Mutations or aberrations of the von Hippel-Lindau gene are responsible for the hereditary neoplastic syndrome that bears the same name, as well as for the majority of sporadic clear cell renal cell carcinomas. The discovery of this gene and subsequent clarification of its mechanism of action have led to a series of targeted treatments for advanced kidney cancer and have dramatically changed how we manage this disease. The discovery of the \( VHL \) gene is a prime example of how discoveries at the bench can inform and revolutionize therapeutics at the bedside. In this review, the authors trace this illuminating tale, from the cloning of the \( VHL \) gene, to elucidating its biologic function, to the development of novel therapeutics that have dramatically changed the paradigm of managing advanced renal cell carcinoma. \( \textit{Cancer} \ 2008;113(7 \text{ suppl}):1768–78. \) © 2008 American Cancer Society.

**KEYWORDS:** von Hippel-Lindau gene, renal cell carcinoma, clear cell renal cell carcinoma, kidney cancer.

**Von Hippel-Lindau disease** is a heritable neoplasia syndrome characterized by the development of benign and malignant tumors in a variety of organ systems.\(^1,2\) It is inherited as an autosomal dominant trait and its incidence in the population is approximately 1 in 36,000 births.\(^3,4\) It has over 90% penetrance by the age of 65 in affected individuals.\(^5\) Hallmarks of the disease are hemangioblastomas of the central nervous system, especially the cerebellum and spinal chord, retinal hemangioblastomas, pheochromocytomas, and renal manifestations including renal cysts and clear cell renal cell carcinomas.\(^1\) The impact of kidney cancer on patients with VHL is profound. In fact, the leading cause of death in patients suffering from von Hippel-Lindau disease is metastatic clear cell renal cell carcinoma (ccRCC).\(^5\)

Germ line mutations of the \( VHL \) gene have been identified as the root cause of this disease.\(^6\) Mutations and/or aberrations of the same gene have been identified in the majority of sporadic, nonfamilial ccRCC, making this a prominent example of a classic tumor suppressor gene as described by Knudson.\(^7-13\) The discovery and characterization of the \( VHL \) gene, and its role in regulating the cell’s response to hypoxia, is a prime example of how discoveries in the basic sciences can revolutionize treatment of human disease. This article will trace the history of this bench-to-bedside tale, from the cloning of the \( VHL \) gene, to elucidating its biologic function, to the development of novel therapeutics that have dramatically changed the paradigm of managing advanced renal cell carcinoma.
The Tumor Suppressor Gene Theory

The paradigm for discovering the VHL gene really started with the development of the tumor suppressor gene theory and the 2-hit hypothesis as described by Knudson. Under this hypothesis, a tumor suppressor gene is anticipated to be one in which both copies of the gene must be disabled in some way or a cancer to develop. In a sporadic, noninherited form of cancer, this requires the development of 2 mutations in the same gene in the same cell. Because this is anticipated to be a relatively rare event, sporadic cancer would be expected to occur later in life, and generally be unifocal. For inherited neoplasia syndromes that are due to a germ-line aberration in a tumor-suppressor gene, the hypothesis would predict that 1, inherited copy of the tumor-suppressor gene is already nonfunctional (the first hit). This means that it only takes 1 further somatic mutation of the same gene (the second hit) in 1 cell for the process of neoplasia to begin. Because it now only requires 1 hit in a given cell to start the process (rather than 2 as in sporadic cancers), this should be a more common event. Thus, one would predict that the affected organs would develop tumors much earlier, and that there would be a much higher probability that they would be multifocal.

By the late 1980s, the basic tenets of this hypothesis had already been applied to retinoblastoma and the Rb gene and then subsequently also applied to neurofibromatosis and the NF-1 and NF-2 genes. Because one of the primary manifestations of von Hippel-Lindau disease is the development of ccRCC, which in other respects was similar to sporadic ccRCC except for its early onset and multifocality, it was postulated that the gene responsible for the inherited disease may also be responsible for the development of the sporadic, noninherited form of the malignancy.

