Abstract

The treatment of advanced renal cell carcinoma (RCC) has undergone a major change with the development of potent angiogenesis inhibitors and targeted agents. Several multitargeted tyrosine kinase inhibitors, sorafenib and sunitinib, have already been approved for the treatment of advanced RCC. Temsirolimus (CCI-779), a mammalian target of rapamycin inhibitor, has shown a survival advantage over IFN in advanced, poor-prognosis RCC patients. Bevacizumab, an antibody targeting vascular endothelial growth factor (VEGF) A, has also shown promising clinical activity. Benefits attributable to these agents have been recognized by high objective response rates (sunitinib), significant increases in progression-free survival (sunitinib, sorafenib and bevacizumab), or improved overall survival (temsirolimus). These agents mediate much of their effect through inhibition of the hypoxia-inducible factor (HIF)-VEGF-VEGF receptor axis. Their inhibitory activity for the signaling of platelet-derived growth factor (PDGF) receptor β or kinases like c-Raf may contribute to the antitumor effects of the multitargeted kinase inhibitors. Nevertheless, all four single agents rarely, if ever, induce complete responses and, at present, all patients develop resistance and, ultimately, progress during therapy. A critical need exists to develop strategies that may increase the degree of the antitumor effects with the hope of inducing more complete responses impeding the onset of or elimination of refractory disease. Combinations of these and other targeted agents may overcome the resistance that develops with single-agent therapy and could be incorporated either as part of initial therapy or later when disease resistance develops. Approaches aimed at combining these agents can be based on the genetics and biology of clear cell RCC. von Hippel-Lindau loss leads to an increase in cellular levels of HIF (HIF-1α or HIF-2α) leading to increased expression of a number of hypoxia-regulated genes critical to cancer progression. Combinations of targeted agents may block several of these mediators (VEGF, epideral growth factor receptor, and PDGF), so-called horizontal blockade. Blockade could also take place at two levels of the pathways (vertical blockade), either at HIF and VEGF or at VEGF and VEGF receptor signaling. Many of the above strategies are ongoing and will require careful phase 1 determination of toxicity and even more rigorous phase 2 analysis before moving onto phase 3 trials.

Both basic science and clinical advances in renal cell carcinoma (RCC) made during the past 20 to 30 years have been recently exploited to improve therapy of this disease (1). The clinical knowledge that RCC is a highly vascular cancer and the scientific insight into the role of the von Hippel Lindau (VHL) protein in sporadic RCC have made antiangiogenic strategies an attractive approach (2). New agents have largely targeted the endothelium and their supporting stromal elements including pericytes (3, 4). The approach of targeting the renal cancer cell itself has thus far been largely unsuccessful. The agents that target renal cancers and their critical stromal elements fall into several categories (Fig. 1).

