Renal Cell Carcinoma: New Developments in Molecular Biology and Potential for Targeted Therapies

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. List the most frequent genetic abnormalities involved in RCC and explain how they lead to abnormal response to hypoxia, cell survival, and angiogenesis.

2. Interpret the current literature concerning the treatment of RCC, and correlate therapeutic agents with their targets and underlying biological processes that drive the disease.

3. Identify the limitations of current agents used in the treatment of RCC and the challenges that need to be overcome in developing therapies to improve the outcome of patients with advanced disease.

ABSTRACT

Renal cell carcinoma (RCC) affects 38,000 individuals in the U.S. yearly. Seventy-five percent of cases are clear-cell carcinomas, and a majority is driven by dysfunction of the von Hippel-Lindau (VHL) gene. VHL loss of function and other non-VHL pathways leading to RCC share aberrant activation of the hypoxic response, such as upregulation of vascular endothelial growth factor (VEGF) and consequent neoangiogenesis. Metastatic RCC has been notoriously resistant to therapy. For decades, its treatment has been based on nephrectomy and limited use of toxic and often inefficient immunotherapy with interleukin-2 or interferon-α. However, new biologic agents are beginning to break the resistance barrier. Small-molecule multikinase inhibitors that target VEGF receptors (sunitinib and sorafenib) have a favorable toxicity profile and can prolong time to progression and preserve quality of life when used in newly diagnosed or previously treated patients. The anti-VEGF antibody bevacizumab enhances the response rate and prolongs disease control when added to interferon-α. Temsirolimus, a mammalian target of rapamycin inhibitor, prolongs the survival duration of patients with poor-risk disease. Despite three new drugs being approved for RCC in the past 2 years, responses are mostly partial and of limited du-
Pathogenesis of Renal Cell Carcinoma: Disordered Responses to Hypoxia as a Common Mechanism

Renal cell carcinoma (RCC) comprises 5% of epithelial cancers with ~38,000 new cases diagnosed in the U.S. this year [1]. The incidence of RCC has been steadily rising over the past 30 years [2]. The majority (75%) of cases are clear-cell RCC. These are characteristically associated with loss of function of the von Hippel-Lindau (VHL) gene, resembling the molecular mechanism of VHL syndrome, and a constitutively activated hypoxic response resulting from upregulation of the hypoxia inducible factor (HIF) α subunits HIF-1α and HIF-2α [3]. Most of what we know about the pathogenesis of clear-cell RCC involves VHL and the pathways it regulates. Relatively little is known about RCCs with functional VHL.

HIF activation results in upregulation of HIF target genes, notably those encoding vascular endothelial growth factor (VEGF), transforming growth factor (TGF)-α, Met, stromal cell–derived factor (SDF)-1 (chemokine C-X-C motif ligand 12 [CXCL12]), and chemokine receptor CXCR4, among others [4, 5]. Depending on the series, VHL mutations occur in approximately 40%–60% of RCCs, and epigenetic alterations occur in another 10%–20% [6]. In addition, VHL affects fibronectin deposition [7], integrin maturation [8], downregulation of protein kinase C signaling [9], cilia formation [10], and protection of p53 [11]. Thus, while RCC development almost certainly involves multiple factors, the deregulation of HIF, or HIF target genes, is a striking common feature.

Binding of HIF-1/2α to VHL is regulated by proline hydroxylation in a reaction involving 2-oxoglutarate, ferrous iron, and prolyl hydroxylase [12]. In cells with wild-type VHL, hypoxia leads to HIF-α stabilization through inhibition of prolyl hydroxylase [13], which requires mitochondrial-derived reactive oxygen species (ROS) [14, 15]. Another pathway of HIF accumulation and RCC development involves Krebs cycle enzyme mutations: succinate dehydrogenase (SDH)-B mutations [16] cause clear-cell RCC, while mutations of fumarate hydratase [17] cause type II papillary cancer. In SDH mutations, accumulation of succinate directly inhibits proline hydroxylase and HIF-α levels rise (ROS may also contribute because SDH is a component of the electron transport chain complex II).

