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Metastatic Renal Cancer: What Role for Everolimus?

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Abstract

Metastatic renal cell carcinoma is uncommon (only 3% of cancers worldwide) but of poor prognosis. Renal cell carcinoma has traditionally been treated with cytokines (interferon- α or interleukin-2). More recently, a more clear understanding of the molecular and cellular mechanisms of the disease, involving the VEGF receptor and mTOR, has led to the discovery of novel therapies. Therapeutic options in patients with advanced RCC include the VEGF receptor inhibitors Sunitinib and Sorafenib, the anti-VEGF monoclonal antibody Bevacizumab and the mTORC1 inhibitors Temsirolimus and Everolimus. In 2009, Everolimus was FDA-approved for the treatment of patients with advanced clear cell RCC which had progressed within 6 months of stopping treatment with Sunitinib or sorafenib, or both drugs. Everolimus resulted in a 70% reduction in the risk of disease recurrence or death. The purpose of this review is to update on the current knowledge of the role of Everolimus in metastatic renal cell carcinoma.

Keywords

everolimus; renal cell cancer; mTOR

Introduction

Renal cell carcinomas (RCCs), which originate within the renal cortex, are responsible for 80 to 85 percent of all primary renal neoplasms. It is estimated that RCC accounts for 2 to 3% of all adult malignancies.^{1,2} RCC occurs predominantly in the sixth to eighth decade of life; it is unusual in patients under 40 years of age. RCC presenting as a mass localized to the kidney can be cured by nephrectomy. Metastatic RCC constitutes approximately 60% of all patients with RCC. Of patients with metastatic RCC, 30% of patients are diagnosed at the time of presentation and 30% at follow-up.³ The median survival for patients with stage IV disease (T4 primary tumor, N2 involvement, or distant metastases) is as low as 16 to 20 months. The five-year survival rate of patients with distant metastases is less than 10 percent. Metastatic RCC is generally of poor prognosis because it is highly resistant to conventional forms of chemotherapy.

Disclosures

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Renal cell carcinoma (RCC), like many other cancers, is caused by abnormal cell signaling. RCC has been associated with both the overproduction of vascular endothelial growth factor (VEGF) and the Von Hippel-Lindau tumor suppressor gene. In the face of low response rates to traditional anticancer therapies namely interferon- α and interleukin-2,⁴ new therapeutic agents have emerged based on knowledge of the abnormal cell signaling. Thus, since 2004, studies of new antiangiogenetic molecules, acting on VEGF and its related receptors (p-VHL-HIF, PDGF, tyrosine-kinase receptors) have shown great promise in the treatment of metastatic RCC. Sunitinib and Sorafenib were suggested as first line and second line agents respectively for intermediate-or low-risk patients. When used in combination with Interferon- α , the humanized anti-VEGF monoclonal antibody (bevacizumab) was also proposed as first line agent in patients with good prognosis RCC.⁵ The mTOR inhibitors show substantial promise for the treatment of metastatic RCC. Temsirolimus, an analogue of rapamycin that is administered intravenously, has been approved by the FDA for the treatment of metastatic RCC.^{6,7} Moreover, as suggested by recent studies, Everolimus, an oral derivative of rapamycin, also has a beneficial effect in metastatic RCC.^{1,2,8}

Central Role of mTOR in Renal Cell Carcinoma

mTOR is a highly conserved serine/threonine kinase that regulates signal transduction pathways that promote cancer cell proliferation and survival as well as tumor angiogenesis.⁹ mTOR is a component of two distinct signaling complexes known as mTOR complex 1 (mTORC1) or rapamycin-sensitive complex, and mTOR complex 2 (mTORC2) or rapamycin-insensitive complex. mTORC1 contains a scaffolding protein called raptor (regulatory-associated protein of mTOR), which links mTOR to different downstream signaling pathways leading to cell growth and cell proliferation. mTORC2 has a different protein called rictor (rapamycin-insensitive companion of mTOR) which interacts with mTOR to regulate cell polarity, the actin cytoskeleton and apoptosis. It is important to note that rapamycin inhibits mTORC1 by preventing the interaction of mTOR with raptor.^{10–14} It was previously believed that rapamycin had no effect on mTORC2. However, it has been recently demonstrated that prolonged rapamycin treatment or high dose rapamycin treatment can inhibit mTORC2.^{15–17} Thus targeting mTORC2 may be a future therapeutic strategy in RCC.

