Targeting of the *VHL*-Hypoxia-Inducible Factor-Hypoxia-Induced Gene Pathway for Renal Cell Carcinoma Therapy

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Abstract. Treatment of advanced renal cancer has made little progress in the past 30 yr. Most clinical efforts have incorporated cytokine-based therapy. The presumption has been that the cytokines may trigger a host immune response against the renal cancer. Only IFN-α and high-dose IL-2 seemed to have positive effects on patient outcomes. IFN has prolonged the lives of patients by a few months, and high-dose IL-2 is capable of inducing very prolonged remissions (>5 yr) for a small number of patients. Nephrectomy in the presence of metastatic disease has been established as an effective procedure for select patients, providing palliation and prolonging survival. Finally, enthusiasm has focused on the use of non-myeloablative allogeneic stem cell transplantation and donor leukocyte infusion for the induction of graft *versus* tumor effects. Early results are both provocative and promising. A number of agents that target the critical gene products downstream from *pVHL* and hypoxia-inducible factor-1, such as vascular endothelial growth factor, PDGF, EGF receptor, and TGF-α, have recently become available. The new agents are capable of inhibiting specific cellular targets, and the biologic characteristics of clear cell carcinoma of the kidney support their application. If the correct targets are carefully selected for inhibition in tumors in which the targets are present (clear cell histologic features and loss of *VHL* expression), then results should resemble those others have observed with targeted therapy, such as the use of STI-571 (Gleevec; Novartis Pharmaceuticals, East Hanover, NJ) for treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors or anti-HER2/neu (Herceptin; Genentech, South San Francisco, CA) for treatment of breast cancer.

Renal cancer represents a significant medical problem in the United States, with nearly 32,000 new cases and 12,000 cancer-related deaths in 2002 (1); this accounts for 2 to 3% of cancer incidence and 2% of cancer deaths in the United States. The incidence seems to have increased in the past 50 yr, whereas no such trend has been noted for similarly located (area of the body) cancers derived from the renal pelvis transitional epithelium (2). The environmental factors associated with sporadic renal cancers include cigarette smoking, obesity, hypertension, and acquired renal cystic disease among patients undergoing long-term dialysis (3–5). As described in the other contributions in this series, genetic factors have been well established for syndromes associated with high incidences of renal cancer, such as von Hippel-Lindau (VHL) syndrome and tuberous sclerosis (6,7). The histologic features of sporadic renal cell carcinomas (RCC) can vary greatly but have recently been classified into five subtypes on the basis of growth patterns, cells of origin, and cytogenetic or molecular characteristics (8). These subtypes include clear cell, chromophilic (papillary), chromophobic, oncocytic, and collecting duct types. More than 75% of cases are of the clear cell type, with 15% being of the papillary type and the remaining types being far less frequent. In many prior clinical studies, very little information regarding histologic subtyping was provided, especially in studies addressing the treatment of advanced disease. This may be an important omission, making the interpretation of those studies difficult. Although the 5-yr overall survival rate for renal cancer has steadily improved to $>60\%$ in the United States, this is unlikely to be attributable to better treatment of advanced disease (1). Instead, it is almost certainly the result of earlier diagnoses with better imaging procedures. The diagnosis of many renal cancers at the presymptomatic stage results from abdominal imaging for other indications. In this review, I describe the current state of therapy of disseminated or locally advanced renal cancer. Then I attempt to demonstrate how advances in our understanding of the *VHL*-hypoxia-inducible factor (HIF)-hypoxia-induced gene pathway are bringing newer and more biologically rational treatment options to the clinic and providing greater insight into clear cell-type RCC.

Systemic Therapy of RCC

Chemotherapy

Standard chemotherapy agents have exhibited dismal results in the treatment of RCC (9). Earlier clinical trials suggested that vinblastine had antitumor activity in renal cancer (9). More recently, 5-fluorouracil administered as a prolonged infusion,

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with various modifications of the administration schedule, was suggested to improve clinical responses, with prolonged periods of disease stability and improved overall survival rates (10). Finally, investigators demonstrated clinical outcomes superior to historical control results from a regimen of gemcitabine plus 5-fluorouracil (continuous infusion) (11). None of these chemotherapy-based approaches fulfilled their promise when evaluated in more rigorous clinical trials. In addition, many agents have failed to demonstrate any promise in early phase II testing. There does not seem to be a well defined role for standard chemotherapy agents in the treatment of advanced RCC.

Cytokine-Based Therapy

In part because of the poor results obtained with chemotherapy and the immune phenomena (spontaneous remissions and responses to nonspecific immune-stimulating agents) associated with RCC, investigators have examined cytokine-based therapies for treatment of renal cancer. Initially, in the 1970s and 1980s, IFN was studied for the treatment of advanced RCC (12). In the late 1980s and 1990s, IL-2 therapy played a significant role in the treatment of RCC (13,18). To summarize the effects of IFN on RCC, phase III studies have suggested that IFN-α may have modest effects on survival rates among patients with advanced renal cancer (15,16). For example, a phase III trial comparing IFN-α2a plus vinblastine and vinblastine alone reported a median survival time of 67.6 wk for patients who received IFN-α plus vinblastine, compared with 37.8 wk for patients who received vinblastine alone ($P = 0.0049$) (15). In another trial, which randomized patients with advanced disease to receive either IFN-α or Megace (Bristol-Myers Squibb, New York, NY), there was a 28% reduction in the risk of death for the IFN-α group ($P = 0.017$) and an improvement in the median survival time of 2.5 mo (16). Other IFN, such as IFN-γ, have been inactive (17). Combination of IFN-α with other agents (cytokines or chemotherapeutic drugs) did not improve the effects of the drug.

