Inhibitors of mTOR

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ABSTRACT

Inhibitors of mammalian target of rapamycin (mTOR) have been approved for the treatment of renal cell carcinoma and appear to have a role in the treatment of other malignancies. The primary objective of this drug review is to provide pharmacokinetic and dynamic properties of the commonly used drugs everolimus and temsirolimus. Additionally, informa-

tion on clinical use, mechanism of action, bioanalysis, drug–drug interactions, alterations with disease or age, pharmacogenetics, and drug resistance is given. This overview should assist the treating medical oncologist in adjusting treatment with mTOR inhibitors to individual patient circumstances. *The Oncologist* 2010;15:1262–1269

INTRODUCTION

Inhibitors of mammalian target of rapamycin (mTOR) have anticancer activity. Recently, two mTOR inhibitors were registered in adults for the treatment of cancer: (a) temsirolimus (Fig. 1A, Table 1), for the treatment of advanced renal cell cancer (RCC) as first-line treatment for patients of the unfavorable prognostic group (Temsirolimus is also indicated for the treatment of mantle cell lymphoma [orphan designation].) and (b) everolimus (Fig. 1B, Table 1), for the treatment of RCC as second-line treatment. Everolimus has already undergone exten-

sive clinical testing in the renal and cardiac transplantation settings, and is well tolerated and effective with daily dosing [1, 2]. Everolimus is a derivative of sirolimus (rapamycin), a macrolide antibiotic produced by *Streptomyces hygroscopicus*, an actinomycete isolated in 1975 from soil of Rapa-Nui (Easter Island) [3, 4]. In 1991, TOR was discovered in yeast [5]. The only known homolog in mammals was subsequently cloned and called mammalian TOR, or mTOR [6]. mTOR has different functions depending on whether it binds to regulatory-associated protein of mTOR (RAPTOR, mTOR complex

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Figure 1. Chemical structures of temsirolimus, 42-[3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate] (A), and everolimus, [40-O-(2-hydroxy)ethyl-rapamycin] (B).

1 [mTORC1]) or rapamycin-insensitive companion of mTOR (RICTOR, mTOR complex 2 [mTORC2]). mTORC1 controls translation, suppresses autophagy, and regulates transcription and response to DNA damage through the phosphorylation of its downstream substrates 4E-BPs and S6Ks [7]. Inhibition of mTORC1 by rapalogs leads to hyperphosphorylation of Akt(Ser-473) in many cancer cell lines. Importantly, activation of Akt may lead to survival when mTORC1 is inhibited. mTORC2 regulates actin cytoskeleton and activates Akt through phosphorylation of Ser-473 [8, 9]. Inhibitors of mTOR predominantly work through their effect on mTORC1 [10].

Ongoing studies are investigating the potential anticancer activity of mTOR inhibitors in lymphomas, neuroendocrine tumors, and gastric, breast, lung, and hepatocellular cancer (<http://www.clinicaltrials.gov>). Other mTOR inhibitors under investigation are ridaforolimus (formerly, deforolimus, AP23573) and sirolimus itself, in combination with grapefruit juice. We focus on the European Medicines Agency and U.S. Food and Drug Administration (FDA)-approved drugs temsirolimus and everolimus.

CLINICAL USE

Temsirolimus, a more water-soluble ester derivative of its parent compound sirolimus, is available as a concentrate for solution for i.v. injections (25 mg/ml). The recommended dose is 25 mg weekly [9, 11].

Everolimus is available as oral tablets of 1.5 mg, 5, mg and 10 mg. The daily recommended dose is 10 mg, either with or without food [12]. Preliminary dose-finding studies suggested that the dose of everolimus may need to be reduced in combination therapy, mainly because of cumulative toxicity [13, 14].

Optimal dosing of mTOR inhibitors may be difficult to define based on toxicity [15].

MECHANISM OF ACTION

mTOR is a highly conserved serine–threonine kinase and a key regulatory protein in cancer that recognizes stress signals (e.g., nutrient and energy depletion, oxidative or hypoxic stress, and proliferative and survival signals) via the phosphoinositide 3-kinase (PI3K)–Akt pathway. Signals from growth factor receptors activate PI3K, resulting in Akt activation and, finally, activation of the centrally located downstream mTOR. It has been demonstrated that Akt, mTOR, and S6K1 are phosphorylated (activated) in most cancer types. These data suggest that activation of PI3K/Akt/mTOR is essential for proliferation and survival of malignancies and mTOR might therefore be a promising target in cancer treatment [16, 17].

