Everolimus
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Abstract
Everolimus, an orally administered rapamycin analog, has recently been approved by the U.S. Food and Drug Administration for treatment of renal cell carcinoma (RCC) refractory to inhibitors of vascular endothelial growth factor (VEGF) receptor signaling. Everolimus significantly increased progression-free survival (median PFS for the everolimus treated group was 4.0 months versus 1.9 months for the placebo group), although tumor regressions were observed only infrequently. Although the target for everolimus, [the serine-threonine kinase mammalian target of rapamycin (mTOR)] is well established, the mechanism by which this agent retards tumor growth is not well defined. Further, biomarkers that predict tumor sensitivity are still elusive. The mechanism of action, preclinical antitumor activity, and clinical activity of everolimus against RCC are reviewed. Clin Cancer Res; 16(5); 1368–72. ©2010 AACR.

Preclinical Data
Everolimus [Afinitor, RAD-001 (40-O-(2-hydroxyethyl)-rapamycin)] is a rapamycin analog (rapalog) that is being developed as an antitumor agent. Like rapamycin, everolimus binds the cyclophilin FKBP-12, and this complex binds the serine-threonine kinase mammalian target of rapamycin (mTOR) when it is associated with raptor and mLST8 to form a complex (mTORC1), and inhibits signaling downstream. Importantly, rapalogs are not direct mTOR kinase inhibitors at pharmacologically achievable drug concentrations. mTORC1 lies downstream of phosphatidylinositol 3’ kinase (PI3K), in a pathway that is very frequently activated in human cancers. Hence mTORC1 represents a pivotal target for cancer therapy (Fig. 1). mTORC1 regulates cap-dependent translation, transcription, cell cycle progression, and survival. This complex coordinates cell growth and metabolism by acting as a restriction point in cells under stress conditions (1–5), such as low oxygen tension (hypoxia; refs. 6–8). The best characterized pathways regulated by mTORC1 are phosphorylation and activation of ribosomal S6 kinase-1 (S6K1) and phosphorylation and inactivation of 4E-BP1, the suppressor of the mRNA cap-binding protein eIF4E. mTORC1-mediated phosphorylation of 4E-BP1 leads to its disassociation from eIF4E, and subsequent association of eIF4E with a scaffolding protein (eIF4G), and recruitment of initiation factors to form the pre-initiation translation complex (eIF4F) required for efficient translation of mRNA species that have highly structured 5′-untranslated regions (5′-UTR; ref. 9). These include cell cycle regulators Cyclin D1 and ornithine decarboxylase, required for transit from G1 phase to S phase, and transcription factors c-MYC and HIF-1α (10).

mTOR exists in a second complex (mTORC2) associated with rictor, sin1, and mLST8, which phosphorylates Akt at Ser-473, leading to full activation of Akt. Anecdotally, inhibition of mTORC1 by rapalogs leads to hyperphosphorylation of Akt(Ser473) in many cancer cell lines, probably through stabilization of IRS-1 subsequent to downregulation of the activity of the mTORC1 substrate S6K1, which normally negatively regulates signaling flux through the type-1 insulin-like growth factor receptor (IGF-1R; refs. 11, 12). Importantly, activation of Akt may lead to survival when mTORC1 is inhibited, or potentially increased VEGF production as PI3K/AKT signaling can induce tumor angiogenesis by regulating VEGF. This regulation occurs at both the mRNA and protein levels, and its regulation of
VEGF mRNA seems to occur by both HIF-1α-dependent and -independent mechanisms (13).

Everolimus and other rapalogs potently inhibit growth of numerous human tumor cell lines, with 50% inhibition of growth in the sub-nanomolar concentration range, inhibit proliferation of human umbilical vein endothelial cells, and reduce expression of HIF-1α and VEGF in cultured tumor cells. The in vivo antitumor activity of rapamycin was identified almost 30 years ago in a National Cancer Institute (NCI) screen but for various reasons was not developed for cancer treatment. Everolimus, a hydroxyethyl ether derivative of rapamycin, has superior pharmaceutical characteristics to rapamycin, and was designed for oral administration. Unlike temsirolimus, everolimus is not converted to rapamycin in vivo.