Mutations of MET, a HIF target gene, cause type I papillary renal cancer [18]. In addition, clear-cell RCC with upregulation of HIF-1/2α occurs as a result of mutations in the tuberous sclerosis (TSC1/2) complex [19]. In this case, HIF deregulation occurs at the level of protein translation initiation. Accordingly, TSC2−/− cells demonstrate exaggerated HIF responses, which can be reversed by treatment with the mammalian target of rapamycin (mTOR) inhibitor rapamycin. Although by itself HIF deregulation is probably insufficient to generate tumors [20], HIF continues to play an important role in the tumorigenicity of established RCCs [21]. Disruption of translocation in renal carcinoma, chromosome 8 gene (TRC8) by a constitutional 3;8 translocation results in hereditary clear-cell RCC and nonmedullary thyroid cancer [22, 23]. Two independent RCC families with 3;8 translocations and disruption of the TRC8 gene have now been described [24]. At least in part, Trc8 inhibits growth by affecting the transcription factors sterol regulatory element binding protein (SREBP)-1 and 2, which regulate endogenous cholesterol and lipid biosynthesis [25]. In yeast, the SREBP system functions as an oxygen sensor and is essential for survival in severe hypoxia [26, 27].

Treatment of Advanced RCC

Metastatic RCC has been notoriously resistant to conventional chemotherapy and is almost invariably an incurable condition. However, new biologic agents are beginning to break the resistance barrier, reflected in the approval of three innovative agents for treatment of advanced RCC in the last 2 years. Here, we summarize the results of chemotherapy and immunotherapy in metastatic RCC, review the new generation of RCC drugs, and discuss investigational compounds that may be part of the RCC armamentarium in the future. Characteristics of the main RCC agents are summarized in Table 1.

Nephrectomy

Contrary to what has been the paradigm in many other solid tumors, nephrectomy is often performed in RCC even with evidence of metastatic disease. Nephrectomy contributes to symptom control by preventing pain and urinary bleeding from the diseased kidney, but also seems to have an impact on survival. In a combined analysis of two randomized studies comparing nephrectomy and interferon (IFN)-α...
therapy with IFN-α alone in patients with metastatic RCC, the former group had a longer survival time (13.6 versus 7.8 months) [28].

**Conventional Chemotherapy**

Many conventional cytotoxic agents have been tried in metastatic RCC with poor results (average response rate of 6%) [29]. Newer agents, including gemcitabine, irinotecan, oxaliplatin, paclitaxel, carboplatin, and ixabepilone, are being tested alone, or in combination, in phase II trials. Recently, the combination of capecitabine and gemcitabine produced a 15.8% partial response (PR) rate in 21 patients with metastatic RCC, although no complete responses (CRs) were observed [30]. On the other hand, the combination of doxo-

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**Table 1. Summary of toxicity and efficacy of RCC agents**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Toxicity</th>
<th>Efficacy</th>
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<tbody>
<tr>
<td><strong>Approved</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High-dose</td>
<td>Cytokine; activates T lymphocytes</td>
<td>Hypotension, pulmonary edema, fever, rash, infections, death</td>
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<tr>
<td>interleukin 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorafenib</td>
<td>Small molecule; inhibits B-Raf, VEGFR-2, VEGFR-3, PDGF-β</td>
<td>Skin rash, diarrhea, hypertension</td>
</tr>
<tr>
<td>Sunitinib</td>
<td>Small molecule; inhibits VEGFR-2, PDGF-β, Src, FGFR-1</td>
<td>Thrombocytopenia, leukopenia, skin discoloration, diarrhea, hypertension</td>
</tr>
<tr>
<td>Temsirolimus</td>
<td>Ester of sirolimus; inhibits mTOR kinase activity</td>
<td>Skin rash, stomatitis</td>
</tr>
<tr>
<td><strong>Active</strong></td>
<td></td>
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<tr>
<td>Interferon-α</td>
<td>Cytokine</td>
<td>Fever, chills, thrombocytopenia, leukopenia, mood changes</td>
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<tr>
<td>Bevacizumab</td>
<td>VEGF targeted monoclonal antibody</td>
<td>Hypertension, proteinuria, bleeding, thrombosis</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>Small molecule; inhibits VEGFR-1 to 3, PDGF-α/β</td>
<td>Elevation of liver enzymes, hypertension, diarrhea</td>
</tr>
<tr>
<td>Everolimus</td>
<td>Inhibits mTOR kinase activity</td>
<td>Skin rash</td>
</tr>
<tr>
<td>Axitinib</td>
<td>Small molecule; inhibits VEGFR-1 to 3, PDGFR-β</td>
<td>Hypertension, stomatitis</td>
</tr>
<tr>
<td>Capecitabine plus gemcitabine</td>
<td>Conventional cytotoxic agents</td>
<td>Diarrhea, thrombocytopenia, leukopenia</td>
</tr>
<tr>
<td>Pazopanib</td>
<td>Conventional cytotoxic agents</td>
<td>Leukopenia, thrombocytopenia</td>
</tr>
<tr>
<td><strong>Possible activity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sirolimus</td>
<td>mTOR inhibitor</td>
<td>Skin rash, stomatitis, pneumonitis</td>
</tr>
<tr>
<td>Vorinostat</td>
<td>Histone deacetylase inhibitor</td>
<td>Diarrhea, fatigue</td>
</tr>
<tr>
<td>Lapatinib</td>
<td>Small molecule; dual EGFR/ErbB-2 inhibitor</td>
<td>Skin rash, diarrhea</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>Proteosome inhibitor</td>
<td>Thrombocytopenia, peripheral neuropathy</td>
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Abbreviations: DFS, disease-free survival; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; IFN, interferon; mTOR, mammalian target of rapamycin; NA, not available; OS, overall survival; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PFS, progression-free survival; RCC, renal cell carcinoma; RR, response rate; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.
rubricin and gemcitabine led to an 11% CR rate and a 28% PR rate in a cohort of 18 patients with either sarcomatoid (56%) or rapidly progressing (44%) RCC [31]. Although there is no established role for conventional chemotherapy in the management of advanced RCC, combined chemotherapy may have a role in unusual histologies and in patients who have progressive disease on now accepted standard therapies (i.e., sunitinib, sorafenib, temsirolimus) and should be further studied in clinical trials.

