Recent advances in molecularly targeted therapy in advanced renal cell carcinoma

In the last few years, enhanced understanding of the biology of clear cell renal cell carcinoma has led to the development of molecular signaling inhibitors, which, with their superior anti-tumor activity demonstrated in randomized clinical trials, have led to a paradigm shift in the treatment of advanced renal cell carcinoma from cytokine-based therapy to signaling-inhibitor therapy. Relevant therapeutic signaling pathways that have emerged are VEGF receptor and mammalian target of rapamycin pathways, and signaling inhibitors sunitinib, sorafenib, temsirolimus and, most recently, everolimus are approved by the FDA for treatment of advanced renal cell carcinoma in the USA. Newer agents with promising anti-tumor activity are in continued development, as are efforts to uncover additional therapeutic targets. Currently, the optimal use of available agents, such as sequence and combination strategies, as well as their role in the adjuvant and neoadjuvant setting, remain unclear and investigative efforts are underway.

KEYWORDS: mammalian target of rapamycin ▪ monoclonal antibody ▪ renal cell carcinoma ▪ targeted therapy ▪ tyrosine kinase inhibitor ▪ VEGF receptor ▪ von Hippel Lindau

Jenny J Kim & Brian I Rini†

†Author for correspondence: Cleveland Clinic Foundation, 9500 Euclid Avenue/Desk R35, Cleveland, OH 44195, USA
Tel.: +1 216 444 9567
Fax: +1 216 444 9464
rinib2@ccf.org

Historical perspective

Renal cell carcinoma (RCC) comprises approximately 2–3% of all malignancies in the USA with an estimated incidence of 39,000 cases and 13,000 deaths in 2008 [1]. RCC is comprised of several different histologic subtypes, with clear cell being the most common, comprising approximately 80% of all RCC. Papillary, chromophobe, oncocytic, collecting-duct, medullary and unclassified tumors together comprise the remaining 20% of RCC. Incidence of RCC has steadily increased over the last 50 years at a rate that is threefold higher than that of RCC mortality, mostly owing to increased use of noninvasive imaging studies that lead to incidental detection of early, organ-confined disease amenable to definitive surgical treatment [3–4]. A slower but steady increase has also been seen in the incidence of metastatic disease, although clear etiology remains uncertain [3].

Historically, advanced RCC has been one of the most difficult malignancies to treat for the medical oncologist owing to its resistance to conventional cytotoxic chemotherapy. For several decades, the treatment of choice was immunotherapy, which showed modest benefit in a small, select group of patients. More recently, however, the elucidation of the role of the von Hippel-Lindau (*VHL*) tumor suppressor gene and its biallelic inactivation leading to upregulation of growth factors associated with tumor cell proliferation and angiogenesis have not only provided new insights into clear cell RCC biology but also identification of molecular targets for novel therapeutic strategies [4–9]. Relevant targets that emerged were the VEGF receptor (VEGFR) pathway and the mammalian target of rapamycin (mTOR) pathway for which monoclonal antibodies and/or small molecule inhibitors have been developed, with several new agents with promising efficacy currently on the horizon. With the superior anti-tumor activity of these targeted agents compared with immunotherapy confirmed in randomized clinical trial settings, targeted therapy has moved to the forefront of advanced RCC treatment. Along with continued development of newer agents, additional randomized trials are needed to establish the optimal use of the existing agents in treatment of advanced RCC.

VHL & hypoxia-inducible factor-1 expression: targeting the VEGF pathway

Hypoxia-inducible factor (HIF)-1 regulates oxygen homeostasis in virtually all bodily tissues [10–13]. HIF-1 is a heterodimer comprised of an α and a β subunit, which upon dimerization enters the nucleus and acts as a transcription factor for a wide variety of genes implicated in processes such as cell proliferation, neovascularization and extracellular matrix formation [14–15]. HIF-1β subunit is constitutively synthesized whereas production of HIF-1α is induced