Temsirolimus and the active metabolite sirolimus, when bound to an intracellular protein (FKBP-12), form a protein–drug complex inhibiting the activity of mTOR that controls cell division. Inhibition of mTOR activity resulted in G₁ growth arrest in treated tumor cells. When mTOR is inhibited, the downstream targets p70S6k and S6 ribosomal protein are not phosphorylated. Additionally, temsirolimus and sirolimus are thought to retard tumor angiogenesis, induce apoptosis, reduce expression of hypoxia-inducible factor-1 α , and sensitize cancer cells to apoptosis induction by DNA-damaging agents such as cisplatin [18–20]. In cell lines, mTOR inhibitors are able to sensitize cancer cells to chemotherapy and radiation, and overcome chemotherapy or endocrine therapy resistance [21–24]. Combination therapy studies of mTOR inhibitors with either endocrine therapy or chemotherapy in metastatic cancer patients showed only a moderate additional benefit [13, 25].

BIOANALYSIS

Temsirolimus and everolimus blood concentrations can be measured by validated liquid chromatography–tandem mass spectrometry combination procedures [26].

Simultaneous quantification of comedication is possible

Table 1. Pharmacological features of everolimus and temsirolimus

Summary table		
Generic name	Temsirolimus	Everolimus
Commercial names	CCI-779, Torisel®, CCI-779	SDZ-RAD, RAD001, Certican®, Afinitor®
Mechanism of action	Inhibition of PI3K–Akt–mTOR pathway, resulting in modulation of cellular metabolism, growth, proliferation, and angiogenesis	
Molecular weight	1030.30 g/mol	958.22 g/mol
Cell-cycle specificity	G ₁ phase	
Route of administration	i.v.	Oral, low bioavailability (16%), high-fat meal reduces absorption
Protein binding	~85%	75%
Metabolism	By CYP3A4 to active metabolite sirolimus	By CYP3A4 to main metabolite hydroxy-everolimus
Elimination	Primarily via feces (82%), also via urine (4.6%)	Metabolites via feces (>90%) and urine (2%)
Terminal half-life	9–27 hours	26–30 hours
Main toxicities	Mucositis, thrombocytopenia, rash, asthenia, nausea, edema, anorexia, anemia, hyperglycemia, hyperlipemia, hypertriglyceridemia, elevated alkaline phosphatase, elevated serum creatinine, lymphopenia, hypophosphatemia	
Pharmacogenetics	To be investigated; data for sirolimus, the main metabolite of temsirolimus, show a correlation between <i>CYP3A4</i> *1/*1B polymorphism and sirolimus pharmacokinetics	
Resistance	Activation of IGFR and Akt/PKB results in activation of both PI3K pathway and antiapoptotic signaling	
Unique features	Ability to penetrate the brain–tumor barrier [69]; allergic reaction possible	Extended experience as immune suppressant in drug-eluting stents
Main drug–drug and drug–complementary and alternative medication interactions	50% dose if concurrent strong inhibitors of CYP3A4 ^a	Interaction with CYP3A4, CYP3A5, and CYP2C8 inducers/inhibitors ^a (e.g., rifampin leads to greater CL/F of everolimus, by ~172%)

^aStrong CYP3A4 inhibitors include nelfinavir, ritonavir, clarithromycin, itraconazole, ketokonazole; moderate CYP3A4 inhibitors include aprepitant, erythromycin, fluconazole, grapefruit juice, verapamil; CYP3A4 inducers include barbiturates, glucocorticoids, phenytoin, St. John's wort; CYP3A4 substrates include dexamethasone, paclitaxel, sunitinib, sorafenib, vincristine, irinotecan, docetaxel.

Abbreviations: CL/F, clearance of the absorbed drug fraction; CYP, cytochrome P450; IGFR, insulin-like growth factor receptor; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B.

[27]. The high costs of the mass spectrometry technique and the demanding technical knowledge are disadvantages. An alternative is a validated enzyme-linked immunosorbent assay platform, which has been applied in several studies [28–30].

PHARMACOKINETICS

Absorption

Everolimus absorption is rapid. After ~30 minutes (range, 0.5–1 hour), the maximum concentration ($C_{\max}$) of everolimus is reached ($44.2 \pm 13.3 \mu\text{g/l}$), with an area under the curve (AUC) of $219 \pm 69 \mu\text{g}\cdot\text{h/l}$ [31]. Steady state is reached within 7 days, with a median threefold accumulation of everolimus exposure. Absorption of everolimus is

reduced by about 50% after a high-fat meal. It is therefore recommended to take the drug consistently with or without food [28]. The overall absorption of everolimus, like that of sirolimus, is probably affected by the activity of P-glycoprotein (P-gp) [32–34]. Liver impairment (liver cirrhosis Child-Pugh class B) did not alter absorption of everolimus [35].