The VHL Gene Is on Chromosome 3

The first clues to the identity and location of the VHL gene came from cytogenetic studies of several independent kindreds in whom there was an inherited susceptibility to ccRCC. In the first kindred, there was an inherited balanced translocation of part of the short arm of chromosome 3 to chromosome 8. Only individuals affected by VHL disease, all of whom developed ccRCC by age 50 years, inherited this translocation, whereas none of the family members without this translocation developed ccRCC. This initial report was followed by 2 others, each from a different kindred. One described a kindred in which there was a translocation of chromosome 3 to chromosome 11 in the renal tumors, and another in which there was a translocation of a part of chromosome 3. In all of these reports, the common thread was an abnormality in chromosome 3 associated with the inheritance of familial susceptibility to ccRCC.

This initial clue was further supported by a series of reports using cytogenetics and restriction fragment length polymorphisms (RFLP) analysis to characterize and identify genetic aberrations in sporadic ccRCC tumors and cell lines. Cytogenetics can distinguish larger-scale deletions, translocations, and rearrangements, and these were consistently identified in chromosome 3. RFLP analysis allows for a much finer and detailed mapping of the site of a genetic alteration by looking at sites in the chromosome in which there are known genetic variations between normal individuals in the population. These latter studies, in particular, consistently demonstrated abnormalities in the short arm of chromosome 3 (chromosome 3p) that were present in the ccRCC tumors that were not present in normal tissue, and were not present in patients with other histologic variants such as papillary RCC.

The Discovery of the VHL Gene

These studies strongly suggested there was a tumor suppressor gene on chromosome 3p that was important in the development of sporadic ccRCC. Despite the finer detailed mapping that is possible with RFLP analysis, the area delineated on chromosome 3p was still too large to allow for precise identification of the actual gene itself. The next step forward required the application of Knudson's tumor suppressor gene hypothesis. If the sporadic form of ccRCC was in part caused by an abnormality of a gene, and patients with von Hippel-Lindau disease developed ccRCC only earlier and in multiple locations, then it is reasonable to hypothesize that the same gene that would fit the definition of a tumor suppressor gene was responsible for both. This was a critical cognitive step, because more precise localization of the VHL gene could now be accomplished by carefully studying multiple families with von Hippel-Lindau disease and using a process known as genetic linkage analysis to determine the area on chromosome 3p common to all these families. The work of several different investigative teams ultimately lead to the localization of the VHL gene to a relatively small region on chromosome 3p that spanned 4 centimorgans. This was now a narrow enough region on the chromosome to allow for specific cloning techniques using a combination of yeast artificial chromosomes and cosmid and phage contiguous segments of cloned DNA (contigs) to construct a detailed map...
of chromosome 3p25-26 (also reviewed in Linehan et al, Gnarra et al, Richards et al, and Seizinger). These efforts ultimately allowed the identification of the VHL gene in a seminal article in 1993 by Latif et al. The gene itself is small, 854 nucleotides in 3 exons encoding 284 amino acids. It has been evolutionarily conserved, consistent with its role as an important tumor suppressor gene. Consistent with Knudson’s hypothesis, the majority of sporadic ccRCC tumors were found to harbor aberrations of VHL, strongly suggesting that the same gene was responsible for both the inherited and noninherited forms of the disease. Indeed, in cases where mutations of VHL in sporadic ccRCC were not identified, often other aberrations were noted, such as abnormal hypermethylation of the promoter region of VHL, leading to significant transcriptional down-regulation of the gene and low or absent protein levels.

**VHL and von Hippel-Lindau Disease**

The cloning of the VHL gene had immediate impact on the ability to screen and diagnose individual family members in kindreds affected by von Hippel-Lindau disease. The specific mutation in a given kindred could now be identified and used to specifically determine what family members were at risk of developing the disease thus facilitating early screening and treatment of lesions before they became symptomatic. Furthermore, it became increasingly clear that there was a relationship between the type of genetic mutation present in the VHL gene (the genotype) and the pattern of tumors that developed in that particular family (the phenotype). For example, in general, large-scale deletions of the VHL gene or mutations that result in a truncated or shortened form of the protein tend to be associated with a lower risk of pheochromocytoma in that kindred. Conversely, missense mutations that result in a single amino acid change are more often associated with the development of pheochromocytoma in that particular family. Indeed, there are families in which a particular missense mutation (examples include a change from a tyrosine to histidine at codon 98 or 111) may confer a high risk of developing pheochromocytoma, but a low risk of developing ccRCC. These insights have had significant implications in counseling and risk-stratifying families with members affected by von Hippel-Lindau disease.