part of future medicine fsg

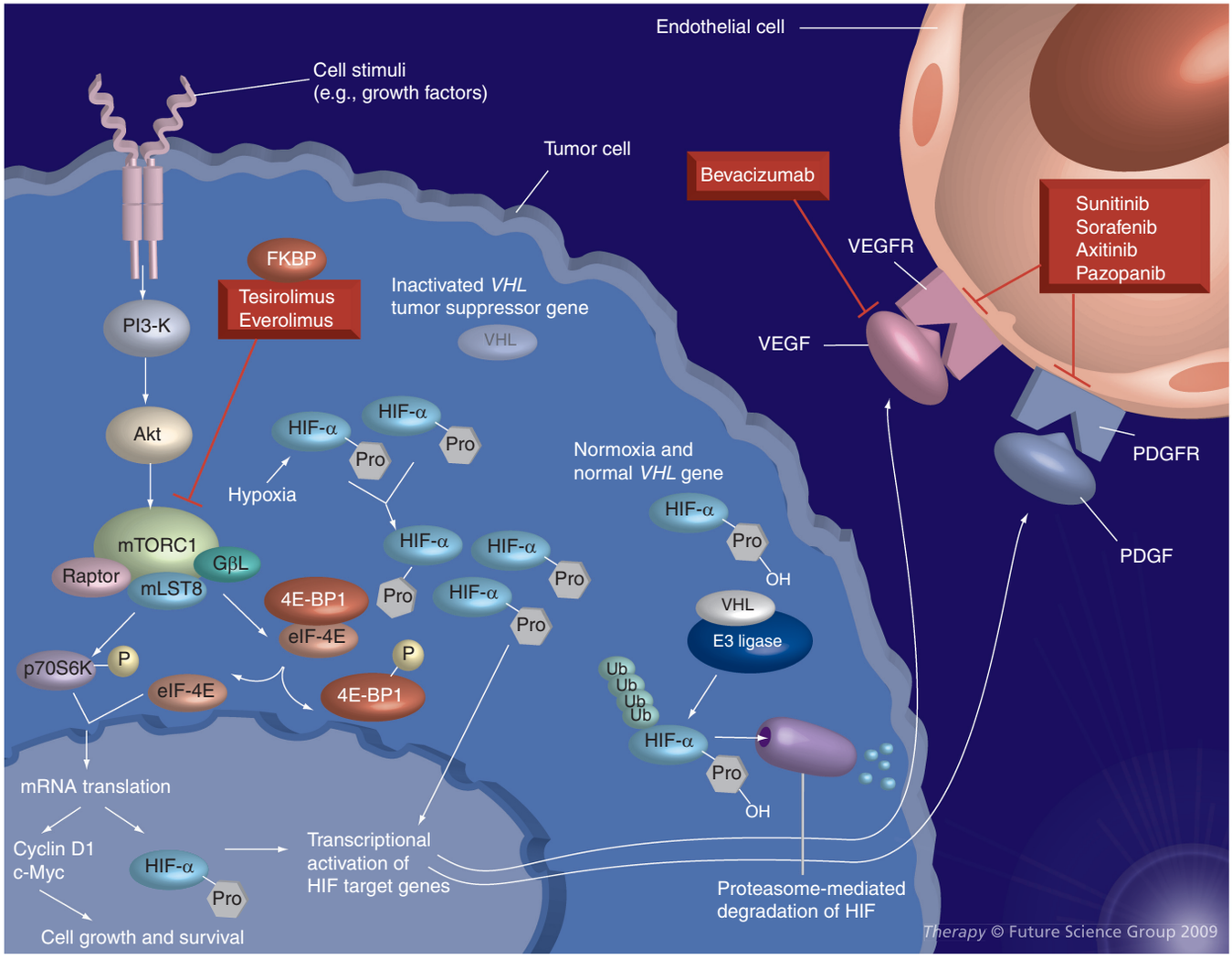

Figure 1. Biologic pathways and therapeutic targets in renal cell carcinoma. Under nomal conditions, VHL protein (pVHL) binds to the E3 ubiquitin ligase complex that targets HIF-α for proteolysis. During cellular hypoxia and/or VHL inactivation, pVHL–HIF interaction is disrupted, leading to stabilization/accumulation of HIF protein that ultimately acts as a transcription factor for hypoxia-inducible genes upon entering the nucleus. Hypoxia-inducible genes include *VEGF* and *PDGF*, which, upon binding their respective receptors on endothelial cells, promote cell migration, proliferation and permeability. HIF accumulation can also result from activation of mTOR, a downstream molecule of the PI3-K/Akt pathway. mTOR phosphorylates and activates p70S6K, leading to enhanced translation of certain proteins including HIF. Activated mTOR also phosphorylates 4E-BP1, which ultimately leads to activation of cell-cycle regulators such as c-myc and cyclin D1. Sites of action of targeted agents are illustrated. Temsirolimus and everolimus bind to FKBP, and the resultant protein–drug complex inhibits the kinase activity of the mTORC1. Bevacizumab is a VEGF antibody. Sunitinib, sorafenib, axitinib and pazopanib are small-molecule inhibitors of multiple tyrosine kinase receptors including VEGFR and PDGFR.
FKBP: FK506 binding protein; HIF: Hypoxia-inducible factor; mTORC1: mTOR complex 1; VHL: von Hippel-Lindau.

by a state of hypoxia [14]. Under normal circumstances, the level of HIF-1α is tightly controlled by the *VHL* gene located on chromosome 3 and its protein product, pVHL. *VHL* is a tumor suppressor gene and its protein product is a component of E3 ubiquitin–protein ligase complex responsible for ubiquitination and subsequent proteosome-mediated degradation of HIF-1α [10,12]. When the *VHL* gene is silenced by deletion, methylation or mutation, HIF-1α is not degraded and the resulting dimerization of HIF-1α with HIF-1β leads to transcription of several factors important in tumor progression, including VEGF, PDGF, βFGF, erythropoietin and TGF-α (Figure 1).

VEGF mediates its effect by binding to VEGF receptor (VEGFR, a receptor tyrosine kinase) on the endothelial cell surface to promote angiogenesis. High levels of VEGF along with microvessel density have been shown in patients with clear cell RCC, which has subsequently led to development of novel therapeutic agents targeting VEGF and VEGFR [6]. Among them are neutralizing antibodies of VEGF ligand (bevacizumab) or small

molecule inhibitor of tyrosine kinase portion of VEGFR (sunitinib, sorafenib). Newer VEGFR inhibitors such as axitinib and pazopanib have shown promising efficacy in Phase II trials and are currently in Phase III development.

Sunitinib

Sunitinib malate (Sutent®, Pfizer, NY, USA) is a small molecule inhibitor of tyrosine kinase portions of VEGFR 1,2 and 3 and PDGFR-a, -b, FLT-3, c-KIT and RET that was shown to have dose-dependent antiangiogenic and antiproliferative effects in preclinical models [16–17]. A Phase I study established the recommended Phase II dose of 50 mg/day for 4 weeks followed by 2 weeks off therapy [18,19]. In 2006, final results of two sequential, single-arm, multicenter Phase II studies were reported, both of which further validated anti-tumor activity of sunitinib in metastatic RCC with partial responses seen in approximately 40% of patients, stable disease for 3 months or more in an additional 27–29% of patients and median time to progression of 8.3–8.7 months [20–21]. The results of these Phase II studies led to the accelerated approval of sunitinib by the US FDA in January 2006 for the treatment of advanced RCC.

Based on this success in treatment of advanced RCC, a large multicenter Phase III trial was designed to evaluate the efficacy of sunitinib in the frontline setting. A total of 750 patients were randomized to receive either IFN-α or sunitinib (50 mg 4 weeks on therapy/2 weeks off therapy schedule). The final analysis reported by ASCO 2008 demonstrated an objective response rate of 39% in the sunitinib cohort (95% CI: 34–44) as compared with 8% in the IFN-α group (95% CI: 6–12); ($p < 0.000001$) by independent review [22]. The median progression-free survival (PFS) was 11 months in the sunitinib group versus 5 months in the IFN-α group with a hazard ratio (HR) of 0.42 (95% CI: 0.32–0.54; $p < 0.000001$). Overall survival (OS) was 26.4 versus 21.8 months for the sunitinib cohort versus IFN-α, respectively, with a HR of 0.82 (95% CI: 0.673–1.001; $p < 0.051$), which was not statistically significant, probably owing to allowance of treatment crossover. When crossover patients were censored, however, a statistically significant OS of 26.4 versus 20 months were seen with a HR of 0.808 (95% CI: 0.661–0.987; $p = 0.0362$). Furthermore, OS analysis of patients who did not have additional treatment after either sunitinib or IFN was 28.1 versus 14.1 months, respectively, suggesting superior efficacy of sunitinib as compared with IFN-α. These data established sunitinib as a robust frontline standard of care in advanced RCC.

The treatment-related toxicity profile differed between the two groups. Fatigue, pyrexia, chills and influenza-like symptoms were predominantly seen in the IFN-treated cohort and diarrhea, nausea, stomatitis, hypothyroidism and hand–foot syndrome were seen in the sunitinib cohort. Grade 3 decline in left ventricular ejection fraction was similarly seen in both groups (2 and 1% in sunitinib and IFN group, respectively) with reversal upon dose modification or discontinuation in the sunitinib group. Overall, patients in the sunitinib group reported a significantly better quality of life than did patients in the IFN-α group ($p < 0.001$). It is unclear whether improved quality of life in sunitinib-treated patients is the result of better control of tumor burden or more favorable treatment-related toxicity with sunitinib, although it is likely that a combination of both factors are in effect. Further analysis to clarify this issue is being pursued by the authors at this time [23].

Another more recently reported side effect of intrigue is the development of macrocytosis in patients treated with sunitinib [24]. Macrocytosis was completely reversible upon sunitinib withdrawl. The mechanism is unclear at this time, although c-KIT inhibition may partly contribute to it, drawing from the reported experience of development of macrocytosis in patients treated with imatinib for gastrointestinal stromal tumors. With chronicity of treatment needed with targeted agents, further studies on long-term effects of sunitinib and other VEGFR tyrosine kinase inhibitors (TKIs) on the bone marrow would be worthwhile.