In rats, the oral bioavailability of everolimus is low (16%) but higher than that of sirolimus (10%) [32]. Steady-state $C_{\max}$, steady-state trough concentration ($C_{\min_{ss}}$), and AUC showed a dose-proportional increase, and $C_{\min_{ss}}$ correlated well with the AUC of everolimus. There is wide interindividual pharmacokinetic (PK) variability for the AUC of 85.4% and an intraindividual interoccasion variability of 40.8%, suggesting a role for therapeutic drug monitoring using $C_{\min_{ss}}$ [36].

Protein Binding

Protein binding of temsirolimus after i.v. injection is ~85% (FDA label). Sirolimus, the major active metabolite of temsirolimus, is approximately 40% bound to the lipoprotein fraction in blood over a sirolimus concentration range of 5–100 ng/ml. Increases in plasma lipoproteins may increase sirolimus plasma concentrations. Patients with Child-Pugh class A or B liver cirrhosis may require a dose reduction by one third [37, 38].

At therapeutic concentrations of everolimus, >75% is partitioned into RBCs and approximately 75% of the plasma fraction is protein bound. The protein binding of everolimus was not influenced by moderate hepatic impairment (liver cirrhosis versus healthy, $73.8\% \pm 3.6\%$ versus $73.5\% \pm 2.4\%$) [35, 36].

Metabolism

Temsirolimus and its primary metabolite, sirolimus, are metabolized by the cytochrome P450 (CYP)3A4 pathway. Sirolimus appears 15 minutes after infusion of temsirolimus with a peak at 0.5–2.0 hours, followed by a monoexponential decrease. Exposure to sirolimus is typically higher than that of temsirolimus, with a mean AUC ratio (sirolimus/temsirolimus) of ~2.5–3.5 (coefficient of variation [CV], 0.2%–69%). Dose-related increases in the sum (sirolimus + temsirolimus) of the AUCs during treatment were significantly less than proportional. The mean terminal half-life for sirolimus was in the range of 61–69 hours (CV, 7%–60%). At doses >34 mg/m², residual concentrations of sirolimus were detectable before the next infusion 7 days later. However, this did not result in a higher AUC of sirolimus after repeated cycles [39].

Everolimus is metabolized mainly in the gut and liver by CYP3A4, CYP3A5, and CYP2C8 [40]. Everolimus and four main metabolites, hydroxy-, dihydroxy-, and demethyl-everolimus and the ring-opened form of everolimus, were found in blood. Hydroxy-everolimus was the most important metabolite, with a dose-normalized AUC of the first 24 hours (AUC₂₄) nearly half that of the parent compound (16.0 ± 6.5 versus 35.4 ± 13.1 $\mu\text{g}\cdot\text{h/l}$), followed by demethyl-everolimus (AUC₂₄, 10.7 ± 15.8 $\mu\text{g}\cdot\text{h/l}$), dihydroxy-everolimus (AUC₂₄, 8.5 ± 5.7 $\mu\text{g}\cdot\text{h/l}$), and ring-opened everolimus (AUC₂₄, 2.3 ± 2.1 $\mu\text{g}\cdot\text{h/l}$). All metabolites appeared relatively soon after administration (time to maximal concentration, 1.2–2.0 hours, versus 1.5 hours for everolimus). The immunosuppressive or toxic activity of everolimus metabolites is unknown [27, 41].

Elimination

The terminal half-life ($t_{1/2}$) of temsirolimus, given at a standard dose of 25 mg weekly, is 13 hours, with a total plasma

clearance (CL) of 16 l/h [42]. Increasing doses of temsirolimus induce significant increases in CL (equivalent to the clearance of the absorbed drug fraction [CL/F]) and decreases in the mean $t_{1/2}$ (CL/F, 19–51 l/h; CV, 14%–32%; $t_{1/2}$, 22 hours following a 34 mg/m² dose to 13 hours following a 220 mg/m² dose; CV, 7%–29%). This suggests autoinduction of factors increasing CL of the drug at higher than clinically used doses [43]. Multiple dosing of temsirolimus has no significant influence on the PK profile [39]. Temsirolimus is excreted predominantly via the feces (78%) and to a minor extent via urine (5%).

About 98% of everolimus is excreted in the bile as metabolites and only 2% of everolimus is eliminated via the urine. The $t_{1/2}$ of everolimus in plasma applied at a dose of 70 mg weekly is approximately 26 hours [44]. For the standard dose of 10 mg everolimus daily in cancer patients, no complete PK data are available yet. Extensive data in non-cancer patients are available [27]. Compared with healthy subjects, patients with moderate hepatic impairment (Child-Pugh class B liver cirrhosis) had a significantly lower CL/F of everolimus, by 53% on average.