**Toward Elucidating the Biology of VHL**

While the discovery of VHL had an immediate impact on families with von Hippel-Lindau disease, its role and function were not initially clear. At the time of its discovery VHL did not have any homology to any known gene. It soon became apparent that VHL could act as a tumor suppressor in kidney cancer cell lines. Several studies demonstrated that if wild type VHL were introduced into RCC cell lines that lacked the protein, then these cells were growth inhibited both in vitro and in vivo. Subsequent studies showed that 2 proteins known to be induced by hypoxia, vascular endothelial growth factor (VEGF) and glucose transporter 1 (GLUT-1), were not regulated normally by VHL-deficient cell lines, and that the normal oxygen-sensitive regulation of these proteins could be restored if wild type VHL were reintroduced. This suggested that VHL was in some way important in the response to hypoxia, but the precise mechanism by which this occurred was not clear.

At approximately the same time that the VHL gene was cloned, an independent series of studies was ongoing by various investigators into the normal cellular response to hypoxia. These studies ultimately lead to the discovery that hypoxia-inducible factor (HIF) was the principle regulator of this process. HIF (both HIF-1 and HIF-2) are members of a family of basic helix-loop-helix transcription factors known as the Per-ARNT-Sim (PAS) family.

It has now been demonstrated that HIF is actually a complex composed of a HIF- subunit that acts as the oxygen sensor in the cell. Recently, it has been demonstrated that HIF-2 is likely the more important of the 2 with respect to ccRCC. Under normoxic conditions, HIF- undergoes a process of hydroxylation and subsequent ubiquitination. This in turn marks HIF- for subsequent degradation by the proteasome. Under hypoxic conditions, levels of HIF- rise, allowing it to heterodimerize with HIF-β. This complex can then bind to specific sequences, known as hypoxia response elements (HRE) in the promoters of a wide array of genes critical in the cell's response to hypoxia (see Fig. 1).

The connection between VHL and HIF was not known at the time VHL was cloned. Over time it was first demonstrated that the VHL protein was part of a complex in a cell with several other proteins including elongin B, elongin C, cullin2, and Rbx. This complex has the ability to ubiquitinate proteins which can then mark these proteins for subsequent
FIGURE 1. Normal biology of the VHL-HIF axis in the setting of (A) normoxia and (B) hypoxia. In normoxic conditions, HIFα is hydroxylated on specific proline residues by prolyl-hydroxylases. VHL acts as the sensor for these hydroxylated proline residues as part of the VHL-E3 ubiquitin ligase. This polyubiquitinates HIFα and marks it for degradation by the proteasome. In hypoxic conditions (or in the presence of aberrant VHL), HIFα is allowed to accumulate in the cell. (C) It associates with HIFβ, and this complex translocates to the nucleus and acts as a transcription factor binding to hypoxia response elements and up-regulating oxygen sensitive genes. These include vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor alpha (TGFβ), glucose transporter-1 (GLUT1), carbonic anhydrase IX (CA-IX), erythropoietin (EPO), and others. Examples of certain ligands’ receptors are given, including VEGF receptor (VEGFR), PDGF receptor (PDGFR), and the receptor for TNFα, epidermal growth factor receptor (EGFR). Shown is the downstream signaling for 1 of these receptors, VEGFR, including through the PI3 kinase (PI3K)/AKT/mTOR, p38 MAP kinase (p38MAPK), and RAS/RAF/MEK/ERK pathways. Examples of agents that impact on this cascade are given, and where they act on the pathway is shown.
degradation by the proteosomal machinery of the cell.\textsuperscript{60,61} This E3 ubiquitin ligase activity is one of the critical mechanisms by which the cell regulates the protein level of key regulatory molecules in the cell, such as HIF-α. The proof that VHL was critical in the process of regulating HIF and marking it for proteolysis in the presence of oxygen came in 1999 in a landmark study by Maxwell et al.\textsuperscript{62} It was subsequently demonstrated that this occurred through the oxygen-dependent poly ubiquitination and subsequent degradation of HIF-α that was critically mediated and regulated by the VHL protein.\textsuperscript{63-67}