Sorafenib

Sorafenib (Nexavar®, Bayer Healthcare Leverkusen, Germany) is an oral inhibitor of Raf kinase, which is a downstream effector molecule of RAS [25,26]. Upon activation by RAS, Raf kinase has been implicated in modulation of gene expression and ultimately cell proliferation via the Raf/MEK/ERK pathway and sorafenib was initially developed as a promising anticancer agent based on its inhibitory effect on Raf kinase [27]. Further characterization of sorafenib, however, demonstrated its dual inhibitory effect against VEGFR-2,-3, PDGFRb, B-Raf, Flt-3 and c-KIT [27] and its activity against tumor angiogenesis was seen in human xenograft models [28,29]. Phase I studies revealed overall tolerability of sorafenib with

establishment of recommended dose of 400 mg twice daily in continuous fashion [30–33]. Based on preclinical studies that showed that the primary effect of sorafenib was inhibition of tumor growth and therefore disease stabilization rather than tumor shrinkage, a Phase II placebo-controlled, randomized discontinuation trial of sorafenib in cytokine-refractory advanced RCC soon followed [34]. Of the 202 patients involved in the trial, 73 patients had tumor shrinkage of 25% or more. A total of 65 patients with stable disease (SD) at 12 weeks were assigned to sorafenib or placebo at the time of randomization. At 24 weeks, 50% of the sorafenib-treated patients were progression free versus 18% of the placebo-treated patients ($p = 0.0077$). Median PFS from randomization was 24 weeks for the sorafenib group versus 6 weeks in the placebo group ($p = 0.0087$). Sorafenib was administered in patients whose disease progressed on placebo and these patients continued sorafenib until further progression for a median of 24 weeks.

With significant disease-stabilizing activity seen in the innovative Phase II randomized discontinuation trial, a Phase III placebo-controlled trial involving 903 cytokine-refractory patients with metastatic RCC, the largest trial involving RCC to date, was conducted and showed statistically significant PFS improvement of approximately 3 months and OS improvement of approximately 4 months when censored for crossover patients [35,36]. Sorafenib was generally well tolerated by most patients, with mainly grade 1 and 2 toxicities that included hypertension, fatigue, gastrointestinal, dermatologic and neurologic symptoms. The side-effect profile was similar to that of sunitinib, including diarrhea, rash, fatigue and hand–foot skin reactions. Based on the results of these studies, sorafenib was approved by the FDA in December 2005 for the treatment of advanced RCC.

However, sorafenib did not meet with similar success in the frontline setting. A smaller Phase II trial was conducted in which 189 previously untreated patients were randomized to receive either sorafenib 400 mg twice daily or IFN-α. Median PFS was comparable between two groups with 5.7 months (95% CI: 5.0–7.4) in the sorafenib cohort versus 5.6 months (95% CI: 3.7–7.4) in the IFN-α cohort [37]. The reason for the lack of significant effect is unclear at this time, although it may be due to weaker inhibition of VEGFR compared with sunitinib [27]. An interesting recent finding with regard to the above Phase II outcome was the recent dose-escalation study of sorafenib in which a response rate of 52% was seen in patients treated with higher doses of sorafenib up to 1600 mg/day [38], suggesting the possibility of a dose increase of sorafenib for maximal benefit. The results of the dose escalation study are intriguing but require further investigation.

Bevacizumab

Bevacizumab (Avastin®, Genentech, CA, USA) is a recombinant human monoclonal antibody directed against all biologically active isoforms of circulating VEGF-A protein [39]. Neutralization of VEGF inhibits its binding to VEGFR, thereby inhibiting tumor angiogenesis [40]. Bevacizumab was the first targeted agent to demonstrate efficacy in metastatic RCC in a Phase II trial in which 116 previously treated patients with metastatic RCC were randomized to placebo, low-dose (3 mg/kg every 2 weeks) or high-dose (10 mg/kg every 2 weeks) bevacizumab. The majority of toxic effects were comprised of hypertension and asymptomatic proteinuria in the high-dose bevacizumab therapy group, which were reversible upon cessation of therapy. Significant prolongation of time to progression was seen with time-to-progression of 4.8 versus 2.5 months in the high-dose bevacizumab group as compared with the placebo group (HR: 2.55; $p < 0.001$), although with a modest objective response rate of 10% [41]. In an attempt to improve its anti-tumor activity, bevacizumab was further evaluated in combination with IFN in a large multicenter study comparing IFN plus placebo versus IFN plus bevacizumab (at 10 mg/kg every 2 weeks) [42]. A significant advantage for the bevacimab-containing arm was demonstrated with objective response rate of 31 versus 13% ($p < 0.0001$), and PFS of 10.2 versus 5.4 months ($p < 0.001$). A second multicenter Phase III trial was conducted through the CALGB, nearly identical in design with the exception of lacking a placebo infusion and not requiring prior nephrectomy [43]. Results were similar, with objective response rate of 25.5 versus 13.1% ($p < 0.0001$) in the bevacizumab-containing arm versus the IFN-alone arm. The most common grade 3 toxicity was fatigue and asthenia. Based on the results of these studies, bevacizumab in combination with IFN has been approved for frontline therapy for metastatic RCC in Europe and approval is pending in the USA. Both trials demonstrated more side effects for the combination arm and, thus, the benefits must be balanced against increased toxicity.

Targeting the mTOR pathway

Mammalian target of rapamycin is a 289 kDa serine/threonine kinase, a downstream molecule of the phosphatidylinositol 3-kinase/Akt activation pathway [44]. Biologic consequences of mTOR activation include production of HIF-1α and HIF-2α. HIF-1α, as discussed previously, is a factor implicated in angiogenesis via transcription induction of growth factors including VEGF [45,46]. mTOR activation also promotes production of cell cycle regulators such as c-myc, cyclin D1 and ornithine decarboxylase, which subsequently lead to tumor cell growth, survival and proliferation [47].

Temsirolimus

Temsirolimus (Torisel®, Wyeth Pharmaceuticals, NJ, USA) is an inhibitor of mTOR. It demonstrated anti-tumor effects across a wide variety of tumor types in preclinical models, especially in those with defective PTEN [48]. Defective PTEN expression results in Akt phosphorylation and activation of downstream molecules including mTOR. A Phase II trial in metastatic RCC randomly assigned 111 patients with treatment-refractory metastatic RCC to receive one of multiple doses of temsirolimus (25, 75 and 250 mg intravenously, weekly). Objective response rate was 7% with an additional 26% of patients showing minor responses [49]. Retrospective assignment of risk criteria showed that patients with three or more poor-risk features demonstrated OS of 8.2 months compared with 4.9 months [49,50].

Based on the Phase II study, which showed significant benefit in poor-risk patients, a multicenter Phase III trial that randomized 626 poor-risk (three or more adverse risk features according to previously established guidelines) [50,51], treatment-naive patients with metastatic RCC to receive 25 mg of temsirolimus weekly, IFN 18MU thrice weekly or combination therapy with 15 mg temsirolimus weekly, plus 6MU IFN-α thrice weekly. Objective response rate in the temsirolimus group was 9% and there was a 49% improvement in OS in the temsirolimus-treated group compared with the IFN-alone group (10.9 vs 7.3 months; $p < 0.0069$) [52]. There was also a benefit in PFS in the temsirolimus group compared with the IFN-alone group (3.8 vs 1.9 months; $p < 0.0001$). No significant survival difference was seen between the combination and IFN-alone groups, possibly owing to the lower dose of temsirolimus administered in the combination group due to toxicity. The most frequent adverse events were rash (76%), mucositis (70%), asthenia (50%) and nausea (43%), which were not shown to be dose dependent. This study not only confirmed the efficacy of temsirolimus in treatment of poor-risk metastatic RCC patients but also established the mTOR pathway as a promising therapeutic target in the treatment of metastatic RCC. The results of the above study led to the approval of temsirolimus by the FDA for treatment of advanced RCC in May 2007. A Phase III trial comparing temsirolimus with sorafenib in the second-line setting is underway in the USA.