Everolimus inhibits the growth of human tumor xenograft models in nude mice even when the cell line in vitro was insensitive to this agent. Similar results have been reported for rapamycin and temsirolimus, and suggest that tumor retardation is a consequence of the agent's anti-angiogenic activity. Consistent with this finding is the reduced blood vessel density observed in several tumor models growing in mice treated with everolimus (14). However, many tumors seem intrinsically resistant to rapalogs, although the mechanism for resistance is as yet unknown. In some human sarcoma xenograft models, rapamycin treatment significantly increased the level of tumor-associated VEGF (15). Of importance is that the spectrum of antitumor activity differs between everolimus and small molecule receptor tyrosine kinase inhibitors that target VEGF receptors (14). At doses in mice that showed effective tumor control (0.5-5 mg/kg/d), everolimus was well tolerated, suggesting that it is a very promising chemotherapeutic agent.

Clinical Studies

The tolerability and efficacy of everolimus has been evaluated in several clinical settings. In the initial phase 1 trial, weekly oral administration at dosages up to 70 mg or daily oral dosing up to 10 mg were examined. Although dose-limiting toxicity was not determined in either arm of this trial, toxicity profiles and frequency were similar between weekly dosing at 70 mg and daily dosing at 10 mg. Most frequent drug-related adverse events were rash, stomatitis and/or mucositis, fatigue, nausea, and vomiting. Although NCI-common toxicity criteria (CTC) events ≥grade 3 were rare, hyperglycemia, hyperglyceridemia, and thrombocytopenia were common to both schedules, the total number of grade 3 adverse events was slightly higher in the daily dosing arm (16). Notably, prolonged disease stabilization of RCC patients (26 months) was observed in both weekly schedules (n = 3) and daily dosing schedules (n = 2), as well as...
other tumors. Pharmacodynamic studies assessing mTORC1 inhibition in peripheral mononuclear cells showed sustained inhibition of S6K1 activity at ≥20 mg daily and ≥5 mg daily.

Phase 2 testing of everolimus was in patients with predominantly clear-cell RCC who had received ≤1 prior treatment, and had progressive measurable metastatic disease. Everolimus was administered orally on a continuous daily schedule, with dose modifications for toxicity. Patients were assessed every 8 weeks (2 cycles) using Response Evaluation Criteria in Solid Tumors (RECIST). The population (n = 37 evaluable patients) was predominantly male (78%), median age 60 years, and good performance status (Zubrod 0-1), and most had received prior therapy (93%). The median progression-free survival (PFS) was 11.2 months, and the median overall survival 22.1 months. Partial responses were observed in 14% patients (7% by independent review), stable disease ≥3 months occurred in 73%, and stable disease lasting ≥6 months in 57% of patients. Frequent toxicities recapitulated those seen in the phase 1 trial with nausea (31%), anorexia (38%), diarrhea (31%), stomatitis (31%), pneumonitis (31%), and rash (26%) being reported. Severe toxicities (≥grade 3) were pneumonitis (18%), elevated transaminases (10%), thrombocytopenia, hyperglycemia, alkaline phosphatase elevation (8%) each, and hyperlipidemia (5%).

The phase 3 registration trial (NCT00410124) was a randomized, double-blind, placebo-controlled trial of everolimus in patients with metastatic RCC whose disease had progressed on sunitinib, sorafenib, or both drugs (17). Patients were randomly assigned in a 2:1 ratio to receive everolimus 10 mg once daily (n = 272) or placebo (n = 138). The primary endpoint was PFS and was assessed by blinded, independent central review.

All 410 patients were included in efficacy analyses. Results from a second interim analysis indicated a significant difference in efficacy between arms. The trial was thus halted early after 191 progression events had been observed [101 (37%) events in the everolimus group, 90 (65%) in the placebo group; hazard ratio 0.30, 95% confidence interval [CI] 0.22-0.40, P < 0.00001]. Median PFS for the everolimus treated group was 4.0 months (95% CI 3.7-5.5) versus 1.9 months (1.8-1.9). The most commonly reported adverse events, stomatitis (everolimus, 40%, versus placebo, 8%), rash (25% versus 4%), and fatigue (20% versus 16%) were mostly mild or moderate in severity. Pneumonitis was detected in 8% of patients receiving everolimus with eight having grade 3 severity. The conclusion from this phase 3 study was that everolimus prolongs PFS in patients with metastatic RCC who had progressed on agents that target VEGF-targeted therapy. Thus, everolimus, like temsirolimus, has significant activity in the setting of RCC, being largely cytostatic and inducing RECIST-defined partial tumor regression only infrequently (1%). Whether the antitumor mechanism of action of everolimus is uniquely directed at the VEGF locus is less certain, although the study supports the nonclinical results indicating that everolimus has antiangiogenic properties distinct from VEGF receptor tyrosine kinase inhibitors (18).