Upstream Effects of mTORC1 in Renal Cell Carcinoma

There is accumulating evidence indicating that external stimuli and factors such as insulin growth factor 1 (IGF-1),^{18,19} epidermal growth factor (EGF)^{20,21} as well as nutrients such as amino acids,^{19,22,23} glucose and oxygen,^{24–26} signal to mTORC1 after diffusion into the cells (Fig. 1). The role of IGF1 in promoting cancer has been investigated for many years. Individuals overexpressing the IGF1 receptor have been shown to be prone to malignancies.^{27,28} Furthermore, elements of the PI3K/AKT/mTOR pathway are constitutionally activated in malignancies^{29–31} including in RCC.³² In the presence of nutrients, the PI3K/AKT/mTOR signaling pathway is initiated by growth factors such as IGF1 through the activation of receptor tyrosine kinases which signals the lipid kinase phosphatidylinositol 3-kinase (PI3K). PI3K in turn phosphorylates the membrane-associated phospholipids

phosphatidylinositol-4,5-bisphosphate (PIP₂) to produce phosphatidylinositol-3-4,5-bisphosphate (PIP₃).³³ The activity of PI3K is negatively regulated by PTEN which by converting PIP₃ back to PIP₂, prevents the inactivation of the tuberous sclerosis complex (TSC) by Akt, the protein kinase B that is directly activated by PIP₃. TSC is a dimer composed of TSC1 (hamartin) and TSC2 (tuberin) and Rheb is a GTPase that directly activates mTORC1. TSC1 is necessary for the inhibitory function of TSC2 on Rheb activity. When the TSC2 function is suppressed, it releases Rheb from the inhibitory effects of TSC and activation of mTORC1 occurs.^{34,35} It has been shown that loss-of-function mutations of PTEN, TSC1 or TSC2 predispose to the development of RCC.^{32,36,37}

Downstream effects of mTORC1 in renal cell carcinoma

Activation of mTORC1 regulates downstream signaling pathways and facilitates coupling of growth stimuli to cell-cycle progression (Fig. 1). A pathway leading to angiogenesis and cell proliferation which is stimulated by hypoxia is also activated by mTORC1. mTORC1 transmits signals to hypoxia-inducible factor-1 α (HIF-1 α) which in turn triggers the expression of vascular endothelial growth factors (VEGF), VEGF receptors (VEGFR), basic fibroblast growth factors (bFGF), platelet derived growth factors (PDGF) and angiopoietin 2, thereby enhancing vascular cell proliferation and angiogenesis.³⁸ Processes that facilitate the degradation of HIF-1 α negatively regulate angiogenesis whereas hypoxemic states including malignancy, which stabilize HIF-1 α , enhance angiogenesis. For instance, in normal individuals, HIF-1 α is only transiently expressed as a result of the high activity of HIF-prolylhydroxylase, the enzyme that degrades HIF. Interestingly, in individuals with RCC, the frequent occurrence of Von Hippel Lindau (VHL) loss-of-function mutations can cause HIF-1 α stabilization with resulting overexpression of growth factors and sustained tumor angiogenesis.³⁹

Other downstream effectors through which mTORC1 acts include 4E-binding proteins (4EBP) and the 70-kD ribosomal S6 kinases (p70S6K).^{10,40} The 4EBP are a family of translation repressor proteins that are necessary for mediating the increases in cell size with progression through the early stages (G0/1) of the cell cycle. Upon phosphorylation of these proteins by mTORC1 the eukaryotic translation initiation factor 4E (eIF4E), which is normally bound and inhibited by the unphosphorylated 4EBP, is freed and released for initiation of mRNA translation.^{10,40-42} mTORC1 also phosphorylates and activates p70S6K, a kinase that enhances the translation and synthesis of proteins essential for the elongation phase of translation.⁴³ Thus, the activation of mTORC1 allows for synergistic actions of 4EBP and p70S6K to promote initiation and elongation phases of mRNA translation.^{10,40}

