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ORIGINAL REPORT

# Phase I Pharmacokinetic and Pharmacodynamic Study of the Oral Mammalian Target of Rapamycin Inhibitor Everolimus in Patients With Advanced Solid Tumors

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## ABSTRACT

**Purpose**
To identify the optimal regimen and dosage of the oral mammalian target of rapamycin inhibitor everolimus (RAD001).

**Methods**
We performed a dose-escalation study in advanced cancer patients administering oral everolimus 5 to 30 mg/wk, with pharmacokinetic (PK) and pharmacodynamic (PD) studies. PD data prompted investigation of 50 and 70 mg weekly and daily dosing at 5 and 10 mg.

**Results**
Ninety-two patients were treated. Dose-limiting toxicity was seen in one patient each at 50 mg/wk (stomatitis and fatigue) and 10 mg/d (hyperglycemia); hence, the maximum-tolerated dose was not reached. S6 kinase 1 activity in peripheral-blood mononuclear cells was inhibited for at least 7 days at doses ≥ 20 mg/wk. Area under the curve increased proportional to dose, but maximum serum concentration increased less than proportionally at doses ≥ 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 ≥ 6 months, including five of 10 patients with renal cell carcinoma.

**Conclusion**
Everolimus was satisfactorily tolerated at dosages up to 70 mg/wk and 10 mg/d with predictable PK. Antitumor activity and PD in tumors require further clinical investigation. Doses of 20 mg/wk and 5 mg/d are recommended as appropriate starting doses for these studies.

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## INTRODUCTION

The mammalian target of rapamycin (mTOR), a highly conserved serine-threonine kinase, is a key regulatory protein in cancer that recognizes stress signals (eg, nutrient and energy depletion, oxidative or hypoxic stress, and proliferative and survival signals) via the PI3K-AKT pathway. mTOR signaling is effected through phosphorylation of substrates p70 ribosomal S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E binding protein 1 (4EBP1). Phosphorylation of 4EBP1 releases eIF-4E, permitting initiation of cap-dependent protein translation. The mTOR kinase also controls angiogenic pathways via hypoxia-inducible factor-1$\alpha$ and vascular endothelial growth factor and via endothelial and smooth muscle cell proliferation.[1,2] mTOR substrates, activating signaling pathways, and mTOR itself have been shown to be dysregulated in a variety of human malignancies, making mTOR an attractive target for anticancer therapy.[3,4]

Everolimus is an orally bioavailable mTOR inhibitor that binds with high affinity to its intracellular receptor FKBP12. The everolimus-FKBP12 complex interacts with mTOR to inhibit downstream signaling events. Preclinical studies show that everolimus inhibits proliferation of a variety of human solid tumors in vitro and in vivo.[5-11]

Optimal dosing of mTOR inhibitors may be difficult to define based on toxicity.[3,12] Thus, before commencing this clinical study, a preclinical model was used to define the biologically active dose of everolimus. Using a syngeneic CA20948 pancreatic rat tumor xenograft model, Boulay et al[8] analyzed the mTOR effectors 4EBP1 and S6K1 in tumor, skin, and peripheral-blood mononuclear cell

(PBMC) extracts after treatment with everolimus. They showed that suppression of tumor growth correlated with inactivation of S6K1 and reduced 4EBP1 phosphorylation. S6K1 could be measured reliably in human PBMCs and, thus, was selected as a surrogate biomarker for study in patients.

Everolimus has already undergone extensive clinical testing in the renal and cardiac transplantation setting, being well tolerated and effective with daily dosing.[13,14] A weekly schedule was selected for the initial phase I study based on persistent antitumor effects and long-term inhibition ($\geq$ 7 days) of S6K1 activity in PBMCs in vivo.[8] Safety, tolerability, and pharmacokinetic (PK) assessment was complemented by PK-pharmacodynamic (PD) modeling to predict an optimal biologically effective dosage.[7]

## METHODS

This study was conducted in two parts. Part 1 was initiated at Institut Gustave-Roussy and Royal Marsden Hospital. Toxicity, antitumor activity, PK, and the relationship of dose to inhibition of S6K1 in PBMCs were evaluated with weekly oral everolimus doses of 5, 10, 20, and 30 mg. The 5-mg starting dose was chosen to provide exposure comparable to that in the transplantation setting, where repeated daily doses of up to 3 mg in combination with cyclosporine (which inhibits CYP3A and increases everolimus concentrations two- to three-fold) are well tolerated. These data, along with blood and tumor drug levels and S6K1 activity from a study in tumor-bearing rats,[8] were used in an exposure-response model to predict drug-target inhibition relationships in tumors.[7] Weekly dosing was justified by the long half-life (30 hours) of everolimus and the prolonged PD effect in preclinical studies.[8]

Part 2 of the study investigated higher weekly doses (50 and 70 mg) and daily administration (5 and 10 mg) because, in the event that a higher dose was required to inhibit mTOR in human tumors, additional safety data were felt to be desirable at higher weekly dose levels. Furthermore, PK-PD modeling was performed using a direct-link model based on rat PBMC and tumor data, as previously described,[7] corrected for human PK. This model proved highly predictive of PD effects in humans (ie, S6K1 inhibition-time profiles in PBMCs; Fig 1) for a given level of drug exposure (Tanaka et al, personal communication, June 2002). Subsequently, the model predicted that daily dosing would provide more sustained target inhibition in tumor tissue, with estimated trough levels of more than 70% inhibition of S6K1 activity (data not shown).[7] This higher dose expansion phase, involving three additional institutions (Queen Elizabeth Hospital, Sarah Cannon Research Institute, and M.D. Anderson Cancer Center), did not involve additional PD studies because the effects in PBMCs were maximal. Patients could remain on therapy for as long as tolerated in the absence of disease progression.