**Pharmacodynamic Studies**

Identifying robust pharmacodynamic markers that predict sensitivity to rapalogs has proven difficult. Phosphorylation of ribosomal protein S6, the most frequent biomarker used, is equally inhibited in cell lines that are sensitive or insensitive to everolimus (18). Similarly, enhanced Akt Ser473 phosphorylation induced by mTORC1 inhibition does not correlate with cell sensitivity, or activation of Akt substrates. Other markers of rapalog sensitivity proposed have been treatment-induced down-regulation of cyclin D1 and CDK4 transcripts, the basal expression of the anti-apoptotic protein BCL-2 and the basal phosphorylation state of Akt. The most comprehensive clinical pharmacodynamic study reported (19) compared markers of mTORC1 inhibition in tumor and skin biopsies. Everolimus was administered either daily (5 or 10 mg) or weekly (20, 50, and 70 mg). There was good concordance between mTORC1 pathway inhibition in tumor and skin biopsies. Inhibition of mTORC1 signaling was observed at all doses and schedules with almost complete inhibition of S6 and eIF4G phosphorylation, although the magnitude of inhibition of 4E-BP phosphorylation was less in tumor than skin. At both dose levels on the daily schedule, proliferation was reduced in tumor and skin, and phospho-Akt increased in approximately half of tumor samples. These pharmacodynamic studies indicated that inhibition of mTORC1 was more robust and prolonged using daily dosing at 10 mg rather than weekly dosing schedules. However, of note was that whereas S6 phosphorylation was suppressed for 5 days after the last dose of everolimus (weekly schedule), hyperphosphorylation of Akt (Ser473) was not maintained. Although these pharmacodynamic studies were valuable for selecting biologically effective dose levels for each schedule (10 mg daily and 50 mg/wk), they did not identify a marker that predicts tumor sensitivity.

**Advantages Over Other Agents**

Three agents were approved for treatment of RCC prior to approval of everolimus. Sorafenib (Nexavar), a rather promiscuous kinase inhibitor with targets including Raf kinase, platelet-derived growth factor (PDGF), VEGF receptor 2 and 3 kinases, and c Kit, the receptor for Stem cell factor, prolongs PFS in patients with advanced clear-cell RCC in whom previous therapy has failed; the median PFS was 5.5 months in the sorafenib group and 2.8 months in the placebo group (hazard ratio for disease progression in the sorafenib group, 0.44; 95% CI 0.35-0.55, P < 0.01).

Sunitinib (Sutent, SU11248), another relatively nonselective inhibitor targeting VEGF receptors, PDGFR, and
Everolimus has established activity for treatment of refractory RCC. Modest activity has been reported for non-small cell lung cancer patients failing chemotherapy or EGFR-targeted treatment (21) or patients with recurrent or metastatic breast cancer (22). Activity has been observed also in hematologic malignancies (23), and everolimus is in phase 2 evaluation for the treatment of childhood malignancies. The agent is largely cytostatic, inducing relatively few regressions, thus the challenge will be in combining everolimus with other targeted agents that together will be cytotoxic: a challenge not unique to this class of agent. Recent studies in childhood sarcoma xenografts suggest that combining rapamycin with an antibody that blocks ligand binding to the insulin-like growth factor receptor leads to synergistic antitumor activity (15). However, clinical trials combining rapalogs with other signaling inhibitors such as erlotinib have generally shown enhanced host toxicity, and combination with standard cytotoxic agents may have variable effects from synergy to antagonism. A second and perhaps more important challenge will be to identify biomarkers that predict response to everolimus. Preferably such biomarkers would be detected in pretreatment biopsies, rather than in response to therapy. The other, largely unanswered question is the biological relevance of Akt hyperphosphorylation following inhibition of mTORC1 signaling. If activation of Akt is important in signaling survival (supported by an extensive literature) one may anticipate superior activity of mTOR kinase inhibitors currently in phase 1 clinical trials, which inhibit both mTORC1 and mTORC2 signaling.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

Original work discussed from this laboratory was supported by PHS awards CA23093 and CA77776 from the National Cancer Institute. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.


References


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*Clin Cancer Res* 2010;16:1368-1372. Published OnlineFirst February 23, 2010.

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