Everolimus

Everolimus (RAD 001) is an oral form of serine-threonine kinase inhibitor of mTOR. The efficacy of everolimus in metastatic RCC was first observed in a Phase II trial involving 41 patients at a dose of 10 mg daily. A total of 12 patients had a partial response (PR), 19 patients had SD for more than 3 months and median OS was more than 11.5 months [53]. The most common side effects included mucositis, anemia and asthenia. Everolimus was also studied in a Phase III randomized, placebo-controlled trial involving 410 patients with sunitinib- and/or sorafenib-refractory mRCC [54]. The results demonstrated statistically significant improvement in PFS of 4.0 versus 1.9 months (HR: 0.3; 95% CI: 0.22–0.40; $p < 0.001$), illustrating the efficacy of mTOR inhibitor in VEGFR TKI refractory patients. Everolimus was recently approved by the US FDA in March of 2009 for the treatment of advanced RCC after sunitinib or sorafenib failure.

Newer agents in development

Axitinib

Axitinib (AG-013736) is an oral receptor TKI of VEGFR1, 2 and 3. It has demonstrated efficacy in cytokine-refractory metastatic RCC patients in a single-arm, Phase II study with an objective response rate of 44.2% (95% CI: 30.5–58.7), PFS of 15.7 months and median OS of 29.9 months [55]. Treatment-related side effects were similar to that of other VEGFR TKIs, including diarrhea, hypertension, fatigue, nausea and hoarseness, and were generally manageable with supportive care or dose modifications. Axitinib has also shown anti-tumor activity in VEGFR TKI-refractory patients in a small Phase II study, with 57% of patients experiencing some degree of tumor regression [56]. Based on the promising results of this Phase II study, axitinib is currently in Phase III development.

Pazopanib

Pazopanib (GW786034) is a selective multi-targeted receptor TKI of VEGFR-1,2,3, PDGFRα/β and c-KIT that has shown potent inhibition of tumor growth and angiogenesis in preclinical as well as clinical models. A Phase II trial involved 225 mRCC patients (67% of patients were treatment naive and 33% had failed either prior cytokine [23%]/bevacizumab [8%] therapy) who were randomized to receive either placebo or pazopanib at 800 mg orally daily for 12 weeks [57]. The final analysis recently reported a response rate of 27%, all of which were PR with the additional 46% of patients showing SD for more than 3 months [55]. The most common side effects were transaminitis, hypertension and diarrhea. During the trial, one patient had a grade 5 adverse event caused by intestinal perforation and and a total of 5% of patients were discontinued from therapy. Based on these results, a placebo-controlled Phase III study is underway.

Cediranib (AZD2171)

Cediranib (AZD2171) is an oral small molecule TKI that inhibits VEGFR, PDGFR, flt-4 and c-kit. Its anti-tumor efficacy has been demonstrated in both as single agent and in combination with gefitinib [58,59]. Several small Phase I and II trials demonstrated promising anti-tumor activity in metastatic RCC with a tumor control rate of up to 84% in patients with previously untreated advanced RCC [60]. A placebo-controlled Phase II trial is ongoing.

Volociximab

Volociximab (M200) is a chimeric monoclonal antibody of a5b1 integrin receptor found on activated endothelial cells, which, upon binding, inhibits angiogenesis by interfering with endothelial cell–cell and endothelial cell–matrix interaction, and was shown to be independent of growth factor stimulus [61]. Volociximab has demonstrated SD in 80% of patients in Phase II setting in treatment-refractory (cytokine and/or antiangiogenic) disease [61]. The most frequent side effects included fatigue in 67.5%, nausea in 35%, dyspnea in 20% and arthralgia in 17.5% of patients. No grade III/IV side effects were seen. Median time-to-progression was 4 months. Median OS has not been reached after 22 months. OS was 79 and 68% at 6 and 22 months, respectively. Based on these results, a randomized, controlled trial is being planned.

Future perspective

The management paradigm for advanced RCC has undergone a transformation within the last several years with the invention of signaling inhibitors that have met with unprecedented success (Table 1). Despite this success, however, the experience with these signaling inhibitors is limited, with many questions remaining to be answered to derive the maximal benefits from these agents. Namely, optimal sequences of these agents have not been established. Similarly, robust clinical and/or biologic predictors of response and resistance to these agents are not defined. Currently, despite the success of signaling inhibitors, treatment of metastatic RCC remains largely palliative, and thus a greater understanding of the risk–benefit ratio with regard to the timing and type of therapy applied to specific patients awaits further investigation. Also, the roles of signaling inhihibitors in the adjuavnt and neoadjuvant setting are yet to be defined, and prospective trials of sorafenib, sunitinib and bevacizumab in the adjuvant setting after resection of high-risk RCC (ECOG-E2805, SORCE, STAR trials) and in the neoadjuvant setting are currently ongoing Furthermore, attempts at combination therapy have met with modest success and it still remains to be defined which signaling pathways exert most influence in RCC progression and thus are the ideal targets for anti-tumor therapy. Development of newer and more potent agents, optimal use of currently available agents and discovery of additional targets for therapeutic consideration remain equally important in the pursuit of increasing complete response rates in a disease with ultimately such a poor outcome.

Sequential therapy

Several small studies have shown promising results of lack of cross-resistance among different agents and therefore are in support of sequential therapy [54,56,62–64]. Everolimus has shown anti-tumor activity in VEGFR TKI refractory setting and can be considered a standard of care in VEGFR TKI-refractory disease [54]. Furthermore, sunitinib has shown significant activity in patients previously treated with bevacizumab in a prospective Phase II trial, suggesting lack of cross-resistance between VEGF and VEGFR inhibitors [62]. Of the 61 patients enrolled in the study, 23% of patients achieved partial response, with an additional 57% demonstrating stable disease. Sorafenib also demonstrated significant anti-tumor activity in the setting of sunitinib or bevacizumab failure in the Phase II setting, with a preliminary analysis of

Table 1. Summary of selected agents and clinical trials in renal cell carcinoma.

Agent	Objective response rate (%)	Progression-free survival (months)		Comments	Ref.
		Tx naive	*Cytokine refractory*		
Sunitinib	30–45	11	8.4	Toxicity: fatigue, mucositis, HFS, diarrhea, HTN and/or hypothyroidism Phase III trial conducted largely in good- and intermediate-risk patients Highest objective response rate as single agent	[20–22]
Bevacizumab plus IFN	26–31	8.5–10.2	4.8	Phase III trial conducted largely in good and intermediate risk patients Enhanced toxicity compared with monotherapy, such as fatigue, asthenia, HTN and proteinuria	[42,43]
Bevacizumab	10–13	8.5	NA	Toxicity: fatigue, anorexia, HTN and/or proteinuria	[41]
Sorafenib	2–10	5.7	5.5	Toxicity: fatigue, mucositis, HFS, diarrhea and/or hypertension Phase III trial conducted largely in good and intermediate-risk patients Possible synergistic effect with IFN-α (ORR = 33%)	[34–37]
Temsirolimus	7–9	3.7	5.8	Toxicity: fatigue, mucositis, rash and/or hypertriglyceridemia/hyperglycemia/hyperlipidemia Phase III trial conducted in poor risk patients Efficacy irrespective of tumor histology (Phase III)	[49,52]
Everolimus	1	NA	4.0	Toxicity: stomatitis, rash and/or fatigue Phase III trial conducted in TKI-refractory patients First agent to demonstrate anti-tumor activity following VEGFR-TKI failure	[54]

HFS: Hand–foot syndrome; HTN: Hypertension; NA: Not available; ORR: Overall response rate; TKI: Tyrosine kinase inhibitor; Tx: Treatment; VEGFR: VEGF receptor.