Immunotherapy

RCC is one of the malignant diseases for which the triggering of presumed immunologic mechanisms by treatment with interleukin (IL)-2, IFN-α, or hematopoietic stem cell transplantation (to induce a graft-versus-tumor response) has repeatedly produced modest, yet beneficial, results.

In the early 1980s, the observation of spontaneous remissions in RCC [32] led to a search for therapeutic agents with potential to improve the immunologic response against RCC tumor cells. This led to IL-2, a molecule produced by type 1 helper T lymphocytes with the ability to cause activation and proliferation of CD4 and CD8 T lymphocytes. Early trials used in vitro stimulation of T cells with IL-2 to produce lymphokine-activated killer (LAK) cells that were coadministered with high-dose IL-2. However, it was later recognized that the therapeutic effect resided predominantly with high-dose IL-2, and the use of LAK cells was abandoned [33]. Although toxic, the response to high-dose IL-2 in metastatic RCC can sometimes be spectacular with long-lasting CRs. However, overall responses are achievable in only about 20% of patients, and complete long-lasting responses occur in only about 5% [34, 35].

The importance of dose intensity of IL-2 for patients with metastatic RCC was clarified in a National Institutes of Health trial that randomized patients to receive high-dose IL-2 (156 patients) or a dose that was 10 times lower (150 patients). There was a significantly higher response rate with high-dose IL-2 than with low-dose i.v. IL-2 (21% versus 13%), but no overall survival difference, and a higher morbidity, as anticipated [36]. This was confirmed in a multi-institutional phase III trial involving 192 patients with metastatic RCC randomized to receive i.v. high-dose IL-2 or low-dose s.c. IL-2 plus IFN-α. The response rate was significantly greater in patients treated with high-dose IL-2 (23.2% versus 9.9%). While there was no significant difference in overall survival (17 versus 13 months), 7% of patients were reported alive and disease free after 3 years of follow-up in the high-dose IL-2 arm versus none in the control arm. As expected, there were more grade 3 and 4 toxicities in the high-dose IL-2 arm, although treatment-related mortality was rare [35]. We conclude that high-dose IL-2 is an acceptable therapy for patients with little or no comorbidities and excellent performance status, for whom the possibility of long-term CR is worth the complexity, risk, and acute toxicity of the treatment. How to best sequence or combine IL-2 with newer drugs is unknown.

IFN-α is produced by macrophages and lymphocytes and induces several biological effects including immunomodulation, antiproliferation, and enhanced expression of cell surface antigens. In phase II studies, recombinant IFN-α was reported to induce response in RCC in up to 29% of cases [37]. However, in contrast to IL-2, IFN-α has no curative potential, and CRs are rare and of short duration. In a randomized trial comparing IFN-α with medroxyprogesterone acetate, IFN-α treatment was associated with a longer survival time, although the benefit was minimal (median survival time, 8.5 versus 6 months), and patients treated with IFN-α had more symptoms and a worse quality of life [38]. Despite being broadly used in practice and often adopted as the control arm in comparative trials with new drugs, IFN-α has never been approved in the U.S. for treatment of RCC.

Stem Cell Transplantation

There have been attempts to achieve graft-versus-tumor effects using nonmyeloablative, allogeneic stem cell transplantation. In an initial report of 19 patients, three obtained a CR and seven obtained a PR. Two patients, however, died of transplant-associated events [39]. Subsequent reports failed to show similar benefit [40]. Considering the complexity and treatment-associated toxicity, allogeneic stem cell transplantation remains an experimental approach for RCC and can only be recommended in the context of a clinical trial.

