Combination Targeted Therapy in Advanced Renal Cell Carcinoma*

Jeffrey Sosman, MD and Igor Puzanov, MD

Several novel therapies have been approved recently in advanced renal cell carcinoma (RCC). These agents inhibit pathways downstream of loss of the von Hippel-Lindau gene VHL. They target the vascular endothelial growth factor (VEGF) ligand, VEGF receptor (VEGFR), mammalian target of rapamycin (mTOR), and other potentially important pathways. Even with improvements in survival, disease progresses in all patients. There is a critical need to increase complete responses (now rare). One such strategy is combining several agents to block different levels of the VEGF-VEGFR axis (vertical blockade). Alternatively, combination of a VEGF-VEGFR inhibitor with an mTOR inhibitor is attractive. Finally, horizontal blockade of VEGFR with epidermal growth factor receptor and/or platelet-derived growth factor receptor, all signaling pathways activated by hypoxia-inducible factor, is another approach. Already trials have revealed difficulties with combination therapy. By combining agents, the toxicity of 1 or both can be enhanced. The authors of this article report their experience with sorafenib plus bevacizumab, which produced increases in hand-foot syndrome, hypertension, and proteinuria, all known toxic effects. Clinical activity was impressive with 25 responses in 48 patients (52% response rate). Other combinations also required dose reductions (sorafenib with temsirolimus) or were intolerable (sunitinib with temsirolimus or sunitinib with bevacizumab). Unexpected toxicity characterized by microangiopathic hemolytic anemia occurred late in treatment with sunitinib and bevacizumab. Toxicity may be more severe in patients with RCC, who frequently have 1 kidney and poor renal function. Once tolerability for combination regimens has been established, it will be critical to design informative phase 2 trials and address the benefit of combination versus sequential therapy. Cancer 2009;115(10 suppl):000–000. © 2009 American Cancer Society.

KEY WORDS: renal cell carcinoma, antiangiogenic, combination therapy, targeted therapy, toxicity.

Previously, clear cell renal cell carcinoma (CCRCC) had been considered a malignancy that was refractory to most therapies with the occasional exception of immunotherapy.1-3 However, during the past decade with the development of effective antiangiogenic agents, treatment has made significant gains for patients with CCRCC. Understanding the essential role of the von Hippel-Lindau gene VHL and its regulation of cellular levels of hypoxia-inducible factor 1 alpha (HIF-1α) and HIF-2α in CCRCC has provided a rationale to evaluate drugs that inhibited the downstream mediators of HIF.4-6 These genes include vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor
(EGF), and EGF receptor (EGFR) as well as a number of other genes, including the erythropoietin gene \( EPO \), the carbonic anhydrase IX gene \( CAIX \), the glucose transporter 1 gene \( Glut-1 \), and the chemokine (C-X-C motif) receptor 4 gene \( CXCR4 \), to name only a few. Currently, 5 drugs, all of which interfere with HIF and/or downstream gene products, have demonstrated effectiveness in phase 3 trials. The antiangiogenic agents include multitargeted kinase inhibitors, sunitinib (inhibiting VEGF receptor [VEGFR], PDGF receptor [PDGFR], c-kit, fms-related tyrosine kinase 3 [Flt3]), sorafenib (inhibiting VEGFR, PDGFR, c-kit, Flt3, c-RAF), bevacizumab (an antibody to VEGF ligand), and temsirolimus or everolimus (both mammalian target of rapamycin [mTOR] inhibitors). \(^7\) \(^\text{-}\) \(^\text{11}\) Several general observations are readily apparent:

1. Sorafenib extended progression-free survival (PFS) by 100% from 2.8 months to 5.6 months in patients who failed on cytokine therapy compared with placebo, \(^8\) but there is no evidence that, in untreated patients, PFS can be extended compared with interferon. \(^12\)

2. Sorafenib has a low objective response rate (Response Evaluation Criteria in Solid Tumors [RECIST], 30%), generally <10%, but many patients have stable disease with some tumor shrinkage or necrosis (but less than the 30% shrinkage required by RECIST criteria). \(^9\) \(^\text{-}\) \(^\text{13}\)

3. Sunitinib can increase PFS in untreated patients compared with interferon from \( \geq 4 \) months to \( \geq 11 \) months. \(^7\)

4. Sunitinib has an objective response rate of 35% to 40% but produces few if any complete responses (CRs). \(^7\) \(^\text{14}\), \(^\text{15}\)