34 patients (27 evaluable) demonstrating clinical activity as measured by tumor burden reduction in 33% of patients [63]. This finding not only suggests the possible sequential use of VEGF inhibitors and VEGFR TKIs, but also suggests the possible sequential use of various agents within the class of VEGFR TKIs as well, thereby further expanding the armamentarium against advanced RCC in individual patients.

Combination therapy

Two different concepts that engendered trials of combination targeted therapy for RCC are 'horizontal blockade' and 'vertical blockade' [64]. Horizontal blockade refers to simultaneous targeting of numerous signaling molecules downstream from HIF α – VEGFR, PDGF receptor and/or EGF receptor – in order to prevent cancer cell proliferation and promote apoptosis while ablating tumor-induced angiogenesis. By contrast, vertical blockade refers to targeting of multiple different signaling molecules at different levels in the same pathway, which, in theory, could overcome resistance that may develop through feedback mechanism.

Several trials have been conducted to evaluate different combinations of available agents with regard to anti-tumor efficacy and tolerability with several others currently in progress [65–72]. In general, combination therapy has shown significant anti-tumor activity, but was frequently poorly tolerated, leading to frequent dose reductions. A Phase I trial of bevacizumab plus sunitinib, while active, showed increasing grade III and IV toxicities with chronic treatment, leading to dose reductions and/or study discontinuation [65]. Another Phase I trial involving sorafenib and bevacizumab combination showed amplified toxicity of sorafenib, again leading to dose reductions [66].

Combination of a targeted agent with IFN also showed increased toxicity. A Phase III, three-arm trial comparing IFN versus temsirolimus versus IFN plus temsirolimus as described previously, showed that the combination arm was inferior to the single-agent temsirolimus group in objective response, PFS and OS [52]. The above results are likely due to the frequent dose reductions and treatment delays that were required in the combination cohort secondary to increased toxicity.

Some success has been met, nonetheless. Bevacizumab in combination with IFN has demonstrated improved response rate and PFS than with either agent alone as discussed in an earlier section [42–43]. Treatment-related toxicities were also enhanced as expected, but remained

manageable. Bevacizumab in combination with IFN is currently approved in the European Community for treatment of advanced RCC in the frontline setting and the same is expected in the USA in the near future.

An attractive combination is that of targeting the VEGF pathway together with the EGF pathway. TGF-α, a ligand for EGFR, had been shown to be elevated in RCC and to serve as a growth factor for RCC in preclinical models, which subsequently provided the rationale for a placebo-controlled Phase II study of bevacizumab with erlotinib versus bevacizumab plus placebo [74]. Combination therapy was tolerated at similar toxicity levels as bevacizumab-alone but did not result in improvement in objective response rate and PFS compared with the bevacizumab alone cohort. Nevertheless, targeting of the EGF pathway in combination with the VEGF pathway or mTOR pathway, with their different mechanism of action as compared with bevacizumab, remains an appealing possibility.

Whether combination regimens, in light of frequently necessary dose reductions due to increased toxicity, would ultimately be favorable compared with sequential single-agent therapy remains unclear. It still remains to be elucidated which pathways exert the most influence in RCC progression, which would provide crucial information in deriving the most effective combination therapy. However, careful evaluation of the toxicity profile will also be needed prior to clinical application. Several trials are ongoing to evaluate efficacy and tolerability of different combinations of agents to determine the optimal combination therapy with highest efficacy to toxicity ratio. For now, combination regimens should only be used in clinical trial settings.

Executive summary

Historical perspective

- Renal cell carcinoma (RCC) comprises approximately 2–3% of all malignancies in the USA with an estimated incidence of 39,000 cases and 13,000 deaths expected in 2008.
- 80% of all RCC is comprised of clear cell subtype.
- Elucidation of von Hippel-Lindau (VHL) biology has led to better understanding of clear cell RCC and subsequent development of signaling inhibitors in treatment advanced RCC.

VHL & HIF-1 expression: targeting the VEGF pathway

- VHL is a tumor suppressor gene and its biallelic inactivation leads to accumulation of HIF-1 which subsequently leads transcription induction of growth factors including VEGF, PDGF, βFGF, erythropoietin and TGF-α.
- Sunitinib is a VEGFR TKI which has shown superior overall response rate (31 vs 6%), progression-free survival (11 vs 5 months) and patient tolerability in a randomized Phase III trial compared with IFN and was established as a frontline treatment in advanced RCC.
- Side effects of sunitinib include hypertension, fatigue, diarrhea, nausea, stomatitis, hypothyroidism, hand–foot syndrome. Congestive heart failure and macrocytosis have been reported.
- Sorafenib is a tyrosine kinase inhibitor of VEGF receptor (VEGFR)-2,-3, PDGFRb, B-Raf, Flt-3 and c-KIT which has shown activity in cytokine refractory advanced RCC. Phase II trial in frontline setting, however, did not show improved PFS.
- Bevacizumab is a monoclonal antibody against all biologically active isoforms of circulating VEGF-A protein which, in combination with IFN, showed superior ORR, and PFS compared with IFN alone and has been approved for treatment in frontline setting in Europe.

Targeting the mTOR pathway

- Activation of mTOR leads to upregulation of HIF, c-myc, cyclin D1 and ornithine decarboxylase, which leads to angiogenesis, tumor growth, survival and proliferation.
- Temsirolimus is an mTOR inhibitor which has exhibited increase PFS compare to IFN in metastatic RCC patients with intermediate- and poor-risk features.
- Everolimus is an mTOR inhibitor that has shown activity in VEGFR TKI refractory patients.

Newer agents in development

- Promising newer agents are currently under development including axitinib, pazopanib, cediranib and voloxicimab.

Future perspective

- Experience with these signaling inhibitors is limited, with many questions remaining to be answered to derive the maximal benefits from these agents.
- Small studies show lack of complete cross resistance between agents which suggests possibility of sequential therapy.
- Different combination regimens are being tested and as expected, enhanced toxicity requires frequent dose reductions. Whether combination regimens will improve outcome as compared with single agent options are currently unclear.
- Effective treatments for advanced non clear cell RCC remain elusive and novel agents are currently under testing.
- Additional signaling inhibitors such as Ang-2/Tie-2 inhibitors (AMG386) and Akt inhibitors (perifosine) have exhibited single agent activity and are currently under development.
- With their success in metastatic setting, sunitinib, sorafenib and bevacizumab are currently under evaluation in adjuvant and neoadjuvant setting.

Agents for non-clear-cell renal cancers

With *VHL* inactivation leading to malignant phenotype applying exclusively to clear cell RCC, effective treatment for non-clear-cell RCC remains elusive. Several trials have evaluated available targeted agents in non-clear-cell histological subtypes of RCC and the results have been somewhat disappointing. Choueiri and colleagues retrospectively reviewed 53 patients with metastatic papillary RCC or chromophobe RCC treated with either sorafenib or sunitinib, which showed modest benefit, with overall response rate of 10%, PFS of 8.6 months and OS of 19.6 months [75]. Interestingly, a subset analysis of the Phase III study comparing temsirolimus, IFN and the combination of both was done to evaluate the outcome in non-clear-cell mRCC. This analysis demonstrated superior OS and PFS in non-clear-cell histology [76] – a finding that warrants further studies to clarify the clinical benefit of temsirolimus in non-clear-cell metastatic RCC. Prospective trials are in progress to evaluate the role of current signaling inhibitors in non-clear-cell RCC, as are efforts in developing other novel agents. XL880 is a cMET inhibitor and mutations of cMET have been associated with inherited and some sporadic papillary RCC. XL880 has shown promising anti-tumor efficacy in patients with papillary RCC in Phase I [77] setting and is currently in Phase II development [78].