Anti-VEGF Antibodies

At least one half of clear-cell RCCs have upregulation of HIF-1α and HIF target genes including VEGF [41]. Bevacizumab (recombinant human monoclonal antibody VEGF; Avastin®; Genentech, Inc., South San Francisco, CA) is a humanized recombinant anti-VEGF antibody that binds all types of VEGF-A isoforms (with an affinity of approximately 1.8 nM) and neutralizes their activities [42].

Bevacizumab was tested in phase I trials in patients with advanced malignant diseases. Among eight RCCs, minor tumor shrinkage was noted in two cases, and stable disease for at least 2 months was observed in five others [43, 44]. In a phase II trial of 116 patients with metastatic clear-cell RCC randomized to placebo or low-dose (3 mg/kg) or high-dose (10 mg/kg) bevacizumab given every 2 weeks, there was a 10% objective PR rate, which was confined to the
high-dose arm. Compared with placebo, there was also a significantly longer time to disease progression (4.8 versus 2.5 months) [45].

In an attempt to improve on its efficacy in RCC, bevacizumab was combined with the small-molecule epidermal growth factor receptor (EGFR) inhibitor erlotinib. Although an early trial with 60 patients reported a 25% objective response rate [46], a subsequent randomized phase II trial of previously untreated patients failed to demonstrate superiority of the combination over bevacizumab alone [47].

Most recently, a phase III trial involving 641 patients with metastatic clear-cell RCC compared IFN-α combined with either bevacizumab or placebo. When compared with placebo, bevacizumab resulted in a significantly longer progression-free survival (PFS) time (10.2 versus 5.4 months) and higher objective tumor response rate (30.6% versus 12.4%). In an interim analysis, there was no significant survival advantage. Common toxicities seen in this and previous trials were hypertension, proteinuria, and a tendency to bleeding and thrombotic events [48].

**Multikinase Inhibitors**

Small-molecule kinase inhibitors that have more than one target (multikinase inhibitors) are generating considerable excitement in the treatment of metastatic RCC. Although these agents have potent activities against specific kinases, the true biologic targets responsible for tumor regression are not precisely known. Sequencing of the human genome has identified 518 putative kinases, of which 90 are tyrosine kinases and 58 are receptor tyrosine kinases [49]. Moreover, the in vivo targets are likely to include kinases present in both the tumor cells and stroma (e.g., endothelial cells). Because deregulation of HIF is an important aspect of RCC development, agents that affect HIF target genes, especially those encoding VEGF and VEGF receptors (VEGFRs), may be particularly useful. Two such VEGFR inhibitors, sorafenib and sunitinib malate, demonstrated sufficient activity to receive regulatory approval for their use in RCC in the U.S. and Europe.

**Sorafenib**

Sorafenib (Nexavar®, Bayer Pharmaceuticals Corporation, West Haven, CT) was initially found in a screen for agents to block the Raf proteins C-Raf and B-Raf (in vitro 50% inhibitory concentration [IC50], 2–22 nM). Further examination demonstrated that this compound also blocked other kinases, including VEGFR-2 and VEGFR-3 (IC50, 10–90 nM), platelet-derived growth factor receptor β (PDGF-β; IC50, 28 nM), as well as the receptors for Flt-3 ligand (Flt-3; IC50, 45 nM) and stem cell factor (c-Kit; IC30, 50 nM) [50, 51]. Enthusiasm for the compound was initially the result of, in part, the high frequency of B-raf mutations in melanoma (~70%) and papillary thyroid cancer (33%). Signaling through many receptors involves activation of Ras, recruitment of Raf to the membrane, and subsequent activation of the mitogen-activated protein kinase (MAPK)–extracellular signal–related kinase (ERK) pathway, which affects a large number of downstream cellular responses. In vitro studies have demonstrated that sorafenib is a potent inhibitor of ERK phosphorylation in many, but not all, cancer cell lines [51]. In particular, cell lines with activating mutations of KRAS appear nonresponsive. However, in vivo growth of these resistant cell lines could still be inhibited by sorafenib, suggesting an antiangiogenic effect.

The effect of sorafenib in RCC was suggested in phase I trials [52] and confirmed in a phase II, randomized discontinuation trial including 202 patients with previously untreated metastatic RCC. Thirty-six percent of patients had tumor shrinkage >25% after 12 weeks. Patients with stable disease randomized to receive sorafenib had a median disease-free survival interval of 24 weeks, significantly superior to the 6 weeks seen in patients randomized to placebo [53].

