mTor inhibition in RCC: proof of concept, clinical use, and future directions

P.G. Aftimos, A.H. Awada, T. Gil

Renal cell carcinoma (RCC) is the most common renal tumour and accounts for approximately 90% of all renal malignancies. Within this group, 75% of cancers have a clear cell histology. The outcome for patients with metastatic RCC (mRCC) is generally poor and incidence of RCC and mRCC are on the rise worldwide. Cytokine therapy was the gold standard systemic treatment for years. However, it is only effective in a relatively small percentage of patients with high toxicity. An improved understanding of the biology of RCC has led to the development of various agents targeting ligands at the molecular level. The rapid development of agents blocking the VEGF pathway and the mTor pathway has established them as the preferred treatment approach in this setting. mTor is a serine/threonine kinase that plays a critical role in regulating cellular processes controlling cell growth, proliferation, cell motility and angiogenesis. Recent randomised phase III trials have determined the role of mTor inhibitors in the treatment of mRCC, and ongoing trials are evaluating targeted agents combinations and testing predictive biomarkers.
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Introduction

Kidney cancer ranks 10th in cancer incidence in the European Union (EU) with 63,300 new cases and 26,400 kidney cancer-related deaths in 2006. Renal cell carcinoma (RCC) is the most common renal tumour and accounts for approximately 90% of all renal malignancies. Within this group, 75% are of the conventional clear cell histology. Twenty-five to 30% of patients with RCC are diagnosed at the metastatic stage while 20-30% treated at an early stage will experience relapse and develop metastases. The outcome for patients with metastatic RCC (mRCC) is generally poor and the incidence of RCC and mRCC is on the rise worldwide with approximately 2% every year, imposing a serious worldwide epidemiological burden. However, mortality rates have been declining in the EU from a peak of 4.8 per 100,000 in 1990-1994 to 4.1 per 100,000 in 2000-2004 (-13.1%) in men, and from 2.1 to 1.8 per 100,000 (-17%) in women. Cytokine therapy was the gold standard treatment of RCC, both in adjuvant and metastatic settings.[1] Immunotherapy is effective in a relatively small percentage of patients but is very toxic. In recent years, understanding of the biology of RCC has improved, leading to the development of various agents targeting ligands at the molecular level. The hypoxia inducible factor-alfa (HIF-1a), vascular endothelial

Authors: P.G. Aftimos MD, A.H. Awada MD PhD, T. Gil MD, Medical Oncology Department, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium.

Please send all correspondence to: P.G. Aftimos MD, Medical Oncology Department, Institut Jules Bordet, 1 Rue Heger-Bordet,1000 Brussels, Belgium, tel.: +32 (0)2 541 3208, E-mail: philippe.aftimos@bordet.be.

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growth factor (VEGF) pathway and mammalian target of rapamycin (mTOR) signal transduction pathway are novel targets. Recent randomised phase III trials have shown an improved outcome in patients with metastatic disease receiving these new agents.

RCC in the pre-targeted therapy era

Kidney cancer remains the internist tumour with only 9% of patients showing the classic triad of flank pain, haematuria and a palpable abdominal mass. Surgery is curative in the majority of patients without metastatic disease. Five and 10-year survivals by Robson stage is 84 and 80% for stage I disease while it is only 46 and 38% for stage III disease.[2] Twenty to 50% of patients with localised tumours experience relapse with lung metastasis being the most common site of distant recurrence, occurring in 50-60% of patients. The median time to relapse after surgery is 1-2 years, with most relapses occurring within 3 years.[3] Another 20-30% of patients is presented with metastatic disease.[1] mRCC is resistant to standard chemotherapy with a literature review of 51 phase II trials testing 33 chemotherapeutic agents in 1347 patients, showing a 0% response rate in 23 trials and ≤6% response rates in 38 trials.[4] As such, chemotherapy is rarely used and RCC is considered an immunogenic disease, specifically after spontaneous remissions have been described.[5] Durable responses can be elicited with cytokine therapy and durable complete remissions have been seen with high-dose interleukin-2 (IL-2) in about 10% of patients; but only a minority of patients experience clinical benefit with problematic adverse events.[1] Secondary treatment is obsolete and urgent alternatives were needed making metastatic RCC an area of unmet need.