Identification of new signaling pathways

Angiopoietin-2 is expressed in remodeling vasculature of human tumors but is limited in normal tissues and is a ligand for the Tie-2 receptor expressed on endothelial cells. Binding of Ang-2 to Tie-2 stimulates angiogenesis and a selective peptibody (AMG386) neutralizing their interaction has shown anti-tumor activity in murine xenograft models [79]. Furthermore, AMG386 has shown enhanced activity in combination with VEGFR inhibitors and trials involving combination therapy are currently underway.

Another target in the signaling cascade is the Akt molecule, an upstream molecule of the mTOR and protein kinase C activation pathway. Perifosine, a synthetic oral alkylphosphocholine, has been shown to inhibit Akt activity and demonstrated single-agent anti-tumor activity in mRCC [72,73]. Enrollment is complete for Phase II trial of combination therapy with TKIs.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Gupta K, Miller JD, Li JZ, Russell MW, Charbonneau C: Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. *Cancer Treat. Rev.* 34(3), 193–205 (2008).

2 Nguyen MM, Gill IS, Ellison LM: The evolving presentation of renal cell carcinoma in the United States: trends from the surveillance, epidemiology and end results program. *J. Urol.* 176(6), 2397–2400 (2006).

3 Chow WH, Devesa SS, Warren JL, Fraumeni JF Jr: Rising incidence of renal cell cancer in the United States. *JAMA* 281(17), 1628–1631 (1999).

4 Herman JG, Latif F, Weng Y *et al.*: Silencing of the *VHL* tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc. Natl Acad. Sci. USA* 91(21), 9700–9704 (1994).

5 Gallou C, Joly D, Mejean A *et al.*: Mutations of the VHL gene in sporadic renal cell carcinoma: definition of a risk factor for VHL patients to develop an RCC. *Hum. Mutat.* 13(6), 464–475 (1999).

6 Schraml P, Struckmann K, Hatz F *et al.*: VHL mutations and their correlation with tumour cell proliferation, microvessel density, and patient prognosis in clear cell renal cell carcinoma. *J. Pathol.* 196(2), 186–193 (2002).

7 Rini BI, Small EJ: Biology and clinical development of vascular endothelial growth factor-targeted therapy in renal cell carcinoma. *J. Clin. Oncol.* 23(5), 1028–1043 (2005)

8 Kaelin WG Jr: The *von Hippel-Lindau* tumor suppressor protein and clear cell renal carcinoma. *Clin. Cancer Res.*13 (Suppl. 2) 680S–684S (2007)

9 Clarkson PE, Cookson MS*:* The *von Hippel-Lindau* gene: turning discovery into therapy. *Cancer* 113 (7 Suppl.), 1768–1778 (2008).

10 Kibel A, Iliopoulos O, DeCaprio JA *et al.*: Binding of the *von Hippel-Lindau* tumor suppressor protein to Elongin B and C. *Science* 269(5229), 1444–1446 (1995).

11 Maxwell PH, Wiesener MS, Chang GW *et al.*: The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature* 399(6733), 271–275 (1999).

12 Cockman ME, Mason N, Mole DR *et al.*: Hypoxia inducible factor-α binding and ubiquitylation by the *von hippel-Lindau* tumor suppressor protein. *J. Biol. Chem.* 275(33), 25733–25741 (2000).

13 Semenza GL: HIF-1 mediates the Warburg effect in clear cell renal carcinoma. *J. Bioenerg. Biomembr.* 39(3), 231–234 (2007).

14 Wang GL, Jiang BH, Rue EA, Semenza GL: Hypoxia-inducible factor 1 is a basic-helix-loop helix-PAS heterodimer regulated by cellular O2 tension. *Proc. Natl Acad. Sci. USA* 1995 92(12), 5510–5514 (1995).

15 Kondo K, Kaelin WG Jr: The *von Hippel-Lindau* tumor suppressor gene. *Exp. Cell. Res.* 264(1), 117–125 (2001).

▪ **Overview of von Hippel-Lindau (*VHL*) suppressor gene.**

16 Schueneman AJ, Himmelfarb E, Geng L *et al.:* SU11248 maintenance therapy prevents tumor regrowth after fractionated irradiation of murine tumor models. *Cancer Res.* 63(14), 4009–4016 (2003).

17 Mendel DB, Laird AD, Xin X *et al.*: *In vivo* antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of pharmacokinetic/pharmacodynamic relationship. *Clin. Cancer Res.* 9(1), 327–337 (2003).

18 Manning WC, Bello CL, Deprimo SE *et al.:* Pharmacokinetic and pharmacodynamic evaluation of SU11248 in a Phase I clinical trial of patients with imatinib-resistant gastrointestinal stromal tumor (GIST). *Program and Abstracts of the American Society of Clinical Oncology 39th Annual Meeting.* Chicago, IL, USA, 31 May–3 June (2003) (Abstract 768).

19 Raymond E, Faivre S, Vera C *et al.:* Final results of a Phase I and pharmacokinetic study of SU11248, a novel multi-target tyrosine kinase inhibitor, in patients with advanced cancers. *Program and Abstracts of the American Society of Clinical Oncology 39th Annual Meeting.* Chicago, IL, USA, 31 May–3 June (2003) (Abstract 769)

20 Motzer RJ, Rini BI, Bukowski RM *et al.*: Sunitinib in patients with metastatic renal cell carcinoma. *JAMA* 295(21), 2516–2524 (2006).

21 Motzer RJ, Dror Michaelson M, Redman BG *et al.*: Activity of SU11248, a multitargeted inhibitor of vascular endothelial growth factor receptor and platelet-derived growth factor receptor, in patients with metastatic renal cell carcinoma. *J. Clin. Oncol.* 24(1), 16–24 (2006).

22 Figlin RA, Hutson TE, Tomczak P *et al.:* Overall survival with sunitinib versus interferon (IFN)-α as first-line treatment of metastatic renal cell carcinoma (mRCC). *Program and Abstracts of the American Society of Clinical Oncology 44th Annual Meeting.* Chicago, IL, USA, 30May–3 June (2008) (Abstract 5024).

▪▪ **Final analysis of Phase III trial of sunitinib versus interferon (IFN) in first-line treatment of advanced renal cell carcinoma (RCC).**

23 Cella D, Li JZ, Cappelleri JC *et al.*: Quality of life in patients with metastatic renal cell caricnoma treated with sunitinib or interferon α: results from a Phase III randomized trial. *J. Clin. Oncol.* 26(22), 3763–3769 (2008).

24 Rini BI, Choueiri TK Elson P *et al.*: Sunitinib-induced macrocytosis in patients with metastatic renal cell carcinoma. *Cancer* 113(6), 1309–1314.

▪ **Sunitinib-induced macrocytosis.**

25 Lyons JF, Wilhelm S, Hibner B, Bollag G: Discovery of a novel Raf kinase inhibitor. *Endocr. Relat. Cancer* 8(3), 219–225 (2001).

26 Hilger RA, Scheulen ME, Strumberg D: The Ras-Raf-MEK-ERK pathway in the treatment of cancer. *Onkologie* 25(6), 511–518 (2002).