The role of mTORC1 in cancer cells has been largely explored but has not been fully elucidated. The best characterized pro-apoptotic molecules known to interact with mTORC1 in cancer cells are BAD, BCL2 and P53. p70S6K, the downstream target of mTORC1, can phosphorylate and inactivate pro-apoptotic BAD by producing a reaction that disrupts BAD's binding to other pro-apoptotic molecules thereby allowing cell survival.^{39,44} On the other hand, in a recent study, Li et al showed that increased expression of anti-apoptotic BCL2 was induced in myeloid progenitor cells upon activation of p70S6K, thereby

promoting cell survival.⁴⁵ Further studies are needed to better understand the effect of rapamycin and its derivatives on apoptosis in various cancer cells.

In summary, mTOR inhibitors affect tumor growth by blocking growth factor stimulation, arresting cell cycle progression, and inhibiting angiogenesis.

VEGF Receptor Inhibitors

VEGF signaling plays an important role in RCC. Molecules that inhibit the kinase activity of VEGF receptors, Sorafenib (Nexavar; Onyx/baker) and Sunitinib (Sutent; Pfizer) were FDA-approved for treatment of advanced RCC in 2005 and 2006, respectively. In a phase 3, randomized, double-blind, placebo-controlled trial of sorafenib in 903 patients with advanced renal-cell carcinoma that was resistant to standard therapy, the median progression-free survival was 5.5 months in the sorafenib group and 2.8 months in the placebo group (P, 0.01).⁴⁶ Sorafenib reduced the risk of death, as compared with placebo (P = 0.02), although this benefit was not statistically significant according to the O'Brien-Fleming threshold.⁴⁶ The conclusion of the study was that sorafenib prolongs progression-free survival in patients with advanced clear-cell renal-cell carcinoma in whom previous therapy has failed.⁴⁶ The anti-VEGF antibody bevacizumab (Avastin; Genentech/Roche) in combination with IFN- α , was approved in Europe for advanced RCC in 2007⁴⁷ and by the FDA in 2009. Temsirolimus (Torisel; Wyeth), a small molecule inhibitor of mTOR, was FDA-approved for treatment of metastatic RCC in 2007.

Rapamycin and Its Analogs in RCC

Rapamycin is a macrolide that was first discovered as a product of the bacterium *Streptomyces hygroscopicus* in a soil sample from Easter Island, an island also known as "Rapa Nui", hence the trade name Rapamycin. Rapamycin was originally developed as an antifungal agent. However, this was abandoned when it was discovered that it had potent immuno-suppressive and antiproliferative properties.^{48,49}

Rapamycin forms an intracellular complex with FK506 binding protein 12 (FKBP-12) that binds and inhibits mTORC1.^{10,41} The chemical properties of rapamycin are similar to those of Everolimus. Both agents display low solubility and therefore are available only for oral formulations whereas temsirolimus and deforolimus are water soluble and may be administered intravenously. In terms of chemical structures, mechanism of action, affinity to target and overall antitumoral activity, there is a striking similarity between rapamycin and all its derivatives. Specifically in renal cell carcinoma, Everolimus has drawn a lot of interest because it provides continuous mTOR inhibition when administered daily.

Everolimus

Everolimus (Affinitor in the USA or Certican in Europe, Novartis Pharmaceuticals), also known as RAD001 was recently approved in 2009 by the FDA and European Medicines Agency (EMA) for treatment of metastatic RCC, at an oral dose of 10 mg once daily, after failure of treatment with sunitinib or sorafenib. Everolimus [42-O-(2-hydroxyethyl)rapamycin] is a rapamycin analogue in which O-alkylation of Rapamycin in

position 40 resulted in a novel, potentially immunosuppressive Rapamycin-derivative with FKBP12 and immuno-suppressive activity as measured by mixed lymphocyte reaction.⁵⁰ Just like Rapamycin, Everolimus selectively binds to FKBP12 to inhibit mTOR kinase. Binding of mTOR with Everolimus prevents mTOR from phosphorylating protein translation factors such as 4EBP1, p70S6K, HIFs, VEGF, hence leading to blockade of the cell cycle at the G1 phase as well as angiogenesis. Rapamycin has immunosuppressive, antifungal and anti-cancer activity. These antineoplastic properties led to investigation of Everolimus as an anticancer therapy in clinical trials.