VHL and anti-angiogenesis targeted therapy

'Treatment Options in Metastatic Renal Carcinoma: An Embarrassment of Riches' is the title of an editorial of the *Journal of Clinical Oncology* in 2006 accompanying the publication of a major trial in the treatment of mRCC.[38] This paper marks the opening of an era of molecularly targeted therapy for advanced renal cell carcinoma based on the increased insight in the molecular mechanisms involved in RCC pathogenesis. Multiple molecular mechanisms are involved and the importance of specific pathways can differ between subtypes. Genetic and molecular defects involved in the conventional clear cell subtype are: loss of heterozygosity (LOH) 3p, mutation of 3p25 (Von Hippel Lindau; VHL), +5q, -8p, -14q, p53 mutation, c-erB-1 oncogene expression.[7] Of the many aetiological factors of RCC, VHL disease is the most common inherited cause. VHL is an autosomal dominant inherited syndrome characterised by the development of cerebellar and spinal hemangioblastomas, retinal angiomas, pheochromocytomas, and renal cysts and tumours. The prevalence is approximately 1 in 36,000 live births and lifetime risk of >70%. The VHL gene maps to the short arm of chromosome 3 (3p25) and mutations were identified in 57% of clear cell renal carcinomas analysed and LOH was observed in 98% of those samples. VHL acts as a tumour suppressor gene (TSG) and inactivation may result from loss, mutation or promoter methylation with a double-hit required in sporadic RCC.[1] Protein products of VHL TSG ($pVHL_{29}$ and $pVHL_{19}$) have the ability to regulate the hypoxia-response genes and this role is closely linked to the development of clear-cell RCC.[8] Angiogenesis has been proven to be a key determinant in the pathogenesis of RCC, which are the most vascularised of all solid cancers. Vascular endothelial growth factor (VEGF) that is overexpressed in most clear-cell RCCs is a key growth factor involved in angiogenesis and VEGF mRNA expression correlates with vascularisation.[9] Disruption of VHL gene function leads to angiogenesis by the accumulation of HIF-1a in conditions of hypoxia or defective/mutated pVHL function.[10] Subsequently, HIF1-a translocates to the nucleus and dimerises with HIF1-ß, resulting in the transcription of several hypoxia-inducible genes among which various growth factors such as VEGF, Platelet Derived Growth Factor (PDGF), basic fibroblast growth factor (bFGF), erythropoietin and Transforming Growth Factor-a (TGF-a).[11] VEGF and PDGF will bind with specific receptors resulting in stimulation of Receptor Tyrosine Kinases (RTKs), leading to endothelial cell proliferation, survival and angiogenesis, and thus contributing to the typical hypervascular histology of clear cell RCC.

mTor and RCC angiogenesis

A second molecular mechanism leading to RCC angiogenesis is the disruption of the mammalian target of rapamycin (mTor) signaling transduction pathway. mTor is a serine/threonine kinase playing a critical role in regulating cellular processes that control cell growth, proliferation, cell motility and angiogenesis.[12] mTor is regulated by both the phosphoinositide-3-kinase (PI3K)/Akt pathway and the mitogen-activated protein kinase (MAPK) pathway (*Figure 1*). Two structurally and functionally distinct mTor complexes are present in humans: mTORC1 and mTORC2. mTORC1 is sensitive and mTORC2 is insensitive to rapamycin.[13] mTOR promotes angiogenesis in 2 different ways: production of angiogenic growth factors by tumour cells through production of the HIF transcription factors and mitogenic signaling in tumour vasculature downstream of angiogenic growth factors.[14] These mTOR functions are relevant to RCC, which is characterised by alterations of the VHL gene, leading to the up-regulation of HIF-a subunits, vascular endothelial growth factor (VEGF), and other molecules that increase angiogenesis.[15] Rapamycin downregulates the mTor pathway by binding to FK-506-binding protein 12 (FKBP12). The FKBP12-rapamycin complex interacts with mTor and inhibits its function.[16]