27 Wilhelm SM, Carter C, Tang L *et al.*: BAY 43–9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. *Cancer Res.* 64(19), 7099–7109 (2004).

▪ **Overview of antitumor activity of sorafenib.**

28 Chang YS, Henderson A, Xue D *et al.*: BAY 43–9006 (Sorafenib) inhibits ectopic and orthotopic growth of a murine model of renal adenocarcinoma predominantly through inhibition of tumor angiogenesis. *Program and Abstracts of American Association for Cancer Reserch 96th Annual Meeting.* Anaheim, CA, USA, 16–20 April (2005) (Abstract 5831).

29 Levy J, Pauloski N, Braun D *et al.*: Analysis of transcription and protein expression changes in the 786-O human renal cell carcinoma tumor xenograft model in response to treatment with the multi-kinase inhibitor sorafenib (BAY 43–9006). *Program and Abstracts of American Association for Cancer Reserch 97th Annual Meeting.* Washington, DC, USA, 1–5 April (2006) (Abstract 906).

30 Awada A, Hendlisz A, Gil T *et al.*: Phase I safety and pharmacokinetics of BAY 43–9006 administered for 21 days on 7 days off in patients with advanced, refractory solid tumors. *Br. J. Cancer* 92(10), 1855–1861 (2005).

31 Clark JW, Eder JP, Ryan D *et al.*: Safety and pharmacokinetics of the dual action Raf kinase and vascular endothelial growth factor receptor inhibitor, BAY 43–9006, in patients with advanced, refractory solid tumors. *Clin. Cancer Res.* 11(15), 5472–5480 (2005).

32 Moore MJ, Hirte HW, Siu L *et al.*: Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43–9006, administered for 28 days on/7 days off in patients with advanced, refractory solid tumors. *Ann. Oncol.* 16(10), 1688–1694 (2005).

33 Strumberg D, Richly H, Hilger RA *et al.*: Phase I clinical and pharmacokinetic study of the novel Raf kinase and vascular endothelial growth factor receptor inhibitor BAY 43–9006 in patients with advanced refractory solid tumors. *J. Clin. Oncol.* 23(5), 965–972 (2005).

34 Ratain MJ, Eisen T, Stadler WM, Flaherty KT *et al.*: Phase II placebo-controlled randomized discontinuation trial of sorafenib in patients with metastatic renal cell carcinoma. *J. Clin. Oncol.* 24(16), 2505–2512 (2006).

▪▪ **Phase II randomized discontinuation trial of sorafenib.**

35 Escudier B, Eisen T, Stadler WM *et al.:* Sorafenib in advanced clear-cell renal cell carcinoma. *N. Engl. J. Med.* 356(2), 125–134 (2007).

▪▪ **Phase III trial of sorafenib in cytokine-refractory advanced clear cell RCC patients.**

36 Bukowski RM, Eisen T, Szczylik C *et al.*: Final results of the randomized Phase III trial of sorafenib in advanced renal cell carcinoma: survival and biomarker analysis. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, June 1–5 (2007) (Abstract 5023).

37 Szcylik C, Demkow T, Staehler M *et al.*: Randomized Phase II trial of first-line treatment with sorafnib versus interferon in patients with advanced renal cell carcinoma: Final results. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, June 1–5 (2007) (Abstract 5025).

38 Amato RJ, Harris P, Dalton M *et al.:* A Phase II trial of inter-patient dose-escalated sorafenib in patients with metastatic renal cell cancer. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, June 1–5 (2007) (Abstract 5026)

39 Ferrara N, Kerbel RS: Angiogenesis as a therapeutic target. *Nature* 438(7070), 967–974 (2005).

▪ **Overview of angiogenesis as a therapeutic target.**

40 Kim KJ, Li B, Winer J *et al.*: Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumor growth *in vivo*. *Nature* 362(6423), 841–844 (1993).

41 Yang JC, Haworth L, Sherry RM *et al.*: A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *N. Engl. J. Med.* 349(5), 427–434 (2003).

42 Escudier B, Pluzanska A, Koralewski P *et al.* Bevacizumab plus interferon α-2a for treatment of metastatic renal cell carcinoma: a randomized, double-blind Phase III trial. *Lancet* 370(9605), 2103–2111 (2007).

▪▪ **Phase III trial of bevacizumab plus IFN versus IFN in first-line treatment of advanced RCC.**

43 Rini BI, Halabi SH, Rosenberg JE *et al.*: Bevacizumab plus Interferon-α versus Interferon-α monotherapy in patients with metastatic renal cell carcinoma: results of CALGB 90206. *J. Clin. Oncol.* 26(33), 5422–5428 (2008).

▪▪ **Phase III trial of bevacizumab plus IFN versus IFN in first-line treatment of advanced RCC.**

44 Schmelzle T, Hall MN: TOR, a central controller of cell growth. *Cell* 103(2), 253–262 (2000).

45 Thomas GV, Tran C, Mellinghoff IK *et al.*: Hypoxia-inducible factor determines sensitivity to inhibitors of mTOR in kidney cancer. *Nat. Med.* 12(1), 122–127 (2006).

▪ **Overview of hypoxia-inducible factor in mammalian target of rapamycin inhibition.**

46 Hudson CC, Liu M, Chiang GG *et al.*: Regulation of hypoxia-inducible factor 1a expression and function by the mammalian target of rapamycin. *Mol. Cell. Biol.* 22(20), 7004–7014 (2002).

47 Fingar DC, Richardson CJ, Tee AR, Cheatham L, Tsou C, Blenis J: mTOR controls cell cycle progression through its cell growth effectors S6K1 and 4E-BP1/eukaryotic translation factor 4E. *Mol. Cell Biol.* 24(1), 200–216 (2004).

48 Neshat MS, Mellinghoff IK, Tran C *et al.*: Enhanced sensitivity of PTEN-deficient tumors to inhibition of fRAP/mTOR. *Proc. Natl Acad. Sci. USA* 98(18), 10314–10319 (2001).

49 Atkins MB, Hidalgo M, Stadler WM *et al.*: Randomized Phase II study of multiple dose levels of CCI-779, a novel mammalian target of rapamycin kinase inhibitor, in patients with advanced refractory renal cell carcinoma. *J. Clin. Oncol.* 22(5), 909–918 (2004)

50 Mekhail TM, Abou-Jawde RM, Boumerhi G *et al.*: Validation and extension of the Memorial Sloan-Kettering prognostic factors model for survival in patients with previously untreated metastatic renal cell carcinoma. *J. Clin. Oncol.* 23(4), 832–841 (2005).

51 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(8), 2530–2540 (1999).

52 Hudes G, Carducci M, Tomczak P *et al.*: Temsirolimus, interferon α, or both for advanced renal-cell carcinoma. *N. Engl. J. Med.* 356(22), 2271–2281 (2007).

▪▪ **Phase III trial of temsirolimus versus IFN versus temsirlimus plus IFN in intermediate and poor risk patients with advanced RCC.**

53 Jac J, Giessinger S, Khan M, Willis J, Chiang S, Amato R: A Phase II trial of RAD001 in patients with metastatic renal cell carcinoma. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, 1–5 June (2007) (Abstract 5107).

54 Motzer RJ, Escudier B, Oudard S *et al.*: Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomized, placebo-controlled Phase III trial. *Lancet* 372(9637), 449–456 (2008).