Antibodies such as bevacizumab [anti–vascular endothelial growth factor (VEGF)] or immunoglobulin G Fc-cytokine receptor molecules (VEGF-Trap) both bind and neutralize VEGF (5, 6). There are agents that act as tyrosine kinase inhibitors for VEGF receptor 2 (VEGFR2), VEGFR1, or VEGFR3 signaling within endothelial cells. More often, however, the tyrosine kinase inhibitors are capable of binding and inhibiting several targets without the specificity initially aimed for in their development. Although these “dirty” receptor tyrosine kinase (RTK) inhibitors may have not been as scientifically clean, they may be more effective in the clinic. These agents include sunitinib, which blocks not only VEGFR2 and platelet-derived growth factor (PDGF) receptor β, but also c-Kit and Flt3 receptor. Additionally, sorafenib was originally thought to bind only Raf kinases; however, with time and the observation of clinical activity in RCC, it was shown to be an effective inhibitor of VEGFR2, PDGFβ, c-Kit, and Flt3, similar to sunitinib (7). Other RTK inhibitors target epidermal growth factor (EGF) receptor (EGFR, ERBB1; erlotinib and gefitinib) alone or combinations of receptors ERBB1 and ERBB2 (lapatinib) or ERBB1 and VEGFR2 (AZD6474; ref. 8). Finally, antibodies have...
been developed that block ligand binding to EGFR1 such as cetuximab and ABX-EGF (9). Certainly, individual antibodies or RTK inhibitors that inhibit the VEGFR signaling pathway and ligand binding are effective in treating RCC. The role of inhibiting PDGF receptor-β and ligand binding are effective in treating RCC. The role of inhibiting PDGF receptor-β signaling in pericytes by both sorafenib and sunitinib or inhibiting Raf in both endothelial and primary RCCs by sorafenib in the clinical antitumor effects has not been definitely established in man. It is more likely that the c-Raf blockade by sorafenib is not a significant factor to its antitumor effects in RCC, but could be important for other cancers. The finding of significant antitumor activity for sunitinib in patients who had progressed on bevacizumab suggests a significant role for PDGFβ blockade by sunitinib. Sunitinib and sorafenib are both approved for treatment of advanced RCC. Additionally, bevacizumab is an active agent based on a phase 2 trial done by Yang et al. (10). Recently, a mammalian target of rapamycin (mTOR) inhibitor, temsirolimus, has been shown to prolong survival in poor-prognosis RCC patients presumably through the inhibition of translation of hypoxia inducible factor (HIF) proteins. A pitfall of these agents is the rare durable complete or near complete response, although as many as 75% of patients treated with these drugs have tumors that remained stable or regressed for periods of 3 to 24 months on average (11). It is hoped that combinations of these agents or combinations of these agents with cytokines may advance treatment further.

Clinical Trial Design

There are numerous questions to be addressed for the successful combination of targeted agents that improve clinical outcome (Table 1). What targets are most important? Do we have agents that can modulate the target? What is the actual goal of therapy with combinations of agents? Is it to enhance the rate and duration of clinical response or to prevent development of clinical resistance? Should we explore how effective the treatment of patients in whom upfront RTK inhibitors have failed to overcome the resistance that has developed? Finally, we need to develop these combinations of drugs, taking advantage of creative trial designs, well-designed administration schedules, and initial doses for escalation.

How does one develop a preclinical basis or rationale to proceed into the clinic with combination therapy? Animal models have great limitations but can still provide in vivo data with human or mouse tumors and allow one to dissect correlative biological effects at tumor sites. One could simply combine these agents based on the pathways activated in RCC and how these pathways interact and complement each other. Lastly, as we observe more resistance to these targeted therapies after prolonged periods of administration, we can begin to understand what are the actual mechanisms of resistance.

Rationale Combinations of Targeted Therapy

Two different concepts of combination targeted therapy for RCC have been put forth by Kaelin and others (Figs. 2 and 3). "Horizontal blockade" is where numerous target molecules downstream from HIF-α are individually or jointly inhibited. The targets thus far generally reside in different cell types such as the tumor cell (EGFR), the endothelial cell (VEGFR2), and the pericyte (PDGFβ). The agents could include specific inhibitors of these pathways such as bevacizumab (VEGF), erlotinib (EGFR), and imatinib (PDGFβ; refs. 2, 12). On the other hand, they could also include multitargeted agents such as sunitinib and sorafenib. Horizontal blockade is intended to prevent cancer cell proliferation and promote apoptosis while ablating tumor-induced angiogenesis. The other popular concept has been labeled "vertical blockade" (Fig. 3). In vertical blockade, the same pathway is targeted at two or more different levels by two or more different agents such as VEGF and its RTK. Approaches to suppress HIF-α by blocking its translation (mTOR inhibitors) or by accelerating its breakdown with heat shock protein antagonists (ansamycins; ref. 13) in combination with inhibition of downstream HIF-α–induced gene products and their functions (bevacizumab, sunitinib, or sorafenib) are another example. Vertical blockade could overcome an aspect of resistance that may develop