Clinical investigations of the use of IL-2 to treat RCC began in the middle 1980s (18). The initial studies were performed with high-dose bolus IL-2 and lymphokine-activated killer cells, on the basis of results from animal studies (18). Dramatic durable responses were reported for a subset of patients. Subsequent clinical studies demonstrated that high-dose bolus IL-2 possesses antitumor activity that is essentially equivalent to that of the combination of IL-2 and lymphokine-activated killer cells. In 1992, high-dose bolus IL-2 received United States Food and Drug Administration approval for use in metastatic RCC, on the basis of data for 255 patients treated in seven clinical trials at 21 institutions. In those studies, recombinant IL-2 (600,000 to 720,000 IU/kg, Proleukin; Chiron Corp., Emeryville, CA) was administered by 15-min intravenous infusion every 8 h on days 1 to 5 and days 15 to 19 (maximum of 28 doses). Treatment was repeated at approximately 12-wk intervals for responding patients, for a maximum of three cycles. Because of considerable toxicity (hypotension, capillary leakage, and central nervous system toxicity) associated with this regimen, treatment needed to be administered in a setting providing intensive care unit-level care and was restricted to carefully selected patients with excellent organ function, who were treated at experienced treatment centers. The Food and Drug Administration was presented with objective responses for 36 of the 255 patients (response rate, 14%). There were 12 complete responses (5%) and 24 partial responses (9%). At that time, the median duration of response was 23.2 mo for all responders (partial responders, 18.8 mo; complete responders, median not reached) (13). The quality and durability of the responses prompted Food and Drug Administration approval, despite the relatively low response rate and the high level of toxicity. Follow-up data for those patients were accumulated through late 1998 (median follow-up period, 8 yr). The clinical results seemed to steadily improve with time. Seventeen patients (7%) were classified as complete responders and 20 (8%) as partial responders. The survival time for the group as a whole was 16.3 mo, with 10 to 15% of patients being estimated to remain alive 5 to 10 yr after treatment with high-dose IL-2. The median response duration for all objective responders was 54 mo (range, 3 to >131 mo). The median response duration for partial responders was 20 mo; that for complete responders had not been reached but was at least 80 mo at the time of that analysis. Eleven patients underwent resection of residual disease either while still responding or after limited-site progression, and nine remained progression-free for a minimum of 65 mo. Therefore, a large percentage of responding patients, particularly those who remained free from progression for >2 yr and those who underwent resection to disease-free status after responding to high-dose IL-2, seemed unlikely to exhibit progression and might actually have been "cured." A large phase III trial comparing high-dose IL-2 with outpatient, subcutaneously administered IL-2 and IFN-α2b enrolled 193 patients, of whom 187 were able to be evaluated with respect to responses (19). Tumor responses were observed for 25 of 97 patients (26%) who received high-dose IL-2, compared with 10 of 90 patients (11%) who received low-dose IL-2 and IFN ($P = 0.01$). High-dose IL-2 produced eight complete responses and nine patients remained progression-free at 3 yr, compared with three complete responses and two patients remaining progression-free at 3 yr with low-dose IL-2 and IFN. These data again support the preferential use of high-dose IL-2 for patients who have access to such treatments and are considered able to tolerate the therapy.

More recently, several interesting observations have been made concerning the histologic features and phenotype of renal cancers that respond to IL-2 and the *pVHL*-HIF pathway. It seems that major responses have been observed predominantly among patients with clear cell carcinoma and not other histologic types (20). In addition, high rates of expression (>85%) of carbonic anhydrase IX (CAIX), a gene product regulated by HIF, are associated with better outcomes and improved response rates with high-dose IL-2 (21). The IL-2 results correlating clinical responses with CAIX expression are very preliminary.

Nonmyeloablative Allogeneic Transplantation

A more purely immunologic approach to the treatment of advanced RCC, *i.e.*, nonmyeloablative allogeneic stem cell transplantation (with donor leukocyte infusions), has gained favor in the research community (14). Such treatment is of great interest, in part because of the definite role that allogeneic immune cells play in the antitumor effects. However, results with nonmyeloablative allogeneic stem cell transplantation are still quite preliminary and the treatment is complicated. Since its therapeutic introduction, allogeneic bone marrow transplantation has evolved from a means of achieving chemotherapeutic dose escalation to a form of adoptive immunotherapy. There is extensive evidence supporting the presence of a graft *versus* malignancy effect with hematologic malignancies, and preliminary data demonstrated the presence of a graft *versus* tumor effect with solid tumors (22,23). Different groups began developing nonmyeloablative allogeneic bone marrow and peripheral blood stem cell transplantation regimens for use for both hematologic and solid tumors (22–26). The goal is to create a conditioning regimen sufficient for proper donor cell engraftment with the least recipient graft ablation possible. Preliminary experiences with nonmyeloablative allogeneic transplantation among patients with RCC have been reported by a number of centers (14,22,24–26). In all studies, the provision existed for posttransplantation donor lymphocyte infusion if complete donor chimerism was not achieved. The largest series, reported by Childs *et al.* (14), demonstrated responses for 10 of 19 patients, with a conditioning regimen of cyclophosphamide and fludarabine. Complete donor chimerism seemed to be a prerequisite for responses, as was some degree of graft *versus* host disease.