5. Bevacizumab plus interferon extends PFS versus interferon from 4 months to >10 months with a 31% response rate. \(^10\)

6. Temsirolimus alone can extend overall survival by 50% in patients with poor prognostic RCC (Memorial Sloan-Kettering Cancer Center [MSKCC] scale), even with an objective response rate <10%. \(^9\) \(^\text{16}\)

7. Everolimus can increase PFS significantly compared with placebo in patients who are refractory to tyrosine kinase inhibitors (sorafenib and/or sunitinib). \(^11\)

8. Few CRs are induced by any of the treatments, and all patients will develop progression usually within 8 to 16 months

9. Overall survival improvements are very likely but difficult to establish because of the large crossover populations. \(^17\), \(^\text{18}\)

10. Some patients have very extensive responses to therapy. In these patients, if it is feasible, the resection of residual metastatic disease may be a reasonable option. This approach may lead to long-term freedom from disease. In addition, in patients who have large primary tumors that remain in place, undergoing a debulking nephrectomy after systemic therapy is an option. This surgery may become easier technically after therapy. This option is being evaluated at an increasing number of centers.

On the basis of the current knowledge, by combining these agents, we hope to improve the benefit to patients with renal cancer. Options for combination therapy include the inhibition of several steps in the sequence along the same pathway (HIF-VEGFR-VEGFR), which has been called vertical blockade. Combination therapy also can target 2 separate pathways with different functions (PDGFR, VEGFR, EGFR) in parallel, which has been called horizontal blockade. Another rational approach is to combine 2 agents, 1 of which can modulate the expression of a target of the second agent, thereby enhancing the efficacy of the second agent. Finally, to make these drugs more effective in combination, each drug individually must target a nonredundant pathway that is critical to cell survival.

What is the benefit we are hoping to achieve? Certainly, current therapy has limitations, and resistance ultimately will occur in all patients after a few months or a few years. An improved outcome may be 1 that increases the number of CRs critical for therapy to be curative. Conversely, therapy could be improved simply by making treatment effective in more patients for longer periods. Long remissions with a median of 2 to 3 years would be a desired outcome. Combinations may require a decrease in the dose of individual drugs to diminish the financial cost.

There obviously are some potential problems. First, sequential therapy may allow for several remissions that, together, last as long or longer than the remissions achieved with combination therapy. There is a growing body of literature on second- and third-line therapy that can be effective after recurrence (Table 1). \(^11\) \(^\text{19}\), \(^\text{21}\) Second, combinations of drugs may increase the toxicity even.
without overlapping single-agent toxicity. This may occur because of changes in metabolism or drug distribution, although this has not been established. However, the spectrum of the single-agent toxic effects to skin, blood pressure, proteinuria, bone marrow suppression, and mucositis involving the entire gastrointestinal tract must be taken into consideration when combining drugs (Table 1).

**Table 1. Prospective Trials of Sequentially Targeted Agents**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Population</th>
<th>No. of Patients</th>
<th>ORR/TS, %</th>
<th>PFS, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunitinib (2008)</td>
<td>Phase 2: Bevacizumab refractory</td>
<td>62</td>
<td>23/75</td>
<td>7.1</td>
</tr>
<tr>
<td>Axitinib (2008)</td>
<td>Phase 2: Sorafenib refractory</td>
<td>62</td>
<td>23/55</td>
<td>7.4</td>
</tr>
<tr>
<td>Sorafenib (Shepard 2008)</td>
<td>Phase 2: Bevacizumab or sunitinib refractory</td>
<td>52</td>
<td>3/38</td>
<td>3.8</td>
</tr>
<tr>
<td>RAD001 (Motzer 2008)</td>
<td>Phase 3: TKI refractory (vs placebo)</td>
<td>410</td>
<td>1/50</td>
<td>4.6 vs 1.8</td>
</tr>
</tbody>
</table>

ORR indicates overall response rate; TS, tumor shrinkage; PFS, progression-free survival; TKI, tyrosine kinase inhibitor; RAD001, everolimus.