Everolimus—pharmacokinetics

The pharmacokinetics of Everolimus was initially studied in kidney transplant patients. Budde et al, found that the bioavailability of the tablet formulation was 2.6-fold higher compared with the capsule, with evidence for dose proportionality over the dose range tested.⁵¹ Because of the variable oral bioavailability and narrow therapeutic index of Everolimus, blood concentration monitoring seems to be important. Oral Everolimus is absorbed rapidly, and reaches peak concentration after 1.3–1.8 hours.

A steady state is reached within 7 days, and steady-state peak and trough concentrations, and area under the concentration-time curve (AUC), are proportional to dosage.^{51,52} The excellent correlation between steady-state AUC and trough concentrations, the variable oral bioavailability and narrow therapeutic index of Everolimus, make its trough concentration a simple and reliable index for monitoring Everolimus levels.

Everolimus—Metabolism, Drug Interactions and Excretion

Isoenzymes responsible for the formation of Everolimus metabolites include the cytochrome P450 (CYP) 3A4, 3A5 and 2C8. The critical role of the CYP3A4 system for Everolimus biotransformation leads to interactions with other inducers of the cytochrome such as rifampin. Dose adjustment should be considered when Everolimus is coadministered with inducers or inhibitors of CYP3A4.^{52,53} Everolimus clearance is reduced in patients with hepatic impairment. Elimination half-life is approximately 30 hours and recovery in the feces and urine is 80% and 5%, respectively.

Clinical Trials of Everolimus in Metastatic RCC

Clinical trials.gov lists 300 studies of Everolimus in many different conditions including autosomal dominant polycystic kidney disease (ADPKD), transplant rejection, solid tumors, breast cancer, prostate cancer, leukemia, lymphoma, neuroendocrine tumors, non small cell lung cancer, hepatocellular cancer, gastric cancer, pancreatic cancer, sarcoma, endometrial cancer and skin cancer in transplant patients.

A phase 1 study of the pharmacokinetics and pharmacodynamics of Everolimus in patients with advanced RCC was performed to identify the optimal regimen and dosage. Everolimus 50 and 70 mg weekly or 5 and 10 mg per day was administered in 92 patients. Dose-limiting toxicity was seen in one patient each at 50 mg/wk (stomatitis and fatigue) and 10 mg/d (hyperglycemia). S6 kinase-1 activity in peripheral-blood mononuclear cells was inhibited

for at least 7 days at doses greater than or equal to 20 mg/wk. Area under the curve increased proportional to dose, but maximum serum concentration increased less than proportionally at doses greater than or equal to 20 mg/wk. Terminal half-life was 30 hours (range, 26 to 38 hours). Partial responses were observed in four patients, and 12 patients remained progression free for greater than or equal to 6 months. The study concluded that Everolimus was satisfactorily tolerated at dosages up to 70 mg/wk and 10 mg/d with predictable pharmacokinetics. Doses of 20 mg/wk and 5 mg/d were recommended as appropriate starting doses in RCC.⁵⁴