The role of sorafenib in RCC was solidified in the Treatment Approaches in Renal Cancer Global Evaluation Trial (TARGET) study, in which 903 patients with previously treated metastatic clear-cell RCC of low or intermediate risk, according to the Memorial Sloan-Kettering Cancer Center (MSKCC) classification [54], were randomized to receive sorafenib or placebo. The median PFS times were 5.5 months in the sorafenib group and 2.8 months in the placebo group, and the objective response rates were 10% in the sorafenib arm and 2% in the placebo arm. Although the difference in survival favoring sorafenib was not statistically significant, it may have been because of early cross-over allowed shortly after an interim analysis showed a difference in PFS. That trial also served to further characterize the side-effect profile of sorafenib. Discontinuation of treatment because of side effects occurred in 10% of patients receiving sorafenib, and 13% required dose reductions because of toxicity. The most important side effects were diarrhea (43%), hypertension (17%), skin rash (40%), and hand–foot syndrome (30%) [55].

With the observed benefit of sorafenib in patients refractory to first-line therapy (mostly immunotherapy) from the TARGET trial, sorafenib was compared with IFN-α in untreated patients in a randomized phase II trial. Contrary to what was expected, the objective response rate was only 5% in patients receiving sorafenib, with no advantage over IFN-α in terms of response rate or PFS duration (5.7 versus 5.6 months) [56]. However, from the large sorafenib open-
access program, there were 224 previously untreated patients for whom the PFS duration was 35 weeks [57].

The potential to expand the benefit of sorafenib therapy resides in dose escalation and combination with other agents. In a phase II trial with 46 patients, intrapatient dose escalation (up to 1,600 mg/day) had acceptable toxicity and induced objective responses in 52% of patients [58]. Sorafenib was combined with IFN-α in 58 previously untreated patients and was associated with a response rate of 19%. Interestingly, most of the toxicities seen with the combination were attributable to IFN-α [59].

Sunitinib
Sunitinib malate (Sutent®; Pfizer, Inc., New York) is also an oral multikinase inhibitor that blocks the activity of VEGFR-2 (IC50, 9 nM) and PDGFR-β (IC50, 8 nM), as well as Src, Abl, insulin-like growth factor receptor-1 and fibroblast growth factor receptor-1 tyrosine kinases [60]. Its effect on RCC became evident with the results of two multi-institutional phase II trials enrolling patients previously treated predominantly with immunotherapy. The first study, including 63 patients with various histologies (although the vast majority had clear-cell carcinoma) of RCC, demonstrated a 40% objective response rate and median time to progression (TTP) of 8.7 months [61]. The second study required prior nephrectomy and included 106 previously treated patients whose tumors were predominantly of the clear-cell type, with a response rate of 34% and median disease-free survival interval of 8.3 months [62].

Despite the broad overlap of targeted kinases between sunitinib and sorafenib, it became evident in these two trials that sunitinib has a distinct profile of side effects, predominantly leukopenia, thrombocytopenia, stomatitis, and transient skin discoloration, with skin rash and diarrhea being less frequent.

The remarkable activity of sunitinib seen in patients with refractory disease justified a large multi-institutional phase III trial randomizing 750 previously untreated patients with RCC with a clear-cell component to receive sunitinib or IFN-α. A higher objective responses rate was seen in the sunitinib arm (31% versus 6%), as was a longer PFS time (11 versus 5 months) and better quality of life. Interestingly, the benefit of sunitinib was demonstrated in all MSKCC risk groups. At the time of the last report, 13% of the patients had died in the sunitinib arm versus 17% in the IFN-α arm; this was not significant in this interim analysis [63]. As a result, sunitinib has emerged as the predominant first-line treatment for metastatic RCC, irrespective of risk category.

Despite the positive impact of the multikinase inhibitors on PFS and quality of life, disease progression eventually occurs. With two approved multikinase inhibitors, it is important to determine the crossresistance between these agents. So far it appears that crossresistance is not complete and objective responses and meaningful disease stabilization are seen when the second multikinase inhibitor (sorafenib or sunitinib) is employed. Absolute resistance to both agents is, in fact, uncommon, and was seen in only 7% of patients in one report [64].

Pazopanib
Pazopanib (GW786034) has a broad spectrum of kinase inhibition including all VEGFRs, PDGFR-α/β, and c-Kit. Pazopanib was tested in an international phase II randomized discontinuation trial in patients with metastatic RCC who had never been treated, or had failed one line of therapy not including a multikinase inhibitor. In an interim analysis, 40% of the 60 patients enrolled had PRs, and an additional 42% of patients had stable disease at 12 weeks. The most common toxicities were liver enzyme elevations, hypertension, and diarrhea. The high rate of disease control led to discontinuation of the placebo randomization, and the study is ongoing as a single-arm trial [65].