Patients were $\geq$ 18 years old and had advanced solid tumors refractory to standard therapy, WHO performance status $\leq$ 2, estimated life expectancy $\geq$ 3 months, adequate bone marrow function (WBC count $\geq 3.5 \times 10^9$/L, absolute neutrophil count $\geq 1.5 \times 10^9$/L, platelets $\geq 100 \times 10^9$/L, and hemoglobin $\geq$ 100 g/L), normal hepatic function (bilirubin $\leq$ upper limit of normal and AST/ALT $\leq 3\times$ upper limit of normal), and normal renal function. Exclusion criteria included primary brain tumor or CNS metastasis, active uncontrolled infection, impairment of GI function likely to modify drug absorption, bleeding diathesis or requirement for anticoagulants, and comedication with a strong inhibitor or inducer of CYP3A4. Prior therapies had to be completed for $\geq$ 30 days for chemotherapy (6 weeks for mitomycin or nitrosoureas), $\geq$ 3 weeks for radiotherapy, and $\geq$ 2 weeks for surgery, with full recovery from toxicity.

Dose-limiting toxicity (DLT), which was assessed using National Cancer Institute Common Toxicity Criteria (CTC) version 2.0, was defined as suspected drug toxicity during the first 4 weeks of therapy, either nonhematologic CTC grade $\geq$ 3 (despite available prophylaxis such as antiemetics) or CTC grade $\geq$ 3 anemia, neutropenia, or thrombocytopenia. Cohorts were expanded to six patients in the event of DLT, with dose escalation permitted either in the absence of DLT in any of four patients or with DLT present in no more than one of six patients. MTD was defined as the dose at which more than one of six patients displayed DLT in cycle 1. Everolimus as 5-mg tablets was taken as a single oral dose and swallowed whole with water in a fasting state or after no more than a light fat-free snack with at least 2 hours before further food intake.

In part 1 of the study, four patients were planned per cohort (5, 10, 20, or 30 mg/wk). In cycle 1 only, after 4 weeks of treatment, week 5 remained treatment free to allow PK and PD sampling. Blood was collected for predose PK and PD sampling during the first 4 weeks. During weeks 4 and 5, sampling was conducted before dose; 1, 2, 4, 6, and 12 hours after dose (PK only); 24, 48, and 72 or 96 hours after dose; and 6 or 7, 9, and 10 or 11 days after the beginning of week 4. Everolimus concentrations were assayed in blood with a liquid chromatography/mass spectroscopy assay as previously described.[15] S6K1 activity was assayed in PBMC extracts as previously described,[8] except that 750 to 800 $\mu$g of total protein extract (in 750 $\mu$L final volume) was first precleared with Protein A-Sepharose beads (Sigma-Aldrich, St Louis, MO) before S6K1 immunoprecipitation. Quality assurance exercises for sample preparation and handling were conducted with the contributing centers. S6K1 assay controls included human embryo kidney (HEK293) protein extracts prepared after transient expression of recombinant S6K1, untreated controls (positive quality control) or everolimus-treated controls (20 nmol/L for 30 minutes; negative quality control). Immunoblotting for $\beta$-tubulin and S6K1 protein acted as internal controls of PBMC extract quality, as described.[8] In part 2 of the study, PK assessments were performed at week 4 in the first four to six patients of each cohort.

At screening, patients had a clinical history assessment, full clinical examination, and documentation of performance status, CBC, fasting biochemistry panel, and urinalysis, as well as baseline ECG, serum pregnancy test, and tumor evaluation. During the intensive 5-week study period, patients were seen weekly for toxicity and standard laboratory panels. Thereafter, visits were monthly if the previous monitoring was stable. Tumor was measured by conventional imaging every 2 months, and change was assessed by Response Evaluation Criteria in Solid Tumors.[16] Positron emission tomography studies were initially incorporated if available at the treating institution. These are not reported here as a result of insufficient data.

The study was conducted in accordance with International Conference on Harmonisation Good Clinical Practice standards, with approval by the ethics committees and health authorities of the participating institutions. All patients provided written informed consent to participate.

## RESULTS

Ninety-two patients were treated; 18 were treated weekly in part 1, and in part 2, 37 were treated weekly, and 37 were treated daily. Patient characteristics are listed in Table 1. All patients are accounted for in the safety analysis with data cutoff 6 months after entry of the last patient.

### *