A double-blind, randomized, placebo-controlled phase 3 trial of the efficacy of Everolimus in advanced RCC was performed in 410 patients from 86 centres in Australia, Canada, Europe, Japan, and the USA.⁵⁵ The study population consisted of adults (aged 18 years and above) with metastatic clear cell RCC which had progressed within 6 months of stopping treatment with sunitinib or sorafenib, or both drugs. Previous therapy with bevacizumab, interleukin -2, or interferon alfa, but not mTOR inhibitors, was also permitted. 26% of patients had previously been treated with both sunitinib and sorafenib and in addition, more than 50% of patients had received cytokine treatment meaning that 65% or more of the patients were third-line or even fourth-line patients. Patients were randomized on a two to one basis to receive Everolimus 10 mg/day or placebo. The primary endpoint was progression-free survival. The study was designed to be terminated after 290 events of progression. The results of the second interim analysis indicated a significant difference in efficacy between Everolimus and placebo and the trial was thus halted early after 191 progression events had been observed. There were 101 [37%] events in the Everolimus group and 90 [65%] events in the placebo group; hazard ratio 0.30, 95% CI 0.22–0.40, $P < 0.0001$; median progression-free survival 4.0 [95% CI 3.7–5.5] vs. 1.9 [1.8–1.9] months). There was a 70% reduction in the risk of disease recurrence or death. Stomatitis rash, fatigue and pneumonitis (any grade) were side-effects in the Everolimus group.⁵⁵ The study supports the anticancer activity of Everolimus in patients with advanced RCC, but does not show that Everolimus can improve overall survival.⁵⁶ The use of progression-free survival as a surrogate for overall survival and clinical benefit is controversial.⁵⁶ The study also did not determine the optimal sequencing of drugs in advanced RCC.⁵⁶

In the large study by Motzer et al⁵⁵ median overall survival had not yet been reached for the everolimus group and data were not yet mature when the study was halted. Updated data from the phase 3 randomized trial of everolimus (RAD001) versus placebo in metastatic renal cell carcinoma showed that overall survival for everolimus was 14.8 months and for placebo was 14.4 months.⁵⁷ The lack of a difference in overall survival between everolimus compared to placebo is due to confounding by crossover: of the 139 patients in the placebo group who progressed, 112 crossed over to everolimus. Thus, it will be impossible to demonstrate an overall survival benefit in this setting without censoring the cross-over patients. In addition, even in the first-line setting, the overall survival benefit of sunitinib, could be shown only after censoring, because some of the new anti-angiogenic drugs will be given to progressing patients after first-line treatment. Thus, overall survival benefits for a single drug in RCC will be increasingly difficult, if not impossible, to demonstrate in the future as was the case in the phase 3 trial.⁵⁵ Updated data from the phase 3 randomized trial

of everolimus versus placebo in metastatic renal cell carcinoma also showed that progression free survival for everolimus was 4.9 months and for placebo was 1.9 months.⁵⁷

In a phase 2 study, 41 patients with metastatic clear cell RCC were treated with a daily regimen of Everolimus to assess the efficacy of daily oral dosing with Everolimus.⁵⁸ Everolimus was administered at a dose of 10 mg daily orally without interruption (28-day cycle) with dose modifications for toxicity. Study patients were evaluated every 2 cycles (8 weeks) using Response Evaluation Criteria in Solid Tumors (RECIST). 83% of patients had received prior therapy. The median progression-free survival (PFS) was 11.2 months and the median overall survival was 22.1 months. Nausea (38% of patients), anorexia (38% of patients), diarrhea (31% of patients), stomatitis (31% of patients), pneumonitis (31% of patients), and rash (26% of patients) were common. Grade 3 or 4 adverse events included pneumonitis (18% of patients); transaminase elevations (10% of patients); thrombocytopenia, hyperglycemia, alkaline phosphatase elevations (8% of patients); and hyperlipidemia (5% of patients). It was concluded that Everolimus demonstrated encouraging antitumor activity against metastatic RCC as indicated by a PFS greater than or equal to 6 months for approximately 70% of patients.

Completed and active clinical trials, that have not yet been published, of Everolimus in RCC are described in Table 1.

Summary

In addition to the VEGF inhibitors (sunitinib, sorafenib and bevacizumab), the mTOR inhibitor (temsirolimus) and immunotherapy with IL-2, everolimus is a new treatment option for patients with renal cell cancer. Everolimus (Affinitor, Novartis) has recently been FDA-approved as the first treatment for patients with advanced RCC, following a failed response to previous therapies using other kinase inhibitor drugs such as sunitinib or sorafenib. The FDA-approval was based mainly on a multinational study of 410 patients with advanced RCC that showed that there was a 70% reduction in the risk of disease recurrence or death in patients treated with Everolimus compared to placebo. Further studies in RCC are needed to determine the optimum sequence of mTOR inhibition, combination therapies with mTOR inhibitors and other targeted agents, biomarkers for appropriate treatment selection, the effect of therapies on overall survival and treatment of histology other than clear cell cancer.