There is a great deal of interest in understanding what antigens on host renal cancer are recognized by donor leukocytes. Such understanding would facilitate specific, more focused approaches that could potentially avoid the toxicity of graft *versus* host disease. It is possible that genes induced by HIF because of the *VHL* mutation represent immune target proteins. CAIX may be an immune target for T lymphocytes (27). There must be other proteins, growth factors, or receptors that could be targeted by donor T lymphocytes, and they might be regulated upstream by *pVHL* and HIF. Finally, the *VHL* mutation itself may represent a unique epitope (peptide including the amino acid mutation) that could be targeted by T lymphocytes. The same issues are important for patients responding to high-dose IL-2. Does the *pVHL*-HIF pathway lead to the expression of important immune targets (antigens)?

Role of Nephrectomy with Systemic Therapy

The primary role for nephrectomy (either standard radical, laparoscopic, or partial) is in the complete resection of primary RCC. Although patients with very large primary tumors (>10 cm), high histologic grade (Fuhrman Grade II to III/III or III to IV/IV), penetration into the perinephric tissues (T3a) or through Gerota's fascia (T4), extensive inferior vena cava involvement below (T3b) or above (T3c) the diaphragm, or lymph node involvement in one (N1) or more (N2) regional sites are at increased risk for recurrence, no adjuvant systemic

therapy has proven effective in preventing recurrences. A number of trials have studied various forms of IFN, and a small trial recently examined high-dose IL-2 therapy. Those trials all failed to demonstrate a definitive benefit with treatment (28,29).

In the setting of advanced disseminated disease, two recent randomized controlled trials demonstrated that nephrectomy followed by IFN-α yielded significantly better overall survival rates than did treatment with IFN-α alone (30,31). How nephrectomy in the setting of disseminated disease improves survival rates is unclear, but the effects are not attributable to a significant increase in the overall frequency of tumor regressions. The effects may be a result of the reduction in tumor bulk and reduction in the source of both immunosuppressive factors, such as vascular endothelial growth factor (VEGF), and tumor growth-enhancing factors.

Advances in Our Understanding of the VHL-*HIF-Hypoxia-Induced Gene Pathway*

The above discussion represents a general overview of the treatment of advanced RCC before the introduction of targeted therapy. We are now aware that biallelic *VHL* inactivation resulting from somatic mutations and/or hypermethylation is observed in 50 to 75% of sporadic clear cell carcinoma cases (32–34). Cells lacking pVHL (such as the majority of sporadic clear cell carcinomas) are unable to suppress the accumulation of hypoxia-inducible genes, including VEGF, under even well oxygenated conditions. In short, whereas normal cells produce hypoxia-inducible mRNA only under hypoxic conditions, cells lacking pVHL produce these mRNA constitutively.

The HIF family of transcription factors plays a central role in maintaining oxygen homeostasis. HIF-α and -β regulate an array of many (>20) HIF target genes that have already been identified, including VEGF, PDGF, TGF-α, EGF receptor (EGFR), and erythropoietin (Figure 1). These protein products play critical roles in cellular and systemic physiologic responses to hypoxia. TGF-α has been demonstrated to be a powerful renal epithelial cell mitogen and may contribute to the development of RCC (35). HIF is a heterodimer composed of α and β subunits. Whereas the HIF-β subunit is constitutively expressed, HIF-α is normally degraded in the presence of oxygen and accumulates only under hypoxic conditions. Degradation is regulated by a 200-amino acid, oxygen-dependent, degradation domain that lies within the central region of HIF-1α (36), as well described in the accompanying discussions. In the absence of oxygen, modification does not take place and pVHL does not bind to HIF; therefore, HIF-1α accumulates.

What Does the VHL-*HIF Pathway Tell Us about Clear Cell-Type RCC?*

Knowledge of the genes induced by high cellular levels of HIF-1α provides a biologic basis for some of the clinical manifestations of renal cancer (41,42). VEGF production likely plays a role in the vascularity that is pronounced in renal cancer. The tendency of these tumors to bleed and their intense vascular networks have been extensively described. Also, the
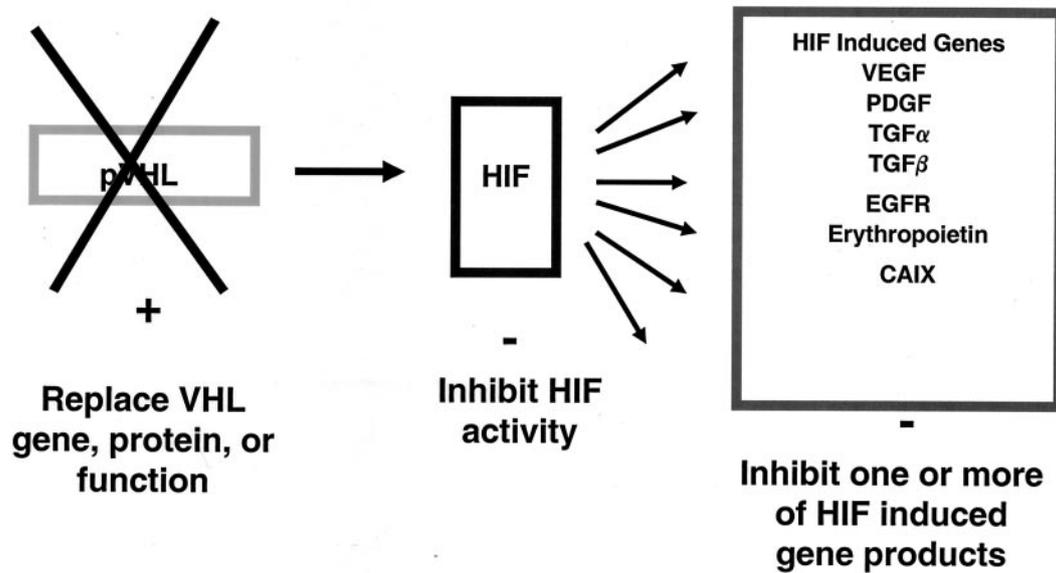
Figure 1. Targeting of the *VHL*-hypoxia-inducible factor (HIF)-hypoxia-induced gene pathway for treatment of renal cell carcinoma. VEGF, vascular endothelial growth factor; EGFR, EGF receptor; CAIX, carbonic anhydrase IX.