**Moving Toward Therapeutics**

With the discovery that the VHL protein is critical in HIF-mediated response to hypoxia, attention became focused on the downstream targets of HIF. Under hypoxic conditions, or if VHL is mutated or nonfunctional as in von Hippel-Lindau disease or ccRCC, HIF-α is not targeted for ubiquitination and thus its levels rise within the cell. As discussed previously, this allows HIF-α to associate with the constitutively expressed and stable HIF-β. This HIF transcriptional regulatory complex then binds to HRE in the promoters of oxygen-sensitive genes. Examples of genes up-regulated by HIF in response to hypoxia include vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), transforming growth factor alpha (TGFα), glucose transporter-1 (GLUT1), carbonic anhydrase IX, erythropoietin (EPO), and others.\textsuperscript{46,68-73} This reproducible response to the loss of VHL protein or to hypoxia suggested that any or all of these downstream molecules and/or their subsequent downstream signaling cascades could be important in the development and/or progression of ccRCC and in turn might be targets for therapy.

The clinical observation that ccRCC is often a hypervascular tumor, combined with the growing awareness that angiogenesis is a critical component for malignant tumor progression stimulated particular interest in 1 member of this signaling cascade, VEGF.\textsuperscript{74-76} The VEGF family of proteins consist of multiple subtypes including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor-1 (PIGF-1).\textsuperscript{77-80} Most of these are regulated by VHL and HIF. These proteins can, in turn, bind to 1 of 3 different cell surface receptors, VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4).\textsuperscript{77-80} Of these, VEGFR-1 and VEGFR-2 are thought to be more important for the process of angiogenesis, whereas VEGFR-3 is thought to be more important for lymphangiogenesis.\textsuperscript{80}

The VEGF receptor family members are cell membrane-associated tyrosine kinases. The binding of VEGF ligand to the receptor results in phosphorylation of critical residues on the cytoplasmic portion of these receptors that in turn leads to activation of downstream signaling events. These downstream events are thought to include signaling through both the Raf-Mek-Erk and the phosphatidylinositol-3 kinase-AKT-mTOR pathways. The activation of these pathways in turn leads to endothelial cell activation, proliferation, migration, and survival.\textsuperscript{77-81}

At this point it is important to reflect back on the state of therapy for advanced renal cell carcinoma at the beginning of this century. For localized disease, the management has always and continues to be based predominantly on surgical excision. However, up to 30% of patients will present with advanced or metastatic disease which is generally chemoresistant.\textsuperscript{82-85} A variety of different chemotherapy regimens have been used in the past, the most extensively studied being fluorouridine and 5-fluorouracil (5-FU). When looked at as a group, these have produced response rates in general of only 13%.\textsuperscript{84,85}

As a result of these poor results, for several decades the systemic therapy for advanced RCC relied on the use of biologic therapy such as immunotherapy. The predominant forms of immunotherapy included interferon α (IFNα) and interleukin-2 (IL-2).\textsuperscript{86} These produced response rates on the order of only 10%-20%, only a minority of which were durable complete responses. For the remaining patients with a partial response the majority were only temporary. Attempts to use a combination of both IFNα and IL-2 did not demonstrate any significant advantage over either therapy alone.\textsuperscript{86} As a consequence, the 5-year survival of metastatic RCC was between 0% to 23%.\textsuperscript{82,87} These results essentially meant that 80%-90% of patients with advanced RCC did not respond to any appreciable degree to the therapy then available. The situation was clearly discouraging and cried out for new and innovative approaches to therapy.