Drug and Complementary and Alternative Medicine Interactions

Potential PK drug–drug interactions for temsirolimus exist with agents that modulate CYP3A4 isozyme activity. Co-administration of 400 mg oral ketoconazole with 5 mg i.v. temsirolimus resulted in a 3.1-fold higher mean AUC than with temsirolimus alone. P450-inducing anticonvulsant agents resulted in 73% and 50% lower C_{max} and AUC values, respectively [45]. If a concomitant strong CYP3A4 inhibitor is necessary, a temsirolimus dose reduction to 12.5 mg weekly should be considered [46]. In vitro studies showed that temsirolimus and sirolimus inhibit the CYP2D6 isozyme ($K_i = 1.5$ and 5 μM , respectively), indicating a potential for PK interaction with agents that are substrates of CYP2D6. However, a single i.v. dose of 25 mg temsirolimus did not alter the disposition of desipramine, widely used as a probe to study potential CYP2D6 drug interactions [47].

Everolimus is a substrate of CYP3A4, CYP3A5, CYP2C8, and the efflux transporter P-gp. Everolimus is, at the same time, a moderate inhibitor of P-gp, a competitive inhibitor of CYP3A4, and a mixed inhibitor of CYP2D6 in vitro [32, 40]. Drug interaction is most likely by drugs influencing activity of the above-mentioned enzyme systems and transporters. Potent or moderately potent inhibitors of CYP3A4, like ketoconazole, erythromycin, or verapamil, will cause a PK interaction, resulting in higher C_{max} and AUC values for everolimus. Concomitant use of potent or moderately potent CYP3A4 inhibitors (e.g., aprepitant,

clarithromycin, diltiazem, erythromycin, fluconazole, grapefruit, itraconazole, ketoconazole, nefazodone, telithromycin, verapamil, voriconazole, and most anti-HIV medication) should be avoided.

Potent inducers of CYP3A4, like rifampin, pharmacokinetically interact with everolimus, leading to lower $C_{\max}$ and AUC values [31]. Concomitant use of potent CYP3A4 inducers (e.g., carbamazepine, dexamethasone, phenobarbital, phenytoin, rifabutin, rifampin) should be avoided. If concomitant use of a potent CYP3A4 inducer cannot be avoided, an increase in everolimus dosage (from 10 mg daily up to 20 mg daily, titrated in 5-mg increments) should be considered based on PK considerations; however, no clinical data on this dosage adjustment in this patient population are currently available. If the potent CYP3A4 inducer is discontinued, the dosage of everolimus should be reduced to the usual recommended dosage.

Neither statins, like atorvastatin or pravastatin, nor anticancer drugs, like letrozole or gefitinib, cause significant alteration of drug exposure [30, 48]. There are no specific data available on the interaction between complementary alternative medicines and mTOR inhibitors.

Alterations with Disease or Age

Efficacy and dosing of temsirolimus are independent of age. A higher incidence of thrombocytopenia was noted in patients with mild hepatic impairment treated with temsirolimus [49]. Moderate and severe hepatic impairment have not been studied in treatment with temsirolimus. Concurrent hemodialysis did not show any influence on temsirolimus and sirolimus PKs, excluding the need for temsirolimus dose adjustments for renal impairment [50]. Interestingly, higher doses of temsirolimus (175 mg weekly for 3 weeks followed by 75 mg weekly) were well tolerated in a phase III study of mantle cell lymphoma [51].

Everolimus PK characteristics did not differ with age, sex, and weight in adults [36]. The dosage of everolimus should be reduced by half in patients with significant hepatic impairment [35].

Only 2% of everolimus is eliminated in the urine; therefore, renal impairment is not expected to influence drug exposure. No dosage adjustment of everolimus is recommended in patients with renal impairment.

PHARMACOGENETICS

Boni et al. [52] found, in a population PK study, that the pharmacogenomic profiling of identified peripheral blood mononuclear cells (PBMCs) altered the expression of ribonucleic acid transcript levels that correlate with exposure.

However, sirolimus pharmacogenetic studies from renal organ transplant show an association between the *CYP3A4**1/*1*B* polymorphism and sirolimus PKs as well as lower dose-corrected trough levels in *CYP3A4**1*B*/*1*B* than in *CYP3A4**1/*1 patients [53]. This was, however, not confirmed by other studies [54]. None of the *CYP3A4*, *CYP3A5*, and *ABCB1* polymorphisms have been associated with sirolimus toxicity or efficacy [53, 55].