Table 1. Targets for RCC therapy

<table>
<thead>
<tr>
<th>What are the targets we want to direct therapy toward?</th>
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<tr>
<td>VHL- or HIF-mediated pathways</td>
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<td>VEGF ligand or receptor signaling</td>
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<td>EGFR</td>
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<td>mTOR</td>
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<td>Non-VHL-mediated pathways</td>
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<td>Raf kinase</td>
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<td>Phosphatidylinositol 3-kinase</td>
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<td>Nuclear factor nB</td>
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Fig. 1. A, relevant endothelial cell pathways. B, relevant RCC pathways.
through feedback mechanisms. Examples of this concept would include an increase in VEGF levels in response to blocking VEGFR2 or an elevation in HIF-α levels in response to inhibition of VEGFR or VEGF (14). These compensatory feedback loops likely represent one mechanism of RCC resistance to antiangiogenesis but by no means the only mechanism.

**Phase 2 Trial of Bevacizumab and Erlotinib**

Levels of transforming growth factor-α, a ligand for EGFR, are usually elevated in RCC and has been shown to be a growth factor for RCC cells independent of stroma (15, 16). Therefore, EGFR is an additional target for therapeutic intervention. In laboratory models, inhibition of EGFR results in suppression of VEGF expression. Elevated expression of VEGF has been considered to be a mechanism for acquired resistance to EGFR blockage. Taken together, these findings make a compelling case for combined blockade of both the VEGF and EGF pathways.

A phase 2 trial we performed was the first attempt to inhibit these two pathways simultaneously in patients with advanced RCC (17). With this combination regimen, inhibition of VEGF is achieved with bevacizumab, and EGFR inhibition is achieved with erlotinib, an EGFR tyrosine kinase inhibitor. Patients were treated with bevacizumab, 10 mg/kg, administered by i.v. infusion every 2 weeks and erlotinib orally at a daily dose of 150 mg, full phase 2 doses of both drugs. From several institutions, 63 patients were enrolled and 58 (92%) were reevaluated for response. The median duration of treatment was >8 months (range, 1-19+ months). Fifteen of 59 (25%) assessable patients (95% confidence interval, 16-37%) had objective clinical responses (1 complete response, 14 partial responses). An additional 36 patients (61%; 95% confidence interval, 48-72%) had stable disease. Of the patients with stable disease, 13 (22%) patients had measurable reduction in tumor size but did not meet Response Evaluation Criteria in Solid Tumors (RECIST) criteria for partial response. Only 8 (14%) patients had progressive renal cancer at the time of first reevaluation. After a median follow-up of 15 months, the median progression-free survival was 11 months, and the progression-free survival at 12 and 18 months was 43% and 26%, respectively. The combination of bevacizumab and erlotinib seemed to be more active than each agent alone and was “well tolerated” for patients with advanced RCC. However, confirmatory data were needed.

**Randomized and Blinded Phase 2 Trial of Bevacizumab with or without Erlotinib in Advanced RCC Multicenter Trial**

Results from a randomized, two-arm, phase 2 trial of bevacizumab plus erlotinib or bevacizumab with placebo was presented at the American Society of Clinical Oncology by Bukowski et al. in 2006. Whereas a placebo was included for erlotinib, toxicity may have made its efficacy in blinding patient and physician poor. One hundred four patients with previously untreated advanced clear cell RCC who had all undergone prior nephrectomy were randomized. Objective response rates were 13% and 14% and progression-free survival was 8.5 and 9.9 months for patients treated with bevacizumab plus erlotinib and those treated with bevacizumab alone, respectively. These differences were obviously not statistically or clinically significant. This shows the difficulty in overinterpreting single-arm phase II trials. The concept of combining an EGFR blocking agent with a VEGF-VEGFR blocking agent has not shown an effect but remains appealing. The results combining erlotinib with either sorafenib or sunitinib may be different due to their different spectrum and mechanism of action on different targets. Additionally, cetuximab or ABX-EGF, a humanized antibody targeting EGFR, has clearly different effects than the RTK inhibitors for EGFR. Antibodies to EGFR are active in colon cancer and head and neck cancer, whereas the RTK inhibitors have no activity. Furthermore, the RTK inhibitors seem to preferentially act against tumors with mutations or amplifications of EGFR itself. The EGFR-directed antibodies seem to function effectively without mutations in EGFR generally required for successful erlotinib activity. Either of these antibodies could be combined with any of the antiangiogenic agents above (sunitinib, sorafenib, and bevacizumab). Finally, it is simply possible that other growth factors and their receptors are more important and dominate in the proliferation and survival of renal cancer cells.

