Tyrosine Kinase Inhibitors in Renal Cell Carcinoma

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ABSTRACT
Current standard treatments for patients with metastatic (stage IV) renal cell carcinoma involve both surgical removal of tumors and treatment with biological agents such as interleukin 2 and/or IFN-α. Unfortunately, such approaches are inadequate for most patients with stage IV disease; the result is a median time to progression of 2 to 4 months and an overall survival of 6 to 17 months. Standard chemotherapies have been uniformly disappointing in this disorder. It is clear that new therapies are needed to approach these patients. Recently, a greater understanding of cancer genetics has led to the successful development of novel therapeutics directed against targets linked to specific types of cancer. During the past decade, researchers have identified the von Hippel-Lindau (VHL) gene as an important tumor suppressor in clear cell carcinoma of the kidney. Elucidation of the VHL gene product (pVHL) and its regulation of hypoxia-inducible factor signaling have created a potential genetic basis for growth factor-targeted strategies in this disease. This review will focus on the potential growth factor targets in clear cell carcinoma, their relation to VHL and hypoxia-inducible factor, and the clinical challenges that face their development.

INTRODUCTION
Renal cell carcinoma represents a significant medical problem in the United States, with ∼35,000 new cases and >12,000 cancer-related deaths in 2003 (1); this accounts for ∼3% of cancer incidence and 2% of cancer deaths in the United States. The standard of care for patients diagnosed as having localized renal cell carcinoma is surgical removal; however, a significant proportion of these patients will experience disease recurrence, whereas others will present with metastatic renal cell carcinoma. Unfortunately, there is currently no standard approach for management of patients with metastatic renal cell carcinoma. Although a small percentage of patients may develop long-term disease-free survival with high-dose interleukin 2 treatment, (2) the morbidity and costs associated with this treatment limit its wide-scale application. IFN-α may offer some modest survival advantage but little hope for durable disease-free survival, and chemotherapy has generally not demonstrated reproducible clinical benefit for most patients. Some other promising strategies remain investigational and are highlighted elsewhere in this issue.

Recently, a better understanding of cancer genetics has helped identify protein targets within certain patient populations in which well-defined and specific therapy has demonstrated a robust clinical response. To date, the most commonly defined protein targets have been growth factor signaling pathways that involve receptor tyrosine kinases (RTKs) on the surface of epithelial or stromal cells and their cognate ligands. In addition, several downstream cytosolic kinases involved in the propagation of RTK signaling have also been identified as potential therapeutic targets. Clinical trials have demonstrated the feasibility and safety of inhibiting RTK using either specific humanized antibodies or small molecular weight molecules. This review will focus on the genetic rationale for inhibiting various RTKs in renal cell carcinoma, as well as the current clinical data.

RTKs as Targets for Antitumor Therapy
RTKs are enzymes that transfer γ-phosphate groups from ATP to the hydroxyl group of tyrosine residues on signal transduction molecules (3). Phosphorylation of signal transduction molecules is a major activating event that leads to significant changes in tumor growth. Some RTKs such as epidermal growth factor receptor (EGFR) can autophosphorylate when activated, as well as phosphorylate other signaling molecules (4). The resulting phosphotyrosine residues in the EGFR cytoplasmic domain serve to additionally activate the tyrosine kinase (TK) activity of the receptor and to act as docking sites for cytoplasmic signal transduction molecules that contain Src homology or phosphotyrosine-binding motifs (3). Strict regulation of TK activity controls the most fundamental cellular processes, such as the cell cycle, proliferation, differentiation, motility, and cell death or survival (5, 6).