Axitinib
Axitinib (AG-013736) is a small molecule that potently inhibits all known forms of VEGFR, PDGFR-β, and c-Kit, with an acceptable side-effect profile consisting predominantly of hypertension and stomatitis [66]. The activity of axitinib in RCC was demonstrated in a phase II trial of 52 cytokine-refractory, nephrectomized patients that obtained an objective response rate of 46%. Axitinib was also recently tested in a cohort of patients with advanced RCC who had progressed on sorafenib; six of 42 had an objective response and 15 of 42 had disease stabilization [67].

Long-Term VEGFR-2 Inhibition
Although the multikinase inhibitors that include VEGFR-2 have clear activity in RCC, and are relatively well tolerated, their toxicity on a long-term basis is unknown. This will become increasingly important as the treatment of RCC improves. Thyroid dysfunction has been reported for both sorafenib [68] and sunitinib [69], and reduced left ventricular ejection fractions have been seen with sunitinib [63]. Interestingly, endothelial cell–specific knockout of VEGF in the mouse was shown to cause cell death, especially following hypoxic stress. In addition, cardiac and bone marrow toxicities were prominent. Development of the circulatory system was surprisingly normal and VEGF levels in the peripheral blood were unaffected [70]. This provocative study indicates that endothelial cells are dependent on autocrine VEGF signaling, and adds further support to the
concept that receptors can be activated by internal ligand binding (intacrine signaling) as part of a normal homeostatic mechanism. Small molecule VEGFR-2 inhibitors have the potential to mimic this VEGF knockout model.

**mTOR Inhibitors**

All mRNAs are not created equal; those with long structured 5′-untranslated regions, often associated with growth and cell-cycle regulatory genes (e.g., those encoding c-Myc, cyclin D1), are poorly translated into protein unless stimulated by growth factor signaling [71]. Akt, MAPK, and mTOR are critical components in this regulation. In at least one form of hereditary RCC, resulting from mutations in the TSC1/2 complex, the mTOR pathway is constitutively activated [72]. Mutations in the tumor suppressor gene phosphatase and tensin homologue deleted on chromosome ten (PTEN), which occur in approximately 5% of RCCs and are associated with advanced-stage aggressive disease, also activate mTOR [73]. As discussed above, VHL loss-of-function mutations lead to HIF and VEGF accumulation. In addition to proteasome-mediated destruction, HIF is also regulated at the level of protein translation initiation, which is controlled by mTOR, thus reinforcing the strong rationale for mTOR inhibitors in RCC.

**Sirolimus**

Sirolimus (Rapamycin, Rapamune®; Wyeth Pharmaceuticals, Inc., Madison, NJ), an immunosuppressant macrolide produced by Streptomyces hygroscopicus, binds FK506 binding protein (FKBP-12) in a molecular complex that involves the subunit regulatory associated protein of TOR (RAPTOR), and specifically inhibits mTOR kinase activity [74]. Inhibition of mTOR by rapamycin leads to downregulation of cyclin–cyclin-dependent kinase (CDK) complexes and p27 (Kip1) accumulation, which blocks cell-cycle progression in late G1/S [75, 76]. In addition, sirolimus is thought to inhibit proliferation of endothelial and vascular smooth muscle cells required for tumor angiogenesis [77]. Despite its excellent bioavailability, favorable side-effect profile, and potent mTOR inhibition at readily achievable levels, sirolimus has not been broadly explored as a RCC agent, and only a few phase II trials exploring sirolimus combinations are currently active.

**Temsirolimus**

Temsirolimus (Torisel®; Wyeth Pharmaceuticals, Inc., Madison, NJ) is a water-soluble ester of sirolimus amenable to i.v. infusion. When administered in a weekly schedule, the plasma concentration of temsirolimus falls to subnanomolar levels in 3–4 days, while sirolimus remains at therapeutic levels, which likely accounts for some, if not all, of the antitumor activity. Major side effects associated with temsirolimus are rash and stomatitis [78].