**Moving From Bench to Bedside**

It is in this context that the discovery of the VHL gene and its critical role in regulating HIF and the cellular response to hypoxia informed the search for new therapeutic targets for advanced ccRCC. Given the connection between aberrations in VHL protein function, leading to accumulation of HIF, and subsequent up-regulation of VEGF, it was logical to explore the use of an inhibitor of VEGF as a novel approach to treating ccRCC.

**Targeting VEGF**

One approach to inhibiting VEGF is through the use of an antibody against the VEGF ligand. To date, the
The median time to progression in the high-dose arm compared with the placebo arm (4.8 vs 2.5 months, *P* < .001). On the basis of these promising results, 2 phase 3 prospective trials were initiated and have already completed enrollment comparing bevacizumab to IFNα as first-line therapy. Although accrual is completed for both these trials the final results are currently not available.

It is noteworthy that although the response rate for bevacizumab was modest at only 10%, this nevertheless translated into a significant difference in TTP. This type of result is typical for agents that modulate or affect specific downstream targets of molecules important in a specific disease, so-called targeted therapy, such as with bevacizumab and the other agents discussed further below. These approaches do not produce the typical responses seen with more traditional, cytotoxic chemotherapy. As a result, the best way to assess the response to targeted therapeutics is through endpoints such as TTP or, better yet, patient survival. This is a critical point, because had the initial investigators involved in these various agents not appreciated this subtlety, much of the progress that has been made in ccRCC management may have been lost. In many respects, the goal for targeted therapies is not so much the eradication of all visible disease but rather to slow or halt its further progress. This is a recurrent theme in virtually all targeted therapies that are currently in use for a variety of malignancies, including ccRCC.

**Targeting Tyrosine Kinases**

An alternative approach to interfering with signaling events downstream of VHL/HIF is to block the function of the receptor rather than blocking the ligand itself. Because the receptors for VEGF, PDGF, TNFα, and others all depend at least in part on tyrosine kinase activity for their normal signaling function, 1 strategy is to block tyrosine kinase activity. This should then block the subsequent downstream signaling from the receptor. On the basis of the biology of VHL and ccRCC, early attempts focused on inhibitors relatively specific for the VEGF receptors’ tyrosine kinase activity. The results were generally disappointing and these agents have not been actively pursued. However, it soon became apparent that the use of less specific tyrosine kinase inhibitors that affected several different tyrosine kinases may be more effective. This is presumably due to the ability of such drugs to block downstream signaling from more than 1 ligand at once with just a single drug. This conceptual framework has resulted in the development, testing, and now approval of several agents in this drug class recently shown in large-scale randomized trials to effectively treat advanced RCC. There is a rapidly expanding pool of potentially active drugs of this type (eq. AG-013736, GW572016, PTK787/ZK222584) but for the purposes of this article we will focus on 2 with large-scale, published phase 3 trials confirming their clinical utility and which are now both approved for use in metastatic ccRCC (for more in-depth reviews of the other agents see references 81,92,93).

Sorafenib is an orally bioavailable, multitargeted tyrosine kinase inhibitor originally developed as a specific inhibitor of RAF1, a member of the Raf/MEK/ERK pathway that lies downstream of receptors important in the VHL/HIF axis such as VEGFR and PDGFR. It was subsequently found to also block the downstream signaling of a variety of additional tyrosine kinases, including VEGFR, PDGFR, as well as others. As mentioned previously, the use of targeted agents as systemic therapy had raised concerns that traditional endpoints and measures of response may not be appropriate for these types of agents. As a result, the initial phase 2 studies with sorafenib used a randomized, discontinuation trial design to enrich the study for patients who may derive the most benefit from the study.