Approximately 90 RTKs and nonreceptor TKs have been identified thus far: 58 transmembrane receptor types and 32 cytoplasmic nonreceptor types (7). Mutations and overexpression of both types of TKs are regularly found in human malignancies (7, 8). However, clinical agents that specifically inhibit the activity of these molecules have been clinically developed for only a few TKs. Some examples of clinically targeted RTKs include the EGFR, the closely related HER-2 (ErbB-2/Neu), platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and c-kit/stem cell factor receptor (7, 8). These new targeted therapies are designed to take advantage of the molecular differences specific to tumor cells compared with healthy tissues. The goal is to achieve tumor responses with better safety profiles than those associated with cytotoxic chemotherapies.
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In the presence of oxygen, VHL recognizes hydroxylated proline residues on HIF-α and binds them. Once bound, HIF becomes ubiquinated and tagged for proteasome-dependent protein degradation. In the absence of oxygen or with loss of pVHL function, HIF-α is allowed to accumulate, ultimately binding to HIF-β and resulting in increased expression of hypoxia-inducible genes, including VEGF, PDGF, and TGF-α.

ROLE OF RTK IN RENAL CELL CARCINOMA

Individuals who inherit a mutated version of the von Hippel-Lindau (VHL) tumor suppressor gene are predisposed to clear cell renal cell carcinoma, which is the most common form of kidney cancer. Tumor development in this setting is linked to somatic inactivation of the remaining wild-type allele. Moreover, biallelic VHL inactivation due to somatic mutations and/or hypermethylation is observed in >50% of sporadic clear cell carcinoma.

pVHL primarily functions to regulate the degradation of hypoxia-inducible factor under normal oxygen (normoxic) conditions (9). However, when pVHL function is lost, a hypoxia-inducible factor is allowed to accumulate to concentrations that result in increased transcriptional activity and expression of various growth factors, including VEGF, PDGF, transforming growth factor α (TGF-α), erythropoietin, and so on (Fig. 1). These growth factors function in a paracrine loop by binding to specific RTKs on the surface of endothelial cells and vascular pericytes, respectively, and thereby activate a signal transduction cascade that results in cell proliferation, survival, and angiogenesis. Phenotypically, these growth factors likely contribute to the hypervascular histologic structure of renal cell carcinoma. In addition, TGF-α has recently been shown to be a powerful renal epithelial cell mitogen and probably contributes to the development of renal cell carcinoma (10). Overproduction of both TGF-α and its receptor, EGFR, are common in renal cell carcinoma (10, 11).

Small molecules directed against VEGFR, PDGFR, and EGFR have shown preclinical activity in various tumor models, including preclinical models of renal cell carcinoma (12–15). In particular, work by Drevs et al. (12) demonstrated in vivo both delay in disease progression and inhibition of metastatic disease using PTK787/ZK 222584 (Novartis Pharmaceuticals, East Hanover, NJ; Shering AG, Berlin, Germany), a pan-VEGFR inhibitor (12). Likewise, Prewett et al. (13) demonstrated tumor responses in vivo using a chimeric antibody to EGFR. Interestingly, Perera et al. (14) later demonstrated that renal cell lines with intact VHL were more responsive to EGFR inhibition in vitro than those with biallelic loss of VHL, suggesting that overexpression of growth factors associated with loss of pVHL might connote a mechanism of resistance. This observation might explain the relative lack of activity seen by EGFR-targeted strategies alone (see below) while supporting the rationale for combining them with other VHL-regulated growth factor targets. Finally, work by Bergers et al. (16) demonstrate advantages to targeting both VEGF and PDGF pathways in terms of antiangiogenic and antitumoral effects in vivo. Collectively, these data support the hypothesis that inhibition of various hypoxia-inducible factor-regulated growth factors such as VEGF, PDGF, and TGF-α may mimic, in part, the restoration of pVHL function (Fig. 2).