induction of erythropoietin has been associated with polycythemia among patients with renal cancer. In fact, normalization of hemoglobin levels frequently predates clinical regression of disease among responding renal cancer patients. It is also possible that the induction of GLUT1 and CAIX affects glucose metabolism and acidic changes, respectively, within the tumor environment, resulting in barriers to a number of different types of therapy (21). There is enthusiasm regarding the potential of gene microarray technology to provide molecular profiles of different renal cancers. There is currently minimal information, compared with that for other malignancies (such as breast cancer, lymphoma, and lung cancer) (43). However, there is hope that, by analyzing these microarrays, we will be able to identify specific genes that lead to the expression of targets for immunotherapy (cellular or passive immunotherapy with antibodies) or targets for signal inhibitors. Overexpression of genes associated with either a good or poor prognosis may suggest targets for antibodies/vaccines or small-molecule signal inhibitors (such as tyrosine kinase inhibitors). Finally, attempts have been made and will continue to be made to determine the molecular profiles of cancers that have proven responsive to therapy. To date, no therapy has been effective enough to indicate much potential for this approach. In the future, with small-molecule therapy (described below), expression arrays may provide important information. In addition, in attempts to inhibit the *pVHL*-HIF pathway, it will be important to identify changes in other genes within tumors that may affect outcomes.

Targeting of the VHL-*HIF-Hypoxia-Induced Gene Pathway for RCC Therapy*

One of the obvious results of the scientific advances in RCC biologic characterization is the importance of recognizing the differences in the biologic features underlying the pathogenesis of the various histologic subtypes of RCC. This is true for at least clear cell and papillary renal cancers (8). Such findings not only will likely affect how we select patients for future therapy but also should be addressed in assessments of the results of prior therapy with IFN-α or IL-2 or nephrectomy (12,20,30).

The ideal therapeutic targets for treatment of patients with advanced clear cell carcinoma would be genes or gene products critical to the development or maintenance of the cancer, with inhibition of the targets by the therapeutic agents not being life-threatening to the normal cells of the host (Figure 1). Ideally, the defect could be corrected and the function of the lost VHL protein replaced (37). This was elegantly demonstrated in a human renal cell line (37). This has been the goal of most gene therapies, without successful results. Recent efforts have been aimed at targeting HIF-1α itself. Previous screenings of small molecules and the novel pharmacologic activity of a potential antiplatelet agent (YC-1) provided a basis for testing of the effects of HIF-1α inhibition on cancer growth and toxicity in normal tissues (38,39). These efforts have been initiated in preclinical animal models, but clinical evaluations should be undertaken with caution, because of the risk of impairing critical physiologic responses to tissue hypoxia (40).

A number of agents that should allow us to target the actual critical gene products induced by HIF, including VEGF, PDGF, EGFR, and TGF-α, have become available (Table 1). These molecules, in combination, form the critical links between pVHL, HIF, and RCC. VEGF and PDGF are critical for angiogenesis and metastatic potential, whereas EGFR and TGF-α are important in renal cell mitogenesis and antiapoptotic effects. A number of agents used clinically may, alone or in combination, affect these pathways and mediate antitumor effects. The most obvious target has been VEGF, targeted with

Table 1. Therapeutic agents targeting renal cancer proteins regulated by *VHL* and HIF[a]

Therapeutic Agent	Mechanism of Action	Target Molecules	Advantages	Disadvantages	Clinical Status
Anti-VEGF	Humanized antibody to VEGF	VEGF	Specificity	Intravenous	Phase II/III
SU5416	Tyrosine kinase inhibitor	VEGFR1, VEGFR2		Toxicities	Program closed
PTK787	Tyrosine kinase inhibitor	VEGFR2, PDGFR	Oral		Phase II/III
VEGF trap	Cytokine trap (Fc Ig-receptor)	VEGFR1, VEGFR2	Long half-life	Intravenous	Phase I/II
STI-571 (Gleevec)	Tyrosine kinase inhibitor	Bcr/Abl, c-Kit, PDGFR	Oral	Nonspecificity	Phase II/III
C225	Humanized antibody to EGFR	EGFR		Intravenous	Phase II/III
ABX-EGF	Human antibody to EGFR	EGFR		Intravenous	Phase II
ZD1839 (Iressa)	Tyrosine kinase inhibitor	EGFR	Oral		Phase II/III
OSI-774 (Tarceva)	Tyrosine kinase inhibitor	EGFR	Oral		Phase II/III
SU11248	Tyrosine kinase inhibitor	VEGFR2, PDGFR, Flt 3, c-Kit	Oral		Phase II
ZD6474	Tyrosine kinase inhibitor	VEGFR2, EGFR	Oral		Phase I/II

[a] HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; PDGFR, PDGF receptor; EGFR, EGF receptor.