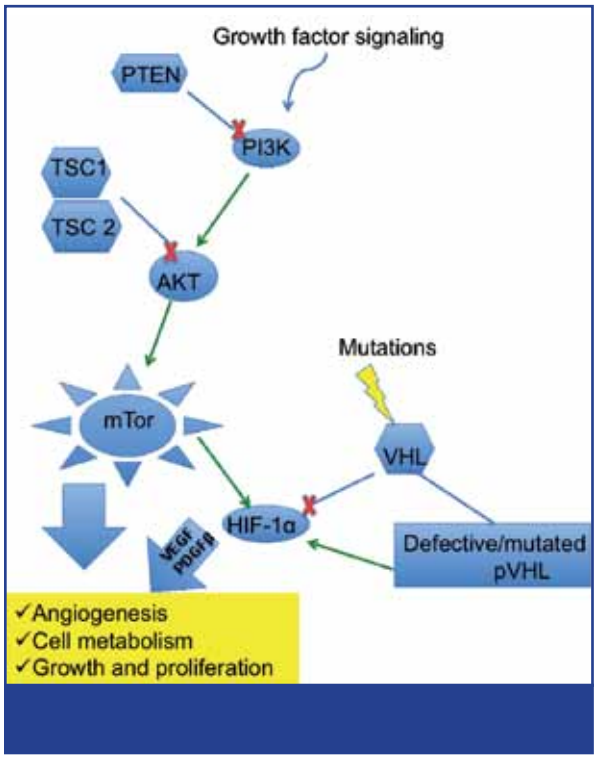

Figure 1. mTor and HIF pathways in RCC.

Rapamycin and agents inhibiting mTor

Sirolimus also known as rapamycin is a macrocyclic lactone, product of the soil bacterium *Streptomyces hygroscopicus*. This agent possesses fungicidal, immunosuppressive as well as antiproliferative properties.[17] Sirolimus derivatives have shown antiproliferative properties in both in vitro and in vivo models: tumour and endothelial cell proliferation inhibition, apoptosis, angiogenesis inhibition.[18,19]

Temsirolimus (CCI-779) is a soluble 42-[2,2-bis (hydroxymethyl)]-propionic ester of rapamycin. It is an intravenously administered mTor inhibitor with sirolimus being its main metabolite, giving this agent a double mTor inhibitory activity.[20]

Cytotoxic activity was deemed moderate in a randomised phase II trial of cytokine pre-treated renal cell carcinoma patients. One out of 3 different doses of temsirolimus (25, 75, or 250 mg, each as a 30-minute IV infusion weekly) were randomly assigned to 111 patients with advanced RCC who either had received previous interleukin or interferon therapy for advanced disease or were ineligible for such therapy.[21] The response rate for the complete population was 7% with 1 complete response and 7 partial responses. A minor response was shown in 26% of patients and 17% had a stable disease for 6 months or longer, suggesting significant anti-tumour activity. Time to progression was 5.8 months and median overall survival (OS) 15 months. Patients were stratified according to prognostic factors of the MSKCC database: low Karnofsky performance status, high lactate dehydrogenase, low serum hemoglobin, high corrected serum calcium, and time from initial RCC diagnosis to start of IFNa therapy of less than 1 year.[22] Patients with an intermediate (1 or 2 risk factors) or poor (3 or more risk factors) prognosis appeared to benefit most with median survivals of 19.3 and 5.8 months respectively. This benefit occurred even though the patients received temsirolimus as second or third-line therapy. Based upon the phase II results, temsirolimus was evaluated in a phase III trial in which 626 previously untreated poor prognosis patients with metastatic or recurrent RCC were randomly assigned to temsirolimus (25 mg IV weekly), the combination of temsiro-

limus (15 mg IV/ week) plus IFNa (escalated up to 6 million units 3 times a week as tolerated), or IFNa as monotherapy (escalated up to 18 million units 3 times a week as tolerated).[23] Single-agent temsirolimus versus IFNa significantly prolonged the median OS (10.9 versus 7.3 months; HR for mortality 0.73, 95% CI 0.58-0.92) and the progression free survival (PFS) (3.8 versus 1.9 months according to investigator and 5.5 versus 3.1 months according to independent radiological assessment). Combination therapy was not superior to single-agent IFNa. A subset analysis showed benefit in patients with non-clear cell histology.[24] Treatment with temsirolimus was generally well-tolerated in these trials. In the phase III trial, temsirolimus was better tolerated than either IFNa alone or the combination of IFNa and temsirolimus. The most serious adverse events (Aes) were asthaenia and anaemia (11 and 20% respectively) with less severe AEs events being rash, peripheral oedema, stomatitis, hyperlipidaemia, hyperglycaemia and hypercholesterolaemia. Temsirolimus was associated with pneumonitis (all grades) in 0.5 to 5% (~1% grade 3/4) of patients enrolled in clinical studies, including rare fatalities. In a secondary analysis of the phase III trial, treatment with temsirolimus was associated with significantly better quality of life than therapy with interferon.[25] By May 30, 2007, the FDA approval on temsirolimus (Torisel™, made by Wyeth, Inc.) had been granted for the treatment of advanced renal cell carcinoma (RCC) and by EMEA in November 2007 for first line treatment of patients with advanced renal cell carcinoma who have at least 3 of 6 prognostic risk factors.