RTK INHIBITION IN RENAL CELL CARCINOMA: CLINICAL TRIAL RESULTS

A number of single agents targeted against the respective RTK pathways (receptors or ligands) have been tested to date (Table 1). The most promising single-target studies have been those focused on the VEGF-VEGFR pathway. As highlighted elsewhere in this issue, the anti-VEGFA antibody bevacizumab (Avastin; Genentech, South San Francisco, CA) demonstrated a statistically significant improvement in overall time to progression compared with placebo in a randomized phase II study (17). In addition, other strategies that target the VEGF signaling pathway, including RTK inhibitors SU11248 (Pfizer, Inc., San Diego, CA), PTK787/ZK 222584 (Novartis Pharmaceuticals and Shering AG), ZD 6474 (AstraZeneca, Wilmington, DE), and BAY 43-9006 (Bayer Corporation, Pittsburgh, PA), as well as cytokine traps (Fc immunoglobulin linked to receptors such as VEGFR 1 and 2), have also demonstrated clinical activity in patients with renal cell carcinoma in phase I studies (18–21). Interestingly, several of these small molecules inhibit two or more of the RTK targets (13, 16, 17).
more RTK targets that are relevant to renal cell carcinoma and might therefore display greater activity than agents that target solely VEGF in future phase II and III studies.

Less clear are the data using single-agent strategies targeted against TGF-α-EGFR. Phase II studies using humanized antibodies directed to EGFR, such as C225 (Erbitux; ImClone, New York, NY) and ABX-EGF (Abgenix, Fremont, CA), have shown few responses (22, 23). In addition, small molecule compounds that inhibit EGFR, including Iressa (AstraZeneca) and Erlotinib (Tarceva; OSI Pharmaceuticals, New York, NY), have generally been disappointing in their single-agent activity (24). Nonetheless, there is still a reasonable rationale for combining these agents with VEGF-targeted strategies in the future.

One promising example of the possible synergistic effect of combining VEGF and EGFR inhibitors was presented at the 2004 Proceedings of the American Society of Clinical Oncology. Hainsworth et al. (25) presented results from a multicenter phase II study of Bevacizumab and Erlotinib in which 12 of 58 (21%) evaluable patients demonstrated a partial response and an additional 38 of 58 (66%) demonstrated minor response or stable disease. These results exceed expected responses by either drug alone (<10% partial response rate) and suggest an additive or possible synergistic effect of the two inhibitors in this disease. Likewise, the toxicity profile with these two agents was not overlapping and treatment was generally as well tolerated as either drug alone. To confirm these results and demonstrate an additive clinical benefit to the combination, a phase III trial of Bevacizumab versus Bevacizumab and Erlotinib is planned.

PDGFR-targeted strategies alone have little published results in renal cell carcinoma but represent a reasonable third target for consideration. Humanized antibodies and imatinib mesylate (Gleevec; Novartis Pharmaceuticals) represent reasonable strategies. With current progress in recent years using VEGF-targeted strategies, a combination approach, either with two agents or a single small molecule that targets both pathways, is perhaps more hopeful. One example of the possible additive effect of blocking both PDGFR and VEGFR may be inferred by the results of SU11248 in patients with cytokine-refractory renal cell carcinoma. Presented at the 2004 American Society of Clinical Oncology meeting, Motzer et al. (26) demonstrated partial response in 21 of 63 (33%) of patients in a multicenter trial with central radiology review. An additional 22 of 63 (37%) of patients demonstrated minor response or stable disease for ≥3 months. Toxicity was moderate with grade 2 fatigue and cytopenias, which became the most commonly seen side effects. These exciting single agent, multi-targeted results have led to a pivotal phase II trial in cytokine-refractory, metastatic renal cell carcinoma that is currently ongoing.

### DEMONSTRATING PROOF OF CONCEPT

One of the biggest obstacles to the successful development and integration of targeted therapy into the treatment of any cancer, including renal cell carcinoma, is establishing the clinical proof of principle that inhibiting a specific target protein results in clinical response. Although several of these initial clinical trials using VEGF-VEGFR–targeted strategies have established some clear albeit modest clinical response, direct measures of VEGF inhibition have been absent. Our clinical results using PTK787/ZK 222584, an RTK inhibitor of VEGFRs 1, 2, and 3, revealed similar results to other agents and strategies against this target. Specifically, we reported a 5% overall partial remission rate, with 14% minor responses, 60% stable disease for ≥3 months, a median time to progression of 5.5 months, and a median survival of 21 months (27). As a surrogate of VEGFR inhibition by PTK787/ZK 222584, we and others have measured effects on blood flow using dynamic magnetic resonance imaging techniques and demonstrated in both renal cell carcinoma and colorectal cancers that decreases in tumor blood flow after treatment correlate with clinical response (28, 29). Such correlations support the principle that directly decreasing tumor blood flow through VEGF signaling can result in clinical response. In contrast, several EGFR–targeted agents have demonstrated direct inhibition of EGFR activation (i.e., phosphorylation) by immunohistochemical analysis in both nontumor tissue (skin) and tumor tissue (30–32). Finally, direct immunohistochemical inhibition of PDGFR has been difficult to determine to date, and no molecular or radiographic surrogates have been validated for isolated PDGFR activity.