In a phase II study [79], 111 patients were treated with varying doses of temsirolimus. The response rate was 7% (including one CR), median time to tumor progression was 5.8 months, and median survival time was 15 months. Interestingly, no dose–response effect was observed, and the lowest dose was chosen for a subsequent phase III trial. A large, international trial randomized 626 previously untreated RCC patients with poor prognostic features (the majority were high risk in the MSKCC classification) to receive treatment with IFN-α, temsirolimus, or the combination, which used a lower dose of temsirolimus. Although there were no significant differences in response rates (i.e., 4.8%, 8.6%, and 8.1%, respectively), temsirolimus alone, but not the combination, was superior to single-agent IFN-α in terms of the PFS (5.5 versus 3.1 months) and overall survival (10.9 versus 7.3 months) times. Temsirolimus was also the safest of the three treatments, having the lowest rates of grade 3 and 4 toxicities [80]. That trial led to the recent approval of temsirolimus for the treatment of advanced RCC in the U.S. Although its benefit was demonstrated in patients with poor prognostic features, its regulatory approval was not restricted to this subgroup of patients.

**Everolimus**

Everolimus (RAD001), an oral mTOR inhibitor, has been tested in RCC patients in phase II trials in which an objective response was seen in 12 of 37 patients, of whom 31 were previously treated. An additional 19 patients had stable disease for >3 months. The toxicity profile seen in that trial was similar to what has been seen with other mTOR inhibitors [81].

**EGFR Kinase Inhibitors**

EGFR belongs to the ErbB family of tyrosine kinase receptors, which includes EGFR itself (ErbB-1/EGFR/HER-1), ErbB-2 (HER-2/neu), ErbB-3 (HER-3), and ErbB-4 (HER-4) [82, 83]. Overexpression of EGFR in RCC is broadly recognized, and EGFR signaling is mitogenic for malignant and normal renal tubular cells [84–87]. TGF-α, an EGFR ligand under transcriptional control by HIF, is constitutively expressed in VHL mutant cells [88]. Downstream consequences of ErbB signaling include activation of the Ras/Raf/MAPK and phosphatidylinositol 3-OH kinase/Akt pathways, as well as Janus kinase/signal transducer and activator of transcription signaling, which mediate a host of cell responses including cell growth and proliferation.

Small-molecule inhibitors of EGFR compete with ATP
binding and block the kinase activity of the receptor. Two of these inhibitors have been tested in patients with metastatic RCC, gefitinib (Iressa®; AstraZeneca Pharmaceuticals, Wilmington, DE) and erlotinib (Tarceva®; Genentech, Inc., South San Francisco, CA). In a phase II study of 21 patients treated with 500 mg gefitinib daily in a 28-day cycle, stable disease was reported in eight (38%), all of whom had previously progressive disease [89]. To our knowledge, no studies with single-agent erlotinib in RCC have been performed. However, as mentioned, erlotinib did not add to the antitumor effect of bevacizumab despite strong biologic rationale [47].

Recently, lapatinib (Tykerb®; GlaxoSmithKline, Philadelphia), a dual EGFR/ErbB-2 kinase inhibitor, was tested in a randomized phase III trial involving 417 patients with EGFR-expressing advanced RCC (of any histology) who received either lapatinib or hormone therapy. The results were essentially negative with no significant difference in TTP or overall survival. However, a subgroup analysis of patients whose tumors strongly expressed EGFR had a longer TTP (15.1 weeks versus 10.9 weeks) and survival time (46.0 weeks versus 37.9 weeks) in the lapatinib arm [90].

Proteosome Inhibitors
The proteosome is a 26S barrel-shaped structure with internally located proteases. Entrance of proteins into the 20S core is sterically inhibited in the absence of a 19S regulatory particle, which consists of a multisubunit proteasome lid and base. Proteins destined for degradation are targeted by specific phosphorylations and subsequently recognized by E3-ubiquitin ligase complexes that add polyubiquitin chains. The polyubiquitylated proteins are bound by the proteasome lid and threaded into the 20S core where they are hydrolyzed. Bortezomib (Velcade®; Millennium Pharmaceuticals, Inc., Cambridge, MA) is a potent, reversible inhibitor of this proteolytic activity.

While proteosome inhibition might be expected to be nonspecific, certain systems appear particularly vulnerable, such as the transcription factor nuclear factor (NF)κB, which is sequestered in the cytoplasm by the inhibitor protein IκB. Because IκB is degraded by the 26S proteosome, proteosome inhibition leads to retention of inactive NFκB in the cytoplasm. Constitutive NFκB activation has been observed in RCC cell lines [91, 92]. Bortezomib is able to induce apoptosis in RCC cell lines with high constitutive NFκB activity in a dose-dependent fashion. Furthermore, the presence a wild-type VHL gene appears to sensitize RCCs to the antitumor effects of bortezomib [93]. Two independent phase II trials of bortezomib have been published, with response rates of 11% and 4%, but a higher proportion of disease stabilization. As expected, neuropathy was the most significant toxicity seen [94, 95].