▪▪ **Everolimus in patients treated with other targeted therapy. First study to establish possibility of sequential therapy in advanced RCC.**

55 Rixe O, Bukowski RM, Michaelson MD *et al.*: Axitinib treatment in patient with cytokine-refractory metastatic renal-cell cancer: a Phase II study. *Lancet* 8 (11), 975–984 (2007).

56 Rini BI, Wilding GT, Hudes G *et al.*: Axitinib in patients with metastatic renal cell cancer refractory sorafenib. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, 1–5 June (2007) (Abstract 5032).

57 Hutson TE, Davis ID, Machiels JP *et al.*: Pazopanib (GW786034) is active in metastatic renal cell carcinoma: interim results of a Phase II randomized discontinuation trial. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, 1–5 June (2007) (Abstract 5031).

58 Sridhar SS, Hotte SJ, Mackenzie MJ *et al.*: Phase II study of the angiogenesis inhibitor AZD2171 in first line, progressive, unresectable, advanced metastatic renal cell carcinoma: a trial of the PMH Phase II Consortium. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, 1–5 June (2007) (Abstract 5093).

59 Van Herpen C, Drevs J, van Cruijsen H *et al.*: Evaluation of AZD2171, an oral, highly potent and selective VEGFR signaling inhibitor, in renal cell carcinioma: combined results from two Phase I studies. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, 1–5 June (2007) (Abstract 3560)

60 Sridhar SS, Mackenzie MJ, Hotte SJ *et al.*: Activity of cediranib (AZD2171) in patients with previously untreated metastatic renal cell cancer. A Phase II trial of the PMH Consortium. *Program and Abstracts of the American Society of Clinical Oncology 44th Annual Meeting.* Chicago, IL, USA, 30 May–3 June (2008) (Abstract 5047).

61 Yazji S, Bukowski R, Kondagunta V, Figlin R: Final results from Phase II study of volociximab, an α5β1 anti-integrin antibody, in refractory or relapsed metastatic clear cell renal cell carcinoma. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, 1–5 June (2007) (Abstract 5094)

62 Rini BI, Michaelson MD, Rosenberg JE *et al.*: antitumor activity and biomarker analysis of sunitinib in patients with bevacizumab-refractory metastatic renal cell carcinoma. *J. Clin. Oncol.* 26(22), 3743–3748 (2008).

63 Rini BI, Hutson TE, Elson P *et al.*: A prospective trial of sorafenib in patients with metastatic clear cell renal cell carcinoma refractory to prior sunitinib or bevacizumab. *ASCO 2008 GU Cancers Symposium.* San Francisco, CA, USA, 14–16 February (2008) (Abstract 346).

64 Sosman JA, Puzanov I, Atkins MB: Opportunities and obstacles to combination targeted therapy in renal cell cancer. *Clin. Cancer Res.* 13(2 Suppl.), 764S–769S (2007).

65 Schmidinger M, Vogl UM, Lamm W, Bojic M, Bojic A, Zielinski CC: Temsirolimus in heavily pretreated patients with metastatic renal cell carcinoma: preliminary results from the austrian compassionate use program. *Program and Abstracts of the American Society of Clinical Oncology 44th Annual Meeting.* Chicago, IL, USA, 30 May–3 June (2008) (Abstract 16125).

66 Feldman DR, Ginsberg M, Baum C *et al.*: Phase I trial of bwvacizumab plus sunitinib in patients with metastatic renal cell carcinoma. *Program and Abstracts of the American Society of Clinical Oncology 44th Annual Meeting.* Chicago, IL, USA, 30 May–3 June (2008) (Abstract 5100).

67 Sosman JA, Flaherty KT, Atkins MB, McDermott DF, Rothenberg ML *et al.*: Updated results of Phase I trial of sorafenib and bevacizumab in patients with metastatic renal cell carncer. *Program and Abstracts of the American Society of Clinical Oncology 44th Annual Meeting.* Chicago, IL, USA, 30 May–3 June (2008) (Abstract 5011).

68 Eto M, Takeuchi A, Ohki T, Tatsugami K, Naito S: *In vitro* and *in vivo* analysis of synergistic antitumor effects of interferon-a and sorafenib in renal cell carcinoma. *Program and Abstracts of the American Society of Clinical Oncology 44th Annual Meeting.* Chicago, IL, USA, 30 May–3 June (2008) (Abstract 16143).

69 Golob JA, Rathmell WK, Richmond TM *et al.*: Phase II trial of sorafenib plus interferon α-2a as first- or second-line therapy in patients with metastatic renal cell cancer. *J. Clin. Oncol.* 25(22), 3288–3295 (2007).

70 Rosenberg JE, Weinberg VK, Claros C *et al.*: Phase I study of sorafenib and RAD001 for metastatic clear cell renal cell carcinoma.

Program and Abstracts of the American Society of Clinical Oncology 44th Annual Meeting. Chicago, IL, USA, 30 May–3 June (2008) (Abstract 5109).

71 Whorf RC, Hainsworth JD, Spigel DR *et al.*: Phase II study of bevacizumab and everolimus (RAD001) in the treatment of advanced renal cell carcinoma *Program and Abstracts of the American Society of Clinical Oncology 44th Annual Meeting.* Chicago, IL, USA, 30 May–3 June (2008) (Abstract 5010).

72 Stephenson J, Schreeder M, Waples J *et al.*: Perifosine, active as a single agent for renal cell carcinoma, now in Phase I trials combined with tyrosine kinase inhibitors. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, 1–5 June (2007) (Abstract 15622).

73 Schreeder MT, Figlin RA, Stephenson JJ *et al.*: Phase I multicenter trial of perifosine in combination with sorafenib for patients with advanced cancers including renal cell carcinioma. *Program and Abstracts of the American Society of Clinical Oncology 44th Annual Meeting.* Chicago, IL, USA, 30 May–3 June (2008) (Abstract 16024).

74 Bukowski RM, Fairooz F, Kabbinavar RA *et al.*: Randomized Phase II study of erlotinib combined with bevacizumab compared with bevacizumab alone in metastatic renal cell cancer. *J. Clin. Oncol.* 25(29), 4536–4541 (2007).

75 Choueiri TK, Plantade A, Elson P *et al.*: Efficacy of sunitinib and sorafenib in metastatic papillary and chromophobe renal cell carcinoma. *J. Clin. Oncol.* 26(1), 127–121 (2008).

76 Dutcher J, de Souza P, Figlin R *et al.*: Effect of temsirolimus versus interferon-a on survival of patients with advanced renal cell carcinoma of different tumor histologies. *ASCO 2008 GU Cancers symposium.* San Francisco, CA, USA, 14–16 February (2008) (Abstract 384).

77 Eder J, Heath E, Appleman L *et al.*: Phase I experience wih c-MET inhibitor XL880 administered orally to patients with solid tumors. *Program and Abstracts of the American Society of Clinical Oncology 43rd Annual Meeting.* Chicago, IL, USA, 1–5 June (2007) (Abstract 3526).

78 Srinivasan R, Choueiri TK, Vaishampayan U *et al.*: A Phase II study of the dual MET/VEGFR2 inhibitor XL880 in patients with papillary renal cell carcinoma. *Program and Abstracts of the American Society of Clinical Oncology 44th Annual Meeting.* Chicago, IL, USA, 30 May–3 June (2008) (Abstract 5103).

79 Oliner J, Min H, Leal J *et al.*: Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2. *Cancer Cell* 6(5), 507–516 (2004).

▪ **Inhibition of angiopoietin-2 in antiangiogenesis therapy.**