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Figure 1.

mTORC1 is a component of mTOR that is directly inhibited by rapamycin and rapamycin derivatives like everolimus. mTORC1 is activated by growth factors and amino acids, which activate PI3K. PI3K phosphorylates PIP₂ to yield PIP₃. PIP₃ phosphorylates and activates Akt which phosphorylates and inhibits the TSC. TSC negatively regulates mTORC1 by inhibiting Rheb which normally activates mTORC1. PTEN, a phosphatase that dephosphorylates PIP₃ to convert it back to PIP₂, negatively regulates PI3K. Upon activation of mTOR, the G1-S cell cycle transition and cell proliferation are stimulated while the apoptosis pathway is inhibited. In addition, mTOR acts as angiogenic agent via activation of HIF to promote tumor growth.

Table 1

Clinical trials of Everolimus in metastatic RCC (completed and active).

Intervention	Study design	Primary outcome measure	Start-finish dates (status)	Sponsor
RAD001/sunitinib phase 1B NCT00788060	Nonrandomized open-label, active control dose single group assignment	Maximum tolerated	2008–2009 (recruiting)	Duke University
Biomarkers in RCC phase 2 NCT00827359	Nonrandomized open-label, active control single group assignment	pAkt and pS6, as markers of RCC in response to Everolimus	2009–2011 (recruiting)	Beth Israel Deaconess Med Center
RAD001 for Non-clear renal RCC Phase 2 NCT00830895	Nonrandomized open-label uncontrolled single group assignment	Progression-free survival	2009–2012 (recruiting)	Seoul National University Hospital
RAD001/sunitinib phase 1 NCT00422344	Nonrandomized open-label, active control single group assignment	Maximum tolerated dose	2006–2010 (recruiting closed)	Memorial Sloan- Kettering Cancer Ctr
RAD001/sorafenib Phase 1/2 NCT00384969	Nonrandomized open-label, active control single group assignment	Maximum tolerated dose	2006–2010 (Completed)	University of California San Fran
RAD001 for Papillary RCC Phase 2 NCT0688753	Nonrandomized open-label single group assignment	Progression-free survival	2009–2011	Novartis
RAD001/bevacizumab interferon alfa-2a/ Bevacizumab phase 2 NCT00719264	Randomized open- label, placebo-controlled Multicenter	Progression-free survival	2008–2012	Novartis
RAD001/imatinib phase 2 NCT00331409	Nonrandomized open-label, active control Multicenter	Progression-free survival	2006–2009 (recruiting closed)	Oregon Health and Science University
RAD001/Bevacizumab Phase 2 NCT00651482	Nonrandomized open-label, active control Multicenter	Progression-free survival	2008–2011 (recruiting)	Stanford University
RAD001 Bevacizumab Phase2 NCT00323739	Nonrandomized open-label, uncontrolled single group assignment	Progression-free survival	2006–2009 (recruiting closing)	Sarah Cannon Research institute
RAD001/BSC vs. BSC/ placebo- controlled phase 3 NCT00410124	Randomized double-blind, crossover, placebo-controlled Multicenter	Progression-free survival	2006–2009 (recruiting closing)	Novartis
RAD001/vatalanib Phase 1 NCT00655655	Treatment	Maximum tolerated dose	2004–2009	Mayo Clinic
RAD001 Phase 2 NCT00529802	Nonrandomized open-label uncontrolled single group assignment	Change in FDG- PET associated with tumor shrinkage	2007–2010 (recruiting)	University of Chicago
RAD001 Phase 2 NCT00446368	Nonrandomized open-label, uncontrolled single group assignment	Timeframe of tumor progression produced by treatment	2005–2008 (Completed)	Methodist Hospital System