### CONCLUSION

In summary, the clinical development of targeted strategies against RTK inhibitors for renal cell carcinoma treatment holds...
significant promise for the future. Single-agent studies using strategies against the VEGF-VEGFR pathways have established the proof of concept in renal cell carcinoma. Combination strategies that target multiple growth factor pathways in the future, including VEGF-VEGFR, TGF-α-EGFR, and PDGF-PDGF, as well as combinations with intracellular targets such as mammalian target of rapamycin and Ras-Raf kinase, represent exciting future possibilities.

OPEN DISCUSSION

Dr. Richard Childs: The initial feeling about imatinib mesylate was that it was going to work and work quickly in patients with chronic myelogenous leukemia. Subsequently, data came out to show that sometimes patients took 8 months to a year to get a cytogenetic remission, and when you looked at data, it came out to show that sometimes patients took 8 months and sometimes 2 years after therapy, suggesting that these responses were ongoing and ultimately could be complete. Have you seen any evidence that the responses are slow and progressive over time and are they durable?

Dr. Robert Motzer: The responses that I’ve seen happen very quickly with SU 011248 and usually you see them within the first cycle. In the first cycle, patients start to respond, and by cycle 2 or 3, if they’re going to be a partial responder, they’ve reached that.

Dr. Daniel George: We have seen some slow, cumulative responses with other similar agents as well. There is a wide variation here in terms of response. It may have to do with the rate of growth of the tumor, the sites of disease, or prior therapies. It’s still a very heterogeneous population.

Dr. Childs: When you say that patients aren’t achieving complete remission with these drugs, are you sure follow-up time is sufficient? I think we have to be cautious because there are several examples where these patients get operated on after a response and are found to have little viable tumor tissue despite their persistent measurable tumor radiographically.

Dr. James Yang: We did noninvasive imaging and dynamic magnetic resonance imaging. The only time we saw differences was when we could also see simultaneous differences on the imaging. Your patient imaged at a month was already showing necrosis. Did you ever look earlier, or did you ever see somebody with a predicted rather than confirmed response?

Dr. George: In four patients, we did arterial spin labeling at day 2, but we didn’t see changes then. With arterial spin labeling, we are measuring purely intravascular flow. Perhaps permeability changes happen quickly, but flow changes may be more delayed. All of our changes in blood flow seemed to happen within 1 month, and then we didn’t see any further regressions in blood flow after that. In fact, we maybe saw some increases, but we had several patients who had continued regression, albeit slowly, all of the way out to over a year. I think they are kinetically disconnected.

Dr. Michael Atkins: There’s going to be some interest in designing neoadjuvant trials because we need to actually measure the ability to hit the target in the tissue. A number of design issues come up. First, when is the best time after you start treatment to determine whether the target has been hit with these types of kinase inhibitors? I think a week is too early because you haven’t reached a steady state yet. Maybe 4 weeks is best. Six weeks is too late because if there is a target, it’s already gone. Have you given any thought to how one would design those trials? Second, is it safe to do surgery in these patients who are getting angiogenesis inhibitors?

Dr. George: I don’t think we can answer the second question except to say that we ought to tread lightly. I probably wouldn’t be dosing these patients intraoperatively but perhaps would dose up to the day of surgery. It depends on whether you are going to look at your target on the surgical specimen.