antibodies such as anti-VEGF (Avastin; Genentech, South San Francisco, CA), kinase inhibitors (SU5416 and PTK787), and cytokine traps (Fc Ig linked to receptors such as VEGF receptors 1 and 2) (44–47). In addition, there are antibodies directed to EGFR, such as C225 (ImClone, New York, NY) and ABX-EGF (Abgenix, Fremont, CA) (48,49). There are compounds inhibiting the EGFR tyrosine kinases, such as Iressa (Astra-Zeneca, Wilmington, DE) and OSI-774, and PDGF receptor tyrosine kinases, such as SU6668 and STI-571 (Gleevec; Novartis Pharmaceuticals, East Hanover, NJ) (50–52). Finally, there are a number of kinase inhibitors, such as PTK787, ZD6474, and SU11248, that exhibit promiscuous activities and inhibit a number of receptor pathways (46,52–54). These offer some potential for simultaneous blockade of several cooperating signaling pathways. There is even evidence that resistance to EGFR inhibition is mediated through enhanced expression of VEGF (55). This suggests that blockade of both pathways (VEGF receptor 2 and EGFR) is important for demonstration of clinically important antitumor effects. SU11248, a novel tyrosine kinase inhibitor targeting VEGF and PDGF receptors, also has the ability to block Flt-3 and c-Kit receptors (54). These agents have largely completed phase I trials. Phase II and III trials have been completed with other cancers, whereas phase II and III trials with RCC are being planned or are underway. To date, the most promising results have been observed with anti-VEGF (Avastin) (56). A recently reported randomized phase II study that enrolled patients with metastatic RCC who had experienced failure of or were not eligible for cytokine therapy randomized the patients to receive either low-dose anti-VEGF (3 mg/kg), high-dose anti-VEGF (10 m/kg), or placebo treatment (56). The study was discontinued prematurely because of a significant delay in disease progression associated with high-dose anti-VEGF, compared with placebo. The trial involved a planned 150 patients, with 50 in each group (10 mg/kg, 3 mg/kg, and placebo). The end points were time to progression, major clinical response, survival time, and toxicity. Only 113 patients were randomized, with 100 able to be evaluated; the study was stopped prematurely

because of large differences in the time to progression, as monitoring rules require. Four patients exhibited partial responses, and 30% were progression-free at 8 mo on high-dose anti-VEGF. Thirty-six of 38 placebo-treated patients exhibited progressive disease, whereas 29 of 37 patients treated with anti-VEGF (10 mg/kg) exhibited progressive disease ($P = 0.001$; hazard ratio, >2.5). Overall survival analysis at 6 mo indicated that 18 of 38 placebo-treated patients were dead as a result of disease and 12 of 37 patients treated with anti-VEGF (10 mg/kg) were dead as a result of disease ($P = 0.20$, crossover allowed). Phase III studies of anti-VEGF in RCC are planned and will be initiated in the near future. At the most recent American Society of Clinical Oncology meeting, the results of a randomized, placebo-controlled trial of anti-VEGF with a standard chemotherapy regimen (irinotecan and 5-fluorouracil plus leucovorin) for treatment of advanced colorectal cancer were presented (57). More than 800 patients were randomized and anti-VEGF was administered intravenously at 5 mg/m^2 every 2 wk. With >400 patients in each treatment group, the results were remarkable, with very significant improvements in median survival time (20.3 *versus* 15.6 mo), median progression-free survival time (10.6 *versus* 6.2 mo), overall response rate (45 *versus* 35%), and duration of response (10.4 *versus* 7.1 mo). The *P* values indicated significance ($P < 0.003$) in each case. This was the first trial that conclusively demonstrated the benefit of an antiangiogenic agent.

Ongoing phase I and II trials in RCC of anti-VEGF with an EGFR tyrosine kinase inhibitor (OSI-774) or with STI-571 (Gleevec), a PDGF receptor tyrosine kinase inhibitor, are generating great excitement, and their results are being eagerly awaited. In addition, small molecules (PTK787, SU11248, and ZD6474) targeting VEGF and PDGF or VEGF and EGF receptor pathways demonstrated evidence of clinical responses in RCC in early phase I studies. Finally, new agents targeting other downstream pathways are of interest. CCI-779 is a rapamycin analog that inhibits *mTOR* downstream of *AKT*, resulting in cell cycle arrest (58). Atkins *et al.* (59) reported the results of a randomized, double-blind, phase II trial examining

three dose levels of CCI-779 among patients with renal cancer who either exhibited refractory disease or were considered to be poor candidates for cytokine-based therapy. Twenty-nine of the 110 patients enrolled (26%) experienced a partial or minor response. The median time to progression and the median survival time for all patients were 6 mo and 15 mo, respectively. The response rate, time to progression, and survival time were considered to be encouraging for this population of patients, most of whom exhibited poor prognostic features.

Overall, the future for the treatment of RCC looks far brighter than ever before. The targeted treatment discussed is firmly based on the biologic features of clear cell cancer of the kidney. However, trials must be carefully performed, must be well controlled, and must have realistic end points. We must carefully confirm that our results are not attributable to patient selection and prognostic factors, such as performance status, prior nephrectomy, serum calcium levels, anemia, and serum lactate dehydrogenase levels (41). If, as clinical investigators, we carefully select the correct molecular targets for inhibition in the tumors and the targets are present (clear cell histologic type, with loss of *VHL* expression), then we will likely begin to observe the results others have experienced with targeted therapy, such as the use of STI-571 (Gleevec) for treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors or anti-HER2/neu (Herceptin; Genentech) for treatment of breast cancer (60–62).