**A Phase 1/2 Trial of Sorafenib with Bevacizumab in Advanced RCC**

The availability of the numerous agents that target different cellular pathways involved in both angiogenesis and tumor cell growth.
growth and death has provided us a challenge to define the best one or several strategies to pursue. Additionally, inhibition of a signaling axis at different levels may provide a strategy to prevent and overcome resistance that ultimately occurs. In other solid tumors, preclinical data support the combination of an EGFR antibody with a RTK inhibitor directed at EGFR signaling. We have recently pursued one of many combinations based on this concept by developing a phase 1/2 trial of sorafenib and bevacizumab in advanced RCC. The phase 1 component is nearly complete and we have already observed significant increase in expected single-agent toxicity with mostly sorafenib toxicities when adding bevacizumab. This is especially true at full-dose 400-mg twice daily sorafenib with the addition of bevacizumab. These patients all had significant toxic effects with a combination of hand-foot syndrome, stomatitis, and anorexia, which led to significant weight loss (>15%), and a major decline in performance status. Twenty-four patients have been enrolled to three dose levels of which one dose level (sorafenib at 200 mg twice daily and bevacizumab at 5 mg/kg i.v. every 2 weeks) included 12 patients of whom 6 were also treated with high-dose pyridoxine (300-400 mg/d) prophylactically for hand-foot syndrome. The trial should proceed to its phase 2 component shortly. We have observed a number of objective responses and others with regression not meeting RECIST criteria. There have been 10 partial responses and 4 patients with 20% to 30% regression among the initial 24 patients. These responders have included 2 patients with sarcomatoid variants of renal cancer. Of the two patients with papillary renal cancer and two with chromophobe, none objectively responded to this combination of agents. Furthermore, among 24 patients on trial for 4 to 14 months, only 3 patients have come off treatment due to progression (<15%). As part of this phase 2 trial, a number of additional ancillary studies will be done, including pharmacokinetics and pharmacodynamics, dynamic contrast-enhanced magnetic resonance imaging, circulating endothelial cells and progenitors, tumor genotyping, and assaying of tumor vascularity density. These correlative studies may provide insight into how well the combination of agents impairs overall angiogenesis and whether there is a correlation between the baseline level of angiogenesis and the degree of inhibition at the tumor site. Measurement of circulating endothelial cells/progenitors may estimate levels of systemic/tumor angiogenesis. It may also provide some information on the profile of tumors more likely to respond to this therapy. The phase 2 study will enroll a minimum of 45 patients. The end points of the phase 2 trial will be both RECIST objective response rate and 6-month progression-free survival.

