) published content from the following publications without attribution:


Accordingly, we are formally retracting this 2005 The Oncologist publication.

See the Editorial by The Oncologist’s Editor-in-Chief, Dr. Bruce A. Chabner, on pages 1347–1348 of this issue for a full background and explanation of the retraction.