Everolimus (RAD001, Novartis), or 42-O-(2-hydroxyethyl) rapamycin, has greater polarity than sirolimus and was developed in an attempt to improve pharmacokinetic characteristics of sirolimus, particularly to increase its oral availability and was extensively studied in the transplant setting.[26] Doses of 5-10 mg per day or 20-50 mg per week were recommended for further studies based on phase 1 studies and a phase II trial evaluated the efficacy and safety of everolimus (10mg daily) in 41 patients with mRCC who had failed no more than one prior therapy.[27] Thirty-seven patients were evaluable for response and five (14%) patients had a partial response and 27 patients (73%) had stable disease for at least 3 months. The median PFS was 11.2 months and the median OS was 22.1 months. Grade 3 out of 4 AEs included pneumonitis (grade 3 in 19% of patients) resolving after treatment delay and not requiring steroids, transaminase elevations (10% of patients), thrombocytopaenia, hyperglycaemia, alkaline phosphatase elevations (8% of patients), and hyperlipidaemia (5% of patients). A further phase II trial then demonstrated the efficacy of everolimus in VEGF receptor tyrosine kinase inhibitors (VEGF-TKI) (sunitinib and sorafenib) pre-treated mRCC patients.[28] No objective responses were observed in 25 evaluable patients but 22 patients had stable disease. Median PFS was 6.53 months and median OS was 16.3 months. A pivotal phase III trial (RECORD-1) randomised 410 patients with clear cell mRCC who failed prior VEGF-targeted therapy in a 2:1 ratio to everolimus (10mg per day) or placebo.[29] All patients had disease progression while on VEGF-TKI or within 6 months after completion of such therapy. The median PFS with everolimus based on independent review was significantly prolonged, compared to placebo (4.9 versus 1.9 months, HR 0.30, 95%CI 0.22-0.40). The benefit extended to all stratification sets (risk group, prior treatment, age, sex, geographic region). There was no statistically significant difference in OS (median 14.8 versus 14.4 months, HR 0.87). However, 80% of patients in the placebo group crossed-over upon disease progression. A recent exploratory analysis of OS from this trial, using a rank preserving structural failure time (RPSFT) model correcting for bias due to crossover, has indicated that everolimus treatment is associated with a beneficial OS compared to placebo (14.8 months versus 10.0 months).[30] Objective responses were rare (1 and 0% with everolimus and placebo respectively), although stable disease was more common (63 and 32% respectively). The most common everolimus-related grade 3 or 4 adverse events included stomatitis (3 versus 0%), fatigue (3 versus <1%), infections (3 versus 0%), and pneumonitis (3 versus 0%). Grade 3 laboratory abnormalities included hyperglycaemia, hypercholesterolaemia, anaemia. Ten percent of patients in the everolimus group discontinued treatment. On March 30th 2009, the FDA approved everolimus tablets for the treatment of patients with advanced renal cell carcinoma after failure of treatment with sunitinib or sorafenib. On June 5th 2007, orphan designation (EU/3/07/449) was granted by the European Com-

Table 1. Algorithm for treating mRCC in 2011 based on phase III trials.

Setting		Therapy	
first-line	low + intermediate risk	sunitinib IFN/Bevacizumab pazopanib	HD IL2 (in special circumstances)
	poor risk	temsirolimus	
second-line	prior cytokine	sorafenib pazopanib	
	prior VEGFR	everolimus	

mission for everolimus in the treatment of renal cell carcinoma. Everolimus was authorised in the EU on August 3rd 2009 for the treatment of patients with advanced RCC, whose disease progressed on or after treatment with VEGF-targeted therapy.