Combinations of Targeted Agents Achieve Vertical or Horizontal Blockade Effects against VEGFR2, PDGF, EGFR, Fibroblast Growth Factor, and mTOR

A number of other combinations of targeted agents are being pursued simultaneously at numerous institutions. These seem to be consistent with several basic principles: First, the combination of two agents affecting the VEGFR2 axis at several levels; an agent such as sunitinib or sorafenib in combination with a VEGF-binding agent capable of sequestering VEGF away from its receptor such as bevacizumab or VEGF-Trap. Additionally, mTOR inhibitors such as temsirolimus and RAD001 that can impair HIF translation are likely to have a major role in RCC. This has become more likely with the phase 3 trial presented by Hudes and colleagues at American Society of Clinical Oncology Annual Meeting in 2006 with temsirolimus alone and with IFN compared directly with IFN upfront in patients with poor-prognosis RCC. A total of 626 advanced, poor-prognosis renal cancer patients were randomized into one of the three arms. Whereas the objective RECIST-defined response rate was similar in all three arms (7-9%) and progression free survival was minimally improved in the temsirolimus arms (1.9 versus 3.7 months), the overall survival in the temsirolimus alone arm was significantly better with a median of 10.9 months versus 7.3 months with IFN alone (P = 0.0069). This was especially impressive based on the high number of risk factors these patients displayed. Combinations of these agents, mTOR inhibitors, with inhibitors of VEGFR2 are under way. Phase 1 trials are needed with a safe dose escalation to define the appropriate doses, starting with low initial doses of both drugs. Trials with one of three agents, bevacizumab, sorafenib, and sunitinib, in combination with CCI-779 are under way. Combinations with the oral mTOR inhibitor RAD001 are soon to be opened.

Recent preclinical studies suggest that fibroblast growth factor up-regulation is important to the development of resistance to antiangiogenic therapy. This suggests that blockade of fibroblast growth factor in combination with inhibitors of VEGF and PDGF may be an approach to prevent development of resistance or for treatment of resistance once it occurs. These dual fibroblast growth factor and VEGF inhibitors are entering early clinical trials, whereas an older already approved agent, IFN-α, has been shown to decrease expression of fibroblast growth factor when administrated to cancer patients (18). Moreover, insulin-like growth factor I–mediated signaling is thought to be involved in the regulation of multiple cellular functions in different tumors, including RCC. Blocking insulin-like growth factor I–mediated signaling by any of the several strategies abolishes or delays the progression of a variety of tumors in animal models. Insulin-like growth factor I–mediated signaling was found to be activated in the presence of mutated VHL tumor suppressor gene. Thus, combination of the insulin-like growth factor receptor blocking antibody with VEGFR axis inhibitors may provide an additional promising approach (19).

The large, cooperative group phase 3 trial of bevacizumab and IFN versus IFN has completed accrual. Furthermore, several groups including Duke University and the University of North Carolina have done a phase 2 trial of sorafenib plus IFN, and their results were a stimulus for a phase 2 Southwest Oncology Group trial. Both studies show mild toxicity (primarily IFN toxicity) and a good response rate from 19% in Southwest Oncology Group up to nearly 40% by Gollob and colleagues.
careful, well-designed trials. Although phase 1 trials may follow standard guidelines of dose escalation, they require special attention to long-term toxicity because of the chronicity of therapy required. Phase 2 studies are much more complicated because many of the beneficial effects of these agents are observed without RECIST-defined objective responses but instead prolonged progression-free survival. Therefore, randomized phase 2 trials including placebo controls may be required to better interpret the results and better judge the value in pursuing a phase 3 trial. Furthermore, the large numbers of agents make it essential to prioritize the clinical efforts and put resources and accrual efforts in trials most likely to succeed or answer critical questions. Combinations of targeted agents need not always be applied in untreated patients but instead may offer an approach to patients who have progressed through treatment with one of the agents. This could help answer whether the combination is effective in settings where the single agent is ineffective.