Histone Deacetylase Inhibitors
Interactions between DNA and histones dictate the degree of DNA condensation. Histone deacetylation makes DNA more compact and prevents access of transcription factors stopping gene expression. In fact, many transcription regulators act in part by recruiting histone deacetylases (HDACs). HDAC inhibitors lead to the upregulation of the CDK inhibitor p21Waf1/Cip1 [96], indirect downregulation of cyclin E and cyclin D1 [97], and transcription-independent upregulation of p53 [98], leading to growth arrest and apoptosis. Vorinostat (suberoylanilide hydroxamic acid [SAHA], Zolinza®; Merck & Co., Inc., Whitehouse Station, NJ) is a broad-spectrum HDAC inhibitor being currently tested, alone and in combination, in the treatment of advanced RCC.

CURRENT QUESTIONS
How Should Trials with Biological Agents Be Performed?
Inevitably, phase II trials with limited accrual are the first and necessary steps to demonstrate clinical efficacy of a new anticancer drug. In the absence of a comparative group, efficacy is mainly judged on response rates following Response Evaluation Criteria in Solid Tumors [99]. The use of such criteria in evaluating responses for the new “targeted” agents has been criticized, because they miss the potentially relevant minimal tumor shrinkage and prolonged disease stabilization often seen with these agents. This has been particularly important in RCC, for which “targeted” agents have become mainstream treatment. A frequently adopted alternative trial design involves randomized discontinuation, intended to identify whether disease stabilization can be attributed to the study treatment. Although such a design often provides valuable information, it has the inconvenience of requiring accrual of a larger number of patients than a typical phase II trial. Moreover, many patients refuse inclusion for not accepting the possibility of having to discontinue an active treatment.

A possible evolution in trial design for biological agents may come with the development of new assessment systems (imaging and biomarkers) for disease activity, moving away from strict size parameters.

How Can Cure Rates Be Improved?
Several new drugs have been approved recently for the treatment of advanced RCC, and many other compounds are undergoing preclinical or early clinical experimentation.
(Table 1). However, except for some patients treated with high-dose IL-2, current treatments have improved quality of life, delayed progression, and extended survival, but have not increased the cure rate for RCC.

One approach has been to bring the advances obtained in the metastatic setting earlier in the course of disease following the paradigm established in breast, colorectal, and lung cancer, where the administration of active agents shortly after primary tumor resection in high-risk patients prevents relapse and saves lives. In this regard, a large ongoing intergroup trial (Adjuvant Sorafenib or Sunitinib for Unfavorable Renal Carcinoma) involves the randomization of high-risk patients who have undergone nephrectomy to receive placebo, sorafenib, or sunitinib for 1 year. With an accrual goal of >1,300 patients, this trial will begin to address this highly relevant question. Two other international adjuvant studies, the STAR (sunitinib trial in advanced renal cancer) and the SORCE (a phase III randomized, double-blind study comparing sorafenib with placebo in patients with resected renal cell carcinoma at high or intermediate risk of relapse) trials, will address the benefit of adjuvant sunitinib and sorafenib, respectively. Another innovative approach currently being tested is the use of neo-adjuvant therapy to increase the resectability of large primary tumors.

How Can Results with Available Agents Be Improved?

In almost every example in oncology, curative chemotherapy consists of a combination of two or more agents having different mechanisms of action and minimally overlapping toxicities. Therefore, improvements in the treatment of advanced RCC are likely to originate from combination of existing drugs, as well as the development of new compounds. In general, targeted agents have fewer side effects than traditional chemotherapeutic agents, which in principle should make them more amenable to combination. Concomitant use of more than one compound may allow interruption of an important cancer pathway at more than one point (e.g., simultaneous blockage of VEGF ligand and receptor) or the synchronous inhibition of a separate but cross-communicating system (e.g., inhibition of growth factor receptors and mTOR). We believe this approach holds more promise than the sequential use of monotherapy because crossresistance between agents is expected and all the RCC agents have very limited durations of benefit. Some combinations currently being tested are bevacizumab plus sunitinib, sorafenib plus everolimus, sirolimus plus erlotinib, and vorinostat plus bevacizumab, among others.

How Do We Choose Among the Current Agents?

Metastatic RCC is a relatively uncommon malignant disease with a dismal outcome, and improvements will be accomplished only by a concerted effort to include patients in clinical trials that are rational and well designed. Ideally, to be most effective, future trials should provide biologic information on why they succeeded or, alternatively, why they failed.

**REFERENCES**

15. Schroedl C, McClintock DS, Budinger GR et al. Hypoxic but anoxic...


Developments in RCC Biology and Targeted Therapy