Combination regimens

Initial studies of dual therapy with mTor inhibitors and conventional cytotoxic therapy suggest enhanced toxicities even at low doses. This limitation could be surpassed by the understanding of the molecular pathogenesis of RCC, opening the door for combinations of targeted agents. Vertical blockade is the blocking of multiple steps in the same pathway and horizontal blockade is the simultaneous inhibition of more than 1 pathway. A phase II study combining everolimus and bevacizumab suggested that this regimen was active and well-tolerated in the treatment of advanced clear cell RCC, either as primary treatment or after treatment with sunitinib and/or sorafenib.[31] RECORD-2 is a large phase II trial investigating the combination of everolimus and bevacizumab as primary treatment for mRCC. Another option is the dual inhibition of the VEGF receptor and mTor which could abrogate potential resistance mechanisms by affecting the hypoxia-inducible factor (HIF)-1a/VEGF angiogenesis pathway as well as cell growth and survival via mTor. The combination of sorafenib and everolimus produced anti-tumour activity and tolerable toxicities in a phase I study with an ORR of 27%.[32] A phase II continuation of this trial has recently been com-pleted and a phase I trial of everolimus plus sunitinib is ongoing.[33] BeST is a 4-arm phase II trial with dual combinations of bevacizumab, sorafenib and temsirolimus planning to compare PFS between patients treated with combination therapies and those receiving bevacizumab alone. RECORD-3 is a phase II trial evaluating the activity of everolimus followed by sunitinib versus the reverse sequence.
Preclinical studies have evaluated the activity of rapamycin in combination with the Akt inhibitor perifosine on RCC cell line[34] and the novel dual PI3-kinase/mTOR inhibitor NVP-BEZ235 induced growth arrest in RCC cell lines both *in vitro* and *in vivo* more effectively than inhibition of TORC1 alone.[35] Furthermore, the crosstalk between the PI3K/Akt/mTOR and Raf/MEK/Erk pathways observed in experimental systems[36] provides a rationale for combining inhibitors of both pathways, such as an mTOR inhibitor with a MEK inhibitor, to achieve antitumour effects greater than can be gained from inhibition of a single pathway.[37]

Current treatment paradigm

Based on positive results from phase III trial, mTor inhibitors have been integrated in the treatment strategy of advanced RCC. While sunitinib (a VEGF receptor tyrosine-kinase inhibitor) is the approved first line treatment of favourable and intermediate risk mRCC, temsirolimus is indicated upfront in poor risk advanced RCC. Sorafenib (a multi-kinase inhibitor) is approved in patients relapsing or progressing after cytokine treatment and everolimus is the indicated agent in the second or third line after anti-VEGF monoclonal antibodies and/or tyrosine kinase inhibitors.

Conclusion

An understanding of the pathogenesis of RCC at the molecular level has resulted in the identification of specific targets for therapeutic intervention. Molecular targeted therapies play a prepon-

Key messages for clinical practice

1. Molecular targeted therapies play an important role in the management of advanced RCC.
2. Temsirolimus is approved for the treatment of advanced RCC that have at least 3 of 6 prognostic risk factors.
3. Everolimus is approved for the treatment of patients with advanced renal cell carcinoma, whose disease has progressed on or after treatment with VEGF-targeted therapy.
4. Treatment-related adverse events are: skin reactions, stomatitis and mucositis, cytopaenias (mainly thrombocytopaenia), hyperglycaemia, hyperlipidaemia, asthaenia, diarrhea and pneumonitis.

derant role in the management of advanced RCC and are being integrated in a treatment algorithm (*Table 1*). The rapid development of agents blocking the VEGF pathway and the mTor pathway has established them as the preferred treatment approach in this setting. Inhibitors of PI3K/Akt/mTOR signaling have proven activity in advanced RCC. These agents are generally well-tolerated, with pneumonitis, fatigue, metabolic abnormalities, stomatitis, diarrhea, and myelosuppression the most common toxic effects. Most of these toxicities are mild to moderate in severity and can be managed clinically by dose modification and supportive measures. How to use mTOR inhibitors most effectively in RCC and other tumours is an active area of clinical research. Approved drugs (temsirolimus and everolimus) have been evaluated in somewhat different clinical settings and no trials provide data about direct comparisons. Studies are under way testing combinations and sequential treatments, and many of the studies testing these newer approaches will include correlations of potential tumour biomarkers with treatment outcomes toward the important goal of determining who will benefit the most from inhibition of PI3K/Akt/mTOR signaling. Molecular markers have the potential to enhance our ability to predict the response of an individual tumour to treatment and to stratify patients into more appropriate risk groups, enabling the move from nonspecific treatments to specific treatments of targeted therapies for enriched patient populations.

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