Dr. Yang: I don’t think we necessarily have the right target to evaluate. First of all, there is going to be data on surgery because patients on bevacizumab may require emergency surgery, and I am unaware of any particular wound healing issues on that.

Dr. Michael Gordon: One should note that in the colorectal study with bevacizumab, there were six patients who had to undergo emergent surgery. Two of those patients had severe complications, including a wound dehiscence. A patient who had a thoracotomy had a dehiscence of their chest lesion and there is another patient who had an abdominal wound complication.

Dr. Yang: We were warned about that, but there are no data on it in renal cancer. So, we didn’t biopsy anyone in our study, but it’s something that I think needs to be elucidated.

Dr. George: That might be one advantage of small molecule inhibitors over antibodies, that you can turn them off quickly to avoid the effects of residual drug.

Dr. Gordon: No question, but it becomes a problem that if you let your drug wash out, then you may well be missing what you want to see.

Dr. Yang: It depends on what size biopsy you’re talking about. Clearly, with small ones you can fix that. The half-life of the drug is the most important part of when to test for the effect. This is a rapid-onset phenomenon. If this drug works, it has to work within hours typically or days at most. I think this would argue for an early biopsy.

Dr. George: The only question will be have you hit steady state? Have you penetrated that tumor or not? I think 2 weeks is enough time.

Dr. Robert Figlin: Are there patient populations with kidney cancer that could, with hypothesis generation, demonstrate target blockade as a way of predicting biology? One such population of patients is those with metastatic disease undergoing nephrectomy. Let’s leave out the initial biopsy for a second. In general, it takes a couple of weeks before those patients go to surgery. If you could find a way to biopsy those patients and test for the tyrosine kinase up front and treat them for a couple of weeks with inhibitors, you might be able to understand biology. The time between when a person is diagnosed as having metastatic disease and when he gets treated with IFN post nephrectomy is ~6 to 8 weeks. The median time to treatment on the SWOG study was ~4 weeks post nephrectomy. So, could you look at patients with metastatic disease whose primary tumors are in place and try to understand the biology before definitive therapy? That is a window that many of us have not looked at in kidney cancer.
Dr. Atkins: We’ve thought about this and have gone as far as designing a clinical trial. We discussed what the risk of surgery would be, and it is potentially different with an agent that has a shorter half-life than with bevacizumab.

Dr. Figlin: We’re going to do a similar neoadjuvant study with an mTOR inhibitor because mTOR inhibition to my knowledge should not be a risk with respect to surgery. You could also restart the therapy very quickly after surgery because there is no expectation of delayed wound healing. At least we will learn if there is any biology that is going to be inhibited. I would encourage all of us to think about models that ask questions in a way that is a bit different so we begin to understand biology better. We are doing that now with prostate cancer because with prostate cancer, there is a window of opportunity between biopsy and definitive surgery to understand biology. The thing that I am struggling with in renal cancer is the need for tissue pretreatment. Some people view that as standard of care. On the other hand, others feel that there is no need to diagnose pretreatment because patients are going to get diagnosed at the time of surgery. Has that been a barrier for anyone else?

Dr. W. Marston Linehan: We have not had complications from renal biopsies. It might delay surgery but not too much. The other question is, “How long a therapy are you talking about?” If, hypothetically, patients had clear cell cancer and if people felt strongly that the Food and Drug Administration-approved drug is interleukin 2-based therapy, how could you argue for delaying that?

Dr. Figlin: At some point post surgery, you have to offer people standard of care. That is why I think the window has to be a biology window, which would not interfere with what would otherwise be considered standard of care.

Dr. Atkins: The real obstacle to doing some of these studies is the proliferation in the community of debulking nephrectomy as the standard of care. These patients aren’t coming to the major medical centers before they have their surgery anymore. They are having a community urologist perform the operation. We are trying to get the word out that doing the biopsy to a big surgical specimen. With biopsies, you could fixate differences. The best way to design these trials is to give the patient’s pathology or the phosphorylation status of a critical protein may be what is important in trying to predict who should continue on therapy.

REFERENCES
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