In this phase 2 study, 202 patients were enrolled who represented a mix of patients with no prior systemic therapy and in whom the majority (though not all) had ccRCC histology. All patients at the outset received 400 mg of sorafenib by mouth twice per day for 12 weeks, and then response was assessed. The 73 patients who had ≥25% tumor shrinkage were continued on sorafenib, whereas the 64 patients with >25% tumor growth were considered to have progressive disease and taken off study. This left 65 patients with stable disease who were then randomized to either continue sorafenib or switch to placebo. The primary endpoint was progression-free survival (PFS), which was found to be significantly higher in the sorafenib arm compared with the placebo arm (24 weeks vs 6 weeks).
This trial was important for 2 reasons. It confirmed the potential clinical utility of sorafenib in RCC that lead to a larger-scale phase 3 trial as noted below. It also demonstrated the validity of using the randomized discontinuation phase 2 trial design in testing compounds whose benefit is likely to be the stabilization of disease and prevention of disease progression as opposed to an absolute response in the more traditional sense used for cytotoxic chemotherapeutic agents.

The promising results of the phase 2 trial in turn lead to a large-scale, multicenter, international, randomized, prospective trial that enrolled 903 patients with ccRCC who had failed at least 1 prior systemic therapy. Patients were randomized to either oral sorafenib, 400 mg twice per day, or placebo. Overall survival was the primary endpoint of the trial. In January 2005, a planned interim analysis was undertaken that demonstrated a significant improvement in median (PFS) in the sorafenib arm compared with the placebo arm (5.5 months vs 2.8 months). On the basis of this analysis, the data safety monitoring committee made the decision to unblind the study and allow crossover to sorafenib for all the study participants. In May 2005, an analysis of overall survival revealed that sorafenib was associated with a lower risk of death compared with placebo (hazard ratio [HR], 0.72; 95% confidence interval [CI], 0.54-0.94; P = .02), although, technically, the effect was not statistically significant. Partial response rates were generally low in both arms (10% in the sorafenib arm, 2% in the placebo arm). In the sorafenib arm, rates were higher for toxicities, which included diarrhea, rash, fatigue, and hand-foot skin reactions. Hypertension and cardiac ischemia were the 2 rare, but potentially serious, side effects noted in the sorafenib arm. Based upon these results, sorafenib is now FDA approved for the management of advanced RCC.

Sunitinib is also a multitargeted tyrosine kinase inhibitor that is orally bioavailable. It has been shown to block the downstream signaling from several receptors important in RCC, such as VEGFR (types 1, 2, & 3) as well as PDGFR. Recently, the promising results of a phase 1 study using this agent were confirmed in 2 phase 2 studies in patients who had failed prior systemic cytokine therapy followed by a large, prospective, randomized phase 3 trial in patients who had not received prior systemic therapy. The first phase 2 study enrolled 63 patients with progressive RCC after failed cytokine-based therapy. Patients received oral sunitinib in repeated 6-week cycles, 50 mg per day for 4 weeks followed by a 2-week rest period. The primary endpoint of the study was objective response rate. A partial response was demonstrated in 40% of patients, while another 27% had stable disease for at least 3 months. The median TTP was 8.7 months. Toxicity was generally mild to moderate and manageable. A second phase 2 trial with a similar design followed shortly after this and the results were equally encouraging. This trial enrolled 106 patients with advanced RCC, again focused on those with progressive disease after failed cytokine therapy. The same treatment regimen was used. The partial response rate among patients in this trial was 34% while the median TTP was 8.3 months.

These very promising results lead to a large-scale, international, multicenter, prospective, randomized, phase 3 trial that enrolled 750 patients with ccRCC who had not received prior systemic therapy. Patients were randomized to receive either sunitinib at the same dosing regimen as the phase 2 studies or IFNα. The primary endpoint of the trial was PFS. The objective response rate (all of which were partial responses) for sunitinib was 31%, which was significantly better than the 6% for patients who received IFNα. The overall median PFS was significantly better in patients who received sunitinib (11 months) compared with 5 months for those who were randomized to IFNα. This was also true when analyzed across all risk substrata of patients. Toxicity was generally mild to moderate and manageable. There was more fatigue among patients treated with IFNα, while those treated with sunitinib experienced more diarrhea. Sunitinib was discontinued secondary to an adverse event in under 10% of patients. For overall survival, the data were not mature at the time of publication; however, a trend in favor of sunitinib was noted. It is noteworthy that when the initial analyses were confirmed, the study then allowed crossover to the sunitinib arm, which may mask any ultimate overall survival benefit. These results strongly support the contention that in the first-line setting, oral sunitinib is substantially better than IFNα for advanced RCC. On the basis of these results, it is FDA approved for use in advanced RCC along with sorafenib.