References

1. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ: Cancer statistics, 2002. *CA Cancer J Clin* 52: 23–47, 2002
2. Chow WH, Devesa SS, Warren JL, Fraumeni JF Jr: Rising incidence of renal cell cancer in the United States. *JAMA* 281: 1628–1631, 1999
3. Yu MC, Mack TM, Hanisch R, Cicioni C, Henderson BE: Cigarette smoking, obesity, diuretic use, and coffee consumption as risk factors for renal cell carcinoma. *J Natl Cancer Inst* 77: 351–356, 1986
4. Matson MA, Cohen EP: Acquired cystic kidney disease: Occurrence, prevalence, and renal cancers. *Medicine (Baltimore)* 69: 217–226, 1990
5. Chow W-H, Gridley G, Fraumeni JF, Jarvholm B: Obesity, hypertension, and the risk of kidney cancer in men. *N Engl J Med* 343: 1305–1311, 2000
6. Lamiell JM, Salazar FG, Hsia YE: von Hippel-Lindau disease affecting 43 members of a single kindred. *Medicine (Baltimore)* 68: 1–29, 1989
7. Bjornsson J, Short MP, Kwiatkowski DJ, Henske EP: Tuberous sclerosis-associated renal cell carcinoma: Clinical, pathological and genetic features. *Am J Pathol* 149: 1201–1210, 1996
8. Storkel S, van den Berg E: Morphological classification of renal cancer. *World J Urol* 13: 153–158, 1995
9. Yagoda A, Petrylak D, Thompson S: Cytotoxic chemotherapy for advanced renal cell carcinoma. *Urol Clin North Am* 20: 303–321, 1993
10. Hrushesky WJ, von Roemeling R, Lanning RM, Rabatin JT: Circadian-shaped infusions of floxuridine for progressive metastatic renal cell carcinoma. *J Clin Oncol* 8: 1504–1513, 1990
11. Rini BI, Vogelzang NJ, Dumas MC, Wade JL III, Taber DA, Stadler WM: Phase II trial of weekly intravenous gemcitabine with continuous infusion fluorouracil in patients with metastatic renal cell cancer. *J Clin Oncol* 18: 2419–2426, 2000
12. Motzer RJ, Bacik J, Murphy BA, Russo P, Mazumdar M: Interferon-α as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. *J Clin Oncol* 20: 289–296, 2002
13. Fyfe G, Fisher RI, Rosenberg SA, Sznol M, Parkinson DR, Louie AC: Results of treatment of 255 patients with metastatic renal cell carcinoma who received high-dose recombinant interleukin-2 therapy. *J Clin Oncol* 13: 688–696, 1995
14. Childs R, Chernoff A, Contentin N, Bahceci E, Schrump D, Leitman S, Read EJ, Tisdale J, Dunbar C, Linehan WM, Young NS, Barrett AJ: Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. *N Engl J Med* 343: 750–758, 2000
15. Pyrhonen S, Salminen E, Ruutu M, Lehtonen T, Nurmi M, Tammela T, Juusela H, Rintala E, Hietanen P, Kellokumpu-Lehtinen PL: Prospective randomized trial of interferon-α2a plus vinblastine *versus* vinblastine alone in patients with advanced renal cell cancer. *J Clin Oncol* 17: 2859–2867, 1999
16. Medical Research Council Renal Cancer Collaborators: Interferon-α and survival in metastatic renal carcinoma: Early results of a randomised controlled trial. *Lancet* 353: 14–17, 1999
17. Gleave ME, Elhilali M, Fradet Y, Davis I, Venner P, Saad F, Klotz LH, Moore MJ, Paton V, Bajamonde A: Interferon-γ1b compared with placebo in metastatic renal-cell carcinoma: Canadian Urologic Oncology Group. *N Engl J Med* 338: 1265–1271, 1998
18. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghausen SE, Matory YL, Skibber JM, Shiloni E, Vetto JT, *et al.*: Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N Engl J Med* 313: 1485–1492, 1985
19. McDermott D, Flaherty L, Clark J, Weiss G, Logan T, Gordon M, Sosman J, Ernstoff M, Urba W, Margolin K, Atkins M: A randomized phase II trial of high-dose interleukin-2 (HD IL-2) *versus* subcutaneous (SC) IL-2/interferon (IFN) in patients with metastatic renal cell carcinoma (RCC). *Proc Am Soc Clin Oncol* 20: 172a, 2001
20. Upton M, Parker RA, Youmans C, Atkins MB: Histologic predictors of renal cell carcinoma (RCC) response to interleukin-2 therapy. *Proc Am Soc Clin Oncol* 22: 851, 2003
21. Bui MHT, Seligson D, Han K, Pantuck AJ, Dorey FJ, Huang Y, Horvath S, Leibovich BC, Chopra S, Liao SY, Stanbridge E, Lerman MI, Palotie A, Figlin RA, Belldegrun AS: Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: Implications for prognosis and therapy. *Clin Cancer Res* 9: 802–811, 2003
22. Ueno NT, Rondon G, Mirza NQ, Geisler DK, Anderlini P, Giralt SA, Andersson BS, Claxton DF, Gajewski JL, Khouri IF, Korbling M, Mehra RC, Przepiorka D, Rahman Z, Samuels BI, van Besien K, Hortobagyi GN, Champlin RE: Allogeneic peripheral-blood progenitor-cell transplantation for poor-risk patients with metastatic breast cancer. *J Clin Oncol* 16: 986–993, 1998
23. Bregni M, Dodero A, Peccatori J, Pescarollo A, Bernardi M, Sassi I, Voena C, Zaniboni A, Bordignon C, Corradini P: Non-myeloablative conditioning followed by hematopoietic cell allografting and donor lymphocyte infusions for patients with metastatic renal and breast cancer. *Blood* 99: 4234–4236, 2002
24. Pedrazzoli P, Da Prada GA, Giorgiani G, Schiavo R, Zambelli A, Giraldi E, Landonio G, Locatelli F, Siena S, Della Cuna GR:

Allogeneic blood stem cell transplantation after a reduced-intensity, preparative regimen: A pilot study in patients with refractory malignancies. *Cancer* 94: 2409–2415, 2002

25. Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, Leitman S, Read EJ, Carter C, Bahceci E, Young NS, Barrett AJ: Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: Full donor T-cell chimerism precedes alloimmune responses. *Blood* 94: 3234–3241, 1999