DRUG RESISTANCE

Rapamycin induces activation of Akt, an oncogenic kinase, in some models [56, 57]. Insulin-like growth factor (IGF)-I and insulin-dependent induction of the PI3K–Akt pathway lead to feedback inhibition of signaling resulting from mTOR/S6K-mediated phosphorylation. Rapamycin-induced Akt activation has been attributed to loss of this negative-feedback loop. The effect of rapamycin on Akt may vary with drug dose, with lower doses leading to greater Akt activation and higher doses leading to less Akt activity [58, 59].

Sirolimus inhibits only mTOR1 and not mTOR2, whereas the latter is responsible for Akt/protein kinase B (PKB) activation via a positive-feedback loop. Activation of IGF receptor and Akt/PKB results in activation of both the PI3K pathway and antiapoptotic signaling [60, 61]. To overcome this problem, dual inhibition of PI3K and mTORC1/mTORC2 signaling by NVP-BEZ235 as a new therapeutic strategy for acute myeloid leukemia has been investigated [62].

In addition, other strategies to downregulate mTOR signaling, such as the use of the antidiabetic drug metformin, an activator of AMP-activated protein kinase, are being pursued in clinical trials [63].

PHARMACODYNAMICS

For mTOR, the two best studied targets are S6K1 and 4E-BP1. Preclinically, rapamycin and its analogs inhibit phosphorylation of 4E-BP1 and S6K1 in tumor, skin, and PBMCs [64, 65].

Time- and dose-dependent inhibition of S6K1 was demonstrated in PBMCs. In preclinical models, a correlation between the antitumor effect of rapamycin and prolonged (≥ 7 days) PBMC-derived S6K1 activity was observed. For everolimus, preclinical simulations suggest that the administration regimen has a greater influence on S6K1 activity in the tumor than in PBMCs, with daily dosing exerting greater activity than weekly doses [66] and sustained S6K inhibition occurring with ≥ 20 mg everolimus weekly and with ≥ 5 mg everolimus daily [44]. These findings highlight that, although PBMC S6K1 activity is often measured as a

pharmacodynamic (PD) marker, it is not a perfect readout of target inhibition in the tumor [67].

In a phase I study of everolimus in solid tumors, pre-treatment and steady-state tumor and skin biopsies were evaluated, showing mTOR signaling inhibition at all dose levels and schedules tested (between 5 mg daily and 70 mg weekly) [68]. Dose- and schedule-dependent inhibition of mTOR was observed, with near complete inhibition of phosphorylated (p)-S6 and p-eIF4G at the 10 mg/day and ≥ 50 mg/week doses. That study demonstrated that inhibition of mTOR signaling may be dependent on dose and schedule, and downstream targets may not always be inhibited concordantly.

The downstream effects of mTOR inhibition in rapamycin-sensitive versus rapamycin-resistant tumors have elucidated rapamycin's mechanism of action. Potential PD markers of response being examined are p-4EBP1, p-PRAS40 (Thr-246), p-Akt, and cyclin D1 levels. In a recent review, it was stated that it is unlikely that any single marker will sufficiently separate responders from nonresponders, and evaluating a panel of rapamycin effectors for PD monitoring has been suggested [67]. Another option is the use of serial biopsies of the tumor, but this is an inconvenient way to determine early signs of response [67]. Molecular imaging with tracers that assess metabolic and proliferative function (^{18}F -fluorodeoxyglucose and ^{18}F -fluorothymidine uptake) has shown promise in preclinical models [67].

PATIENT INSTRUCTIONS AND RECOMMENDATIONS FOR SUPPORTIVE CARE

Oral ulcerations (i.e., mouth ulcers, stomatitis, oral mucositis) are very common in mTOR inhibition. Topical therapy is recommended; however, alcohol- or peroxide-containing mouthwashes should be avoided. Myelosuppression is the second most common toxicity and requires monitoring of serial blood counts. Hyperglycemia and dyslipidemia can worsen, so regular blood tests are warranted, and the use of antidiabetic and antihypertensive medications to optimize blood glucose and blood pressure is recommended. The use of mTOR inhibitors may cause drug-induced pneumonitis, which usually responds well to steroids and withdrawal of the mTOR inhibitor. In cases of dyspnea during treatment, other causes should be excluded. The immunosuppressive activities of mTOR inhibitors are rare, but infections should be treated according to standard of care. For herpes lesions, topical and systemic treatments with antiviral drugs are recommended. A histamine-1 blocker should be given approximately 30 minutes before each weekly temsirolimus infusion as prophylaxis against an allergic reaction.

AUTHOR CONTRIBUTIONS

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