**Targeting mTOR**

Because aberrations of VHL that result in abnormally high levels of HIFα are important in ccRCC, another strategy in interrupting this cascade is to lower the starting levels of HIFα. One of the important regulators of HIFα expression and subsequent protein levels in the cell is the AKT/mTOR pathway. To date, there are several agents that have been studied that inhibit this pathway and act to decrease HIFα, including rapamycin and everolimus (RAD-001,
Novartis AG; see references 104-106). However, the agent that has received the greatest attention and study in advanced RCC is temsirolimus (CCI-779, Wyeth). Temsirolimus is a water-soluble ester of sirolimus which is able to inhibit mTOR’s kinase activity, leading to cell cycle arrest. Phase 2 trials have been conducted evaluating its efficacy both in combination with IFNα and as a single agent in cytokine refractory advanced RCC. Only modest response rates were observed in these trials; however, the PFS rates appeared to be substantially longer than in historical controls suggesting that the agent had substantial activity. These studies, therefore, prompted a large-scale, prospective, randomized, phase 3 trial that enrolled more than 600 patients with high-risk metastatic RCC (based on the Motzer criteria) in a 3-arm trial. Patients were randomized to receive temsirolimus alone, IFNα alone, or the combination of both agents. The results demonstrated that temsirolimus as monotherapy improved not only PFS, but also improved overall survival compared with either IFNα or combination therapy. The toxicity noted in the temsirolimus monotherapy arm was manageable. On the basis of these results, temsirolimus has now joined both sunitinib and sorafenib as approved agents for use in advanced RCC.

Conclusions
The discovery of the VHL gene has, therefore, taken a discouraging and desperate situation in which there were essentially only 2 agents routinely used in the management of metastatic ccRCC (high-dose IL-2 and IFNα) and opened the door to an expanding menu of therapeutic options. We have gone from a frustrating predicament in which >80% of patients did not respond to any of the available agents to one in which we have a menu of proven agents that can arrest the progression of disease and substantially improve overall survival, even in high-risk metastatic ccRCC patients. Despite these remarkable therapeutic discoveries and encouraging clinical results, the overwhelming majority of patients with metastatic RCC will still die to their disease. Thus, although these successes are encouraging and provide hope for our patients with ccRCC, we cannot afford to be complacent. This sobering reminder should serve as a stimulus for continued scientific discovery as we continue to expand our understanding of the tumor biology and subsequent development of more effective targeted therapies.

The intense study of the VHL/HIF signaling axis continues with the expectation that new agents will follow from the 3 newly approved compounds. The cloning of VHL and subsequent elucidation of its central role in ccRCC has opened up a brand new door into the inner workings of this malignancy and is now rapidly expanding the options for patients with this difficult disease. The trail that was blazed by pioneering researchers, dating back more than 30 years now, is an instructive tale on the value and necessity of studying the basic mechanisms underlying the biology of malignant tumors.

The recent progress in managing advanced RCC over the last several years can be directly traced to the early, groundbreaking work that led to the cloning of the gene. This represents, therefore, a gratifying example of taking discoveries at the bench, and turning them into new therapeutics at the bedside. In addition, it must be kept in mind that it took many years and multiple investigators to first clone the VHL gene over 10 years ago. In the last decade, molecular biology and cloning techniques have advanced so rapidly that what previously took years to accomplish now takes only months, or sometimes even weeks. The pace of discovery is accelerating faster and faster (see Fig. 2). It is hoped that, in so...
doing, more of the discoveries at the laboratory bench will become bedside therapies for many people around the world who suffer every day from the ravages of advanced malignancies.

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