26. Rini BI, Zimmerman T, Stadler WM, Gajewski TF, Vogelzang NJ: Allogeneic peripheral blood stem cell transplantation for metastatic renal cell carcinoma. *J Urol* 165: 1208–1209, 2001

27. Hernandez JM, Bui MHT, Han K, Mukouyama H, Freitas DG, Nguyen D, Caliliw R, Shintaku PI, Paik SH, Tso C-L, Figlin RA, Belldegrun AS: Novel kidney cancer immunotherapy based on the granulocyte-macrophage colony-stimulating factor and carbonic anhydrase IX fusion gene. *Clin Cancer Res* 9: 1906–1916, 2003

28. Messing EM, Manola J, Wilding G, Propert K, Fleischmann J, Crawford ED, Pontes JE, Hahn R, Trump D: Phase III study of interferon α-NL as adjuvant treatment for resectable renal cell carcinoma: An Eastern Cooperative Oncology Group/Intergroup trial. *J Clin Oncol* 21: 1214–1222, 2003

29. Clark JI, Atkins MB, Urba W, Figlin R, Dutcher JP, Flaherty L, Sosman J, Logan T, White R, Margolin K: Adjuvant high-dose bolus interleukin-2 in patients with high-risk renal cell carcinoma: A Cytokine Working Group phase III trial. *J Clin Oncol* 21: 3133–3140, 2003

30. Flanigan RC, Salmon SE, Blumenstein BA, Bearman SI, Roy V, McGrath PC, Caton JR Jr, Munshi N, Crawford ED: Nephrectomy followed by interferon-α2b compared with interferon-α2b alone for metastatic renal-cell cancer. *N Engl J Med* 345: 1655–1659, 2001

31. Mickisch GH, Garin A, van Poppel H, de Prijck L, Sylvester R, European Organisation for Research and Treatment of Cancer (EORTC) Genitourinary Group: Radical nephrectomy plus interferon-α-based immunotherapy compared with interferon-α alone in metastatic renal-cell carcinoma: A randomised trial. *Lancet* 358: 966–970, 2001

32. Kenck C, Wilhelm M, Bugert P, Staehler G, Kovacs G: Mutation of the *VHL* gene is associated exclusively with the development of non-papillary renal cell carcinomas. *J Pathol* 179: 157–161, 1996

33. Foster K, Prowse A, van den Berg A, Fleming S, Hulsbeek MM, Crossey PA, Richards FM, Cairns P, Affara NA, Ferguson-Smith MA, Buys CHM, Maher ER: Somatic mutations of the von Hippel-Lindau disease tumor suppressor gene in non-familial clear cell renal carcinoma. *Hum Mol Genet* 3: 2169–2173, 1994

34. Masahiro Y, Minoru Y, Takeshi K, Noboru N, Masaya B, Kazuki K, Takeshi M, Masatoshi M, Yoji N, Yukio N, Yoshinobu K, Kei-ichi K: *VHL* tumor suppressor gene alterations associated with good prognosis in sporadic clear-cell renal carcinoma. *J Natl Cancer Inst* 94: 1569–1575, 2002

35. de Paulsen N, Brychzy A, Fournier MC, Klausner RD, Gnarra JR, Pause A, Lee S: Role of transforming growth factor-α in von Hippel-Lindau (*VHL*)(−/−) clear cell renal carcinoma cell proliferation: A possible mechanism coupling *VHL* tumor suppressor inactivation and tumorigenesis. *Proc Natl Acad Sci USA* 98: 1387–1392, 2001

36. Jaakkola P, Mole DR, Tian YM, Wilson MI, Gielbert J, Gaskell SJ, Kriegsheim AV, Hebestreit HF, Mukherji M, Schofield CJ, Maxwell PH, Pugh CW, Ratcliffe PJ: Targeting of HIF-α to the von Hippel-Lindau ubiquitylation complex by O_2-regulated prolyl hydroxylation. *Science* 292: 468–472, 2001

37. Iliopoulos O, Kibel A, Gray S, Kaelin WG Jr: Tumour suppression by the human von Hippel-Lindau gene product. *Nat Med* 1: 822–826, 1995

38. Rapisarda A, Uranchimeg B, Scudiero DA, Selby M, Sausville EA, Shoemaker RH, Melillo G: Identification of small molecule inhibitors of hypoxia-inducible factor 1 transcriptional activation pathway. *Cancer Res* 62: 4316–4324, 2002

39. Yeo E-J, Chun YS, Cho Y-S, Kim J, Lee J-C, Kim M-S, Park J: YC-1: A potential anticancer drug targeting hypoxia-inducible factor-1. *J Natl Cancer Inst* 95: 516–525, 2003

40. Pili R, Donehower RC: Is HIF-1α a valid therapeutic target? *J Natl Cancer Inst* 95: 498–499, 2003

41. Motzer RJ, Mazumdar M, Bacik J, Berg W, Amsterdam A, Ferrara J: Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. *J Clin Oncol* 17: 2530–2540, 1999

42. Tsui KH, Shvarts O, Smith RB, Figlin RA, deKernion JB, Belldegrun A: Prognostic indicators for renal cell carcinoma: A multivariate analysis of 643 patients using the revised 1997 TNM staging criteria. *J Urol* 163: 1090–1095, 2000

43. Masayuki T, Daniel R, Rhodes K, Furge A, Kanayama H, Kagawa S, Haab B, Tean B: The gene expression profiling of clear cell renal cell carcinoma: Gene identification and prognostic classification. *Proc Natl Acad Sci USA* 98: 9754–9759, 2001