Targeted Agents with High-Dose Interleukin-2

Up until 2005, the only approved drug for RCC in the past 30 years has been high-dose interleukin-2. Its benefit is observed in a small percentage (20-25%) of highly selected good performance status RCC patients (20). However, it is the only agent that has been shown to induce complete remissions that are very durable, leading to "cures" in 5% to 10% of patients. How to improve its efficacy has been a constant frustration and largely unsuccessful. Bevacizumab is unique not only in terms of its effects on tumor endothelial expansion but also in terms of its effects on the immune system. VEGF has been shown to suppress immune function both through T lymphocytes and dendritic cells (21–23). VEGF seems to block maturation of myeloid cells into mature dendritic cells and actually induce the immature myeloid cells to suppress normal T-cell responses. Therefore, anti-VEGF may reverse these immunosuppressive effects. Finally, interleukin-2 induced profound hypotension through vascular permeability and nitric oxide production. Anti-VEGF can block these effects and has the ability to induce hypertension alone. The question of whether anti-VEGF could block the hypotension so that interleukin-2 can be administered at full or higher doses needs to be addressed.

Summary

The future is bright for RCC patients. The number of active drugs is increasing, and patients will be living longer with a greater chance for improvement in their quality of life. Presently, the field is putting a great emphasis on combinations of agents to overcome resistance and produce a greater degree of regression and more durable responses. This may ultimately be the future, but we will need to be careful about toxicity and to appropriately interpret the benefit of these combinations.

Open Discussion

Dr. Figlin: I would like to see phase 1 trials of combinations performed with good pharmacokinetic and pharmacodynamic data. Phase 1 trials that do not take into consideration the pharmacokinetic and pharmacodynamic aspects of the combination miss the opportunity to understand the biology. Are you planning on doing pharmacokinetic and pharmacodynamic studies of the combination?

Dr. Sosman: We will get that data with the phase 2 part of the trial at a single dose/schedule.

Dr. Figlin: We should also resist emphasizing clinical end points without the biology and pharmacokinetic and pharmacodynamic data as a measure of how to combine drugs. The one thing that could kill combination targeted agents is using clinical end points that do not have any biology-driven variables.

Dr. Sosman: Our goal could be to define a dose that reaches a pharmacokinetic end point that we think is critical.

Dr. Flaherty: One pharmacodynamic effect may be hand-foot syndrome. We are seeing this at a level and consistency that we have not seen with sorafenib alone.

Dr. Figlin: If that is the case, you would argue pharmacokinetically that bevacizumab is modulating the sorafenib area under the curve.

Dr. Flaherty: We have not tested that, but obviously that is hypothesis number one. Hypothesis number two is that we are blocking the recovery.

Dr. Figlin: Searching for validation of biomarkers usually follows clinical activity.

Dr. Flaherty: We are already seeing clinical activity.

Dr. Rini: Do we have to do randomize phase 2 trials for every doublet that we test? I worry that we are placing too much value on the randomized phase 2 trial, which is not a perfect design and can miss small but clinically meaningful effects.

Dr. Atkins: The experience with bevacizumab and erlotinib is perhaps informative. A single-arm phase 2 trial prompted physicians all over the country to start prescribing bevacizumab/erlotinib for patients with renal cell carcinoma. A quick randomized phase 2 trial established that the favorable outcome was entirely due to an unanticipated degree of bevacizumab activity.

Dr. Figlin: The question is how do you intelligently design a phase 2 trial with a specific primary aim that makes sense and that can be appropriately powered?

Dr. Rini: It may be with these new agents that progression-free survival is the best end point and the null hypotheses for what to expect for progression-free survival with these agents are just now being defined.

Dr. Sosman: If you say that we cannot do randomized phase 2 trials in every combination, the two choices then are to try initial phase 2 trials without randomization or go on to phase 3 trials. These are not good choices.

Dr. Rini: We have successfully developed drugs in oncology without randomized phase 2 trials. We have done single-arm phase 2 trials, seen a signal of activity, then moved on to a phase 3 trial to test the hypothesis of benefit.

Dr. Atkins: You need validation after the phase 2 trial is done. You cannot just say this is better based on your results. You have to test it against the single agents or some standard to make sure that the combination is truly better.
References


Opportunities and Obstacles to Combination Targeted Therapy in Renal Cell Cancer

Jeffrey A. Sosman, Igor Puzanov and Michael B. Atkins

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