**Table 2. Combination Drug Therapy: Selected Adverse Events With Agents**

<table>
<thead>
<tr>
<th>Rash or Hand-Foot Reaction</th>
<th>Hypertension</th>
<th>Cytopenia</th>
<th>Proteinuria</th>
<th>Gastrointestinal-Mucosal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunitinib</td>
<td>Yes</td>
<td>Yes</td>
<td>—</td>
<td>Yes</td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Yes</td>
<td>—</td>
<td>—</td>
<td>Yes</td>
</tr>
<tr>
<td>Bevacizumab</td>
<td>—</td>
<td>Yes</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Temsirolimus</td>
<td>Yes</td>
<td>—</td>
<td>Yes</td>
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</table>

ATTEMPTS AT HORIZONTAL BLOCKADE

Initial efforts at horizontal blockade were pursued by Hainsworth and his colleagues (Fig. 1). Attempts were made to block the VEGF pathway with bevacizumab and to block the EGFR pathway with erlotinib, which is an EGFR tyrosine kinase inhibitor. Initial phase 2 findings in a 2-center trial were promising with what appeared to be a better response than what was reported with bevacizumab alone. Of 58 evaluable patients, 12 patients (21%) had objective partial remissions, 38 patients (66%) had stable disease, and 12 additional patients had significant regression but did not meet RECIST criteria. Only 8 patients (13%) initially developed disease progression after 2 4-week cycles. Attempts to add a PDGFR tyrosine kinase inhibitor (imatinib) to the combination was not tolerable. Bukowski and colleagues attempted to confirm those results in a randomized placebo-blinded study in which all patients received bevacizumab and were randomized to receive either placebo or erlotinib. The response rate was between 10% and 15%. The addition of erlotinib added toxicity but did not obviously improve the response rate or PFS. Theoretically, horizontal blockade still is an attractive approach, when combined with the inhibition of other pathways (PDGFR and VEGFR) by sunitinib and sorafenib. In vitro at the cellular level, RCC is highly responsive to EGFR inhibition. CCRCC has high expression of EGFR, but this has not translated into a therapeutic benefit. Could K-ras mutations in CCRCC explain resistance to EGFR inhibition, as it does in part for lung cancer and colon cancer? This may be a topic for further studies.

Attempts at Vertical Blockade

We and others have pursued different combination regimens, including drugs that block HIF translation (the mTOR inhibitors temsirolimus and everolimus), inhibit VEGF (bevacizumab), or inhibit VEGFR (sunitinib or sorafenib). Other regimens inhibit the VEGF ligand (bevacizumab) plus the VEGFR kinase (sunitinib or sorafenib). Elevation in VEGF and HIF levels are likely to occur in response to the hypoxic environment. Escape from sunitinib and sorafenib blockade of the VEGFR and mTOR inhibition of HIF translation may occur if levels of VEGF exceed a threshold that can overcome the blockade. Levels of VEGF are known to increase in blood after...
therapy with both sunitinib and sorafenib, but their role in resistance is not established. Inhibition of these pathways at another level, such as VEGF ligand or HIF with mTOR inhibitors, in some cases could either prevent or circumvent the developing resistance. The phase 1 trials are extremely small, and some have been restricted to patients with RCC alone or others have been open to all patients with solid tumors (Table 3).\textsuperscript{27-33} Toxicity has been variable. Although in, some combinations, both agents seem to be tolerable when used in full doses (temsirolimus or everolimus with bevacizumab),\textsuperscript{28,29} other not safe or feasible as a singular agent combinations have required dose reductions (sorafenib with bevacizumab, temsirolimus with sorafenib) or are not safe to be given together (temsirolimus with sunitinib, bevacizumab with sunitinib).\textsuperscript{32} There appeared to be a difference in toxicity when the combinations were administered to patients with RCC alone or to a diversity of cancer patients in some phase 1 studies. This was most apparent with the sunitinib and bevacizumab combination.\textsuperscript{30,31} The phenomena that causes the enhanced toxic effects in patients with RCC may be related to their limited renal reserve or even may be an aspect of RCC biology. The increased toxicity observed with some regimens has been studied only in a limited fashion, with a few studies demonstrating no drug-drug interactions or changes in pharmacokinetics. Sunitinib in combination with either bevacizumab or temsirolimus has been difficult to administer safely to
patients with RCC. Limited experience in the first few patients revealed dose-limiting toxic effects even at low doses of sunitinib and temsirolimus. Conversely, combining sunitinib with bevacizumab in patients with RCC appeared to be safe at full doses until long-term follow-up revealed that numerous patients had developed microangiopathic hemolytic anemia with hemolysis, schistocytes in blood, elevated lactate dehydrogenase, low platelet counts, renal insufficiency, and neurologic toxic effects with seizures and confusion. This toxicity has not been observed in a phase 1 trial that enrolled predominantly non-RCC patients at the Cleveland Clinic.

Our own experience in patients with RCC who received sorafenib plus bevacizumab has been remarkable in terms of the enhanced toxic effects observed from combining the drugs. This has been characterized by the sorafenib-related toxic effects of severe hand-foot syndrome and severe functional stomatitis and the bevacizumab-related toxic effects of hypertension, which was difficult to control even with multiple antihypertensive medications, and persistent proteinuria (protein level >2 g). The combination required lowering the bevacizumab dose in nearly all patients to 5 mg/kg intravenously every 2 weeks and lowering the sorafenib dose to 200 mg orally once daily. Patients who started treatment at higher doses had to interrupt their therapy rapidly and then reduce their dose within the initial 2 to 4 weeks.

In terms of the clinical activity of these combinations, the data are limited up to this point. We have observed a 52% response rate among all 48 patients who were enrolled on the sorafenib plus bevacizumab phase 1 trial. The overall PFS was 14 months, and 11 patients still continue to receive therapy without disease progression at 13 to 33 months. In 2008, Whorf et al presented a phase 2 trial of everolimus and bevacizumab in 59 patients with RCC. Twenty-nine of those patients had received prior therapy with sunitinib, sorafenib, or both, and 30 patients were untreated for metastatic disease. Untreated patients had a response rate of 23% (7 of 30 patients), whereas patients who had received prior multitargeted kinase inhibitor therapy had a response rate of 17% (5 of 29 patients), and approximately 60% of patients initially were stable in both groups. The PFS was 12 months and 11 months for untreated and previously treated patients, respectively. Feldman and colleagues at MSKCC observed an excellent response rate of >50% in patients who received sunitinib and bevacizumab, although long-term toxic effects made this combination untenable because of disabling adverse effects (microangiopathic hemolytic anemia).

| Table 3. Combination Drug Therapy: Phase 1 Trials With Antiangiogenic and Mammalian Target of Rapamycin Inhibitors |
|--------------|----------------|-----------------|-----------------|----------------|
| **Trial**    | **Enrollment** | **Regimen (Full Dose)** | **MTD/RPTD** | **No.** | **ORR** |
| Sosman 200827 | RCC only     | Bevacizumab (10 mg/kg) and sorafenib (400 mg) twice daily | 5/200 q d | 48 | 52% |
| Merchan 200729 | RCC only       | Bevacizumab (10 mg/kg) and temsirolimus (25 mg) | 10 q d/25 q w | 12 | 67% |
| Whorf 200828  | RCC only; both untreated and previously treated (MTKI) | Bevacizumab (10 mg/kg) and everolimus (10 mg) | 10/10 q d | 59 | Untreated, 23%; previously treated, 17% |
| Feldman 200830 | RCC only      | Bevacizumab (10 mg/kg) and sunitinib (50 mg) every 2 w and daily 4/6 weekly | Intolerable | 23 | 56% |
| Cooney 200831 | All solid tumors | Bevacizumab (10 mg/kg) and sunitinib (50 mg) every d | 10/50 every 2 w and daily 4/6 weekly | 37 | 29% (9 Patients; 3/7 RCC); 2 PRs (TCC), 2 PRs (melanoma, adrenal, thyroid) |
| Fischer 200832 | RCC only       | Temsirolimus (25 mg) and sunitinib (50 mg) | Intolerable at 15/25 daily 4/6 | 3 | IE |
| Patnaik 200733 | All solid tumors and lymphomas | Temsirolimus (25 mg) and sorafenib (400 mg) weekly b.i.d. | 25/200 weekly b.i.d. | 33 | 1 PR (NHL), 1 PR (thyroid) |

MTD indicates maximum tolerated dose; RPTD, recommended phase 2 dose; ORR, overall response rate; RCC, renal cell carcinoma; MTKI, multitargeted kinase inhibitor; PRs, partial responses; TCC, transitional cell carcinoma; IE, inevaluable; NHL, non-Hodgkin lymphoma.
Clinic performed a short phase 1 trial with temsirolimus and bevacizumab that was able to quickly dose escalate to full doses of each agent.29 Also impressive were the 8 responses among only 11 evaluable patients.

**Other New Agents in RCC**

Currently, there is a plethora of other targeted agents that have not yet been approved by the Food and Drug Administration but that quickly are undergoing clinical evaluation initially as single agents. VEGF or VEGFR remain good targets for axitinib, pazopanib, and AZD2171, all of which inhibit VEGFR; whereas VEGF ligand inhibition includes the VEGF Trap and IM1121B.34-36 There also are Tie-2 inhibitors (AMG 386) and inhibitors to the PI3/Akt kinase pathway (Perifosine) that provide different targets to inhibit alone or in combination.37 Better preclinical models are needed to develop combinations of drugs more rationally. Animal models of renal cancer use a very limited number of long-term human RCC cell lines. These xenografts require an immunosuppressed host, and both the stroma and tumor vascularity are murine in origin. A tumor model driven by the VHL transgene may provide much greater insight into human CCRCC. These models would help define biomarkers that can be used for defining clinical benefit before imaging confirmation. Everolimus, an oral mTOR inhibitor, probably will be approved rapidly based on its strong results in patients who failed on multiple prior regimens, including multtargeted kinase inhibitors. This provides an opportunity to expand the clinical evaluation of this agent in combination therapy.11

**Planned Randomized Clinical Trials**

Two large trials recently have started enrollment or soon T4 will be underway. The 2 trials are outlined in Table 4. The BeST (ECOG 2804) trial is a randomized phase 2 trial examining doublets of sorafenib, bevacizumab, and temsirolimus plus an arm with bevacizumab alone. This 4-arm trial will enroll a minimum of 90 patients per arm and is designed to determine whether any arm appears promising compared with bevacizumab. It is estimated that the PFS for bevacizumab is 9 months, and a doublet arm would be promising if the PFS was 15 months. The other trial is a large randomized phase 3 trial of the AVO-REN phase 3 arm of bevacizumab plus interferon versus bevacizumab plus temsirolimus based on Merchan’s small phase 1 experience.10,29 This trial will enroll >400 patients per arm worldwide and hopes to achieve an extension of PFS from 10.2 months with bevacizumab plus interferon to 13.5 months with bevacizumab plus temsirolimus. Additional phase 2 experience with bevacizumab plus temsirolimus will be available before the initiation of this trial.

**Combination Targeted Drug Therapy: Lessons and Recommendations**

Many agents are available for combination therapy of CCRCC, but many have redundant mechanisms of action. Adverse event profiles can compromise drug dose delivery, exacerbate toxic effects, and even reduce efficacy compared with a drug’s single-agent clinical activity. Still, we have limited information on toxicity and how it may differ, depending on the cancer undergoing therapy and the drug used. We need to obtain more experience with long-term administration of some combination treatments. We still need to prioritize studies that have as their objective a better understanding of the mechanisms of resistance and how to prevent or circumvent resistance. It will be important to develop new targets for therapy that are critical for cancer cell survival and not part of a redundant pathway. Finally, we need to identify the biologic

**Table 4. Ongoing and Planned Randomized Combination Trials in Renal Cell Carcinoma**

<table>
<thead>
<tr>
<th>Trial</th>
<th>Phase randomized</th>
<th>Enrollment</th>
<th>Design</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOG E2804 (BeST), 360 patients, only prior cytokine therapy</td>
<td>2</td>
<td>360 (90/Arm)</td>
<td>1) Bevacizumab vs 2) bevacizumab+temsirolimus vs 3) bevacizumab+sorafenib vs 4) sorafenib+temsirolimus</td>
</tr>
<tr>
<td>Wyeth (temsirolimus)</td>
<td>3</td>
<td>822 (411/Arm)</td>
<td>Bevacizumab+interferon vs bevacizumab+temsirolimus</td>
</tr>
</tbody>
</table>

ECOG indicates Eastern Cooperative Oncology Group; BeST, phase 2 trial examining doublets of bevacizumab, sorafenib, and temsirolimus plus an arm with bevacizumab alone.
characteristics of tumors that respond to specific agents and identify biomarkers to define responses early in treatment. There still is significant clinical work needed to define the role for combination therapy, but we have developed the basis to move forward.

Conflict of Interest Disclosures

The program was made possible by educational grants provided by Genentech, Novartis Pharmaceuticals, Pfizer, Inc., and Wyeth Pharmaceuticals. Program management and CME sponsorship were provided by InforMEDical Communications, Inc., Carlisle, Massachusetts.

Jeffrey A. Sosman has served as a consultant for Genentech and Wyeth and has received honoraria and research funding from Bayer/Onyx, Genetech, and Wyeth.

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Combination Targeted Therapy in Advanced Renal Cell Carcinoma

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There are several newly approved antiangiogenic agents that are effective in patients with advanced renal cell cancer that clearly have improved the outcome for these patients. Because these agents are not curative, there is a significant opportunity to improve on them; combination therapy is 1 approach being pursued with early success but in the face of unexpected toxic effects at this time.