44. Kim KJ, Li B, Winer J, Armanini M, Gillett N, Phillips HS, Ferrara N: Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth *in vivo*. *Nature* 362: 841–844, 1993

45. Fong TA, Shawver LK, Sun L, Tang C, App H, Powell TJ, Kim YH, Schreck R, Wang X, Risau W, Ullrich A, Hirth KP, McMahon G: SU5416 is a potent and selective inhibitor of the vascular endothelial growth factor receptor (Flk-1/KDR) that inhibits tyrosine kinase catalysis, tumor vascularization, and growth of multiple tumor types. *Cancer Res* 59: 99–106, 1999

46. Wood JM, Bold G, Buchdunger E, *et al*.: PTK787/ZK222584, a novel and potent inhibitor of vascular endothelial growth factor receptor tyrosine kinases, impairs vascular endothelial growth factor-induced responses and tumor growth after oral administration. *Cancer Res* 60: 2178–2189, 2000

47. Huang J, Frischer JS, Serur A, Kadenhe A, Yokoi A, McCrudden KW, New T, O'Toole K, Zabski S, Rudge JS, Holash J, Yancopoulos GD, Yamashiro DJ, Kandel JJ: Regression of established tumors and metastases by potent vascular endothelial growth factor blockade. *Proc Natl Acad Sci USA* 100: 7785–7790, 2003

48. Perrotte P, Matsumoto T, Inoue K, Kuniyasu H, Eve BY, Hicklin DJ, Radinsky R, Dinney CP: Anti-epidermal growth factor receptor antibody C225 inhibits angiogenesis in human transitional cell carcinoma growing orthotopically in nude mice. *Clin Cancer Res* 5: 257–265, 1999

49. Wang P, Friedlin P, Davis CG, Yang XD: Therapeutic potential of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for the treatment of renal cell carcinoma. *Proc Am Soc Clin Oncol* 21: 191a, 2002

50. Bergers G, Song S, Meyer-Morse N, Bergsland E, Hanahan D: Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. *J Clin Invest* 111: 1287–1295, 2003

51. Laird AD, Cherrington JM: Small molecule tyrosine kinase inhibitors: Clinical development of anticancer agents. *Expert Opin Invest Drugs* 12: 51–64, 2003

52. Ciardiello F, Caputo R, Damiano V, Caputo R, Troiani T, Vitagliano D, Carlomagno F, Veneziani BM, Fontanini G, Bianco AR, Tortora G: Antitumor effect of ZD6474, a small molecule VEGF receptor tyrosine kinase inhibitor, with additional activity against the EGF receptor tyrosine kinase. *Clin Cancer Res* 9: 1546–1556, 2003

53. Laird AD, Vajkoczy P, Shawver LK, Thurnher A, Liang C, Mohammadi M, Schlessinger J, Ullrich A, Hubbard SR, Blake RA, Fong TA, Strawn LM, Sun L, Tang C, Hawtin R, Tang F, Shenoy N, Hirth KP, McMahon G, Cherrington J: SU6668 is a potent antiangiogenic and antitumor agent that induces regression of established tumors. *Cancer Res* 60: 4152–4160, 2000

54. Mendel DB, Laird AD, Xin X, Louie SG, Christensen JG, Li G, Schreck RE, Abrams TJ, Ngai TJ, Lee LB, Murray Lesley J, Carver J, Chan E, Moss KG, Haznedar JO, Sukbuntherng J, Blake RA, Sun L, Tang C, Miller T, Shirazian S, McMahon G, Cherrington JM: *In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: Determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res* 9: 327–337, 2003

55. Viloria PA, Crombet T, Jothy S, Hicklin D, Bohlen P, Schlaeppi JM, Rak J, Kerbel RS: Acquired resistance to the antitumor effect of epidermal growth factor receptor-blocking antibodies *in vivo*: A role for altered tumor angiogenesis. *Cancer Res* 61: 5090–5101, 2001

56. Yang JC, Haworth L, Sherry RM, Hwan P, Schwartzentruber DJ, Tapolian SL, Steinberg SM, Chen HX, Rosenberg SA: A randomized trial of Bevacizumab, an anti-vascular endothelial growth factor antibody for metastatic renal cancer. *N Engl J Med* 349: 427–434, 2003

57. Hurwitz L, Fehrenbacher T, Cartwright J, Hainsworth W, Heim J, Berlin S, Griffing W, Novotny E, Holmgren F, Kabbinavar H: Bevacizumab (a monoclonal antibody to vascular endothelial growth factor) prolongs survival in first-line colorectal cancer (CRC): Results of a phase III trial of bevacizumab in combination with bolus IFL (irinotecan, 5-fluorouracil, leucovorin) as first-line therapy in subjects with metastatic CRC. *Proc Am Soc Clin Oncol* 22: 3646, 2003

58. Hidalgo M, Rowinsky EK: The rapamycin-sensitive signal transduction pathway as a target for cancer therapy. *Oncogene* 19: 6680–6686, 2000

59. Atkins MB, Hidalgo M, Stadler W, Logan T, Dutcher JP, Hudes G, Park Y, Marshall B, Boni J, Dukart G: A randomized double-blind phase 2 study of intravenous CCI-779 administered weekly to patients with advanced renal cell carcinoma. *Proc Am Soc Clin Oncol* 2002

60. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S, Sawyers CL: Efficacy and safety of a specific inhibitor of the Bcr-Abl tyrosine kinase in chronic myeloid leukemia. *N Engl J Med* 344: 1031–1037, 2001

61. Demetri GD, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silberman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CD, Joensuu H: Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 347: 472–480, 2002

62. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J, Norton L: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 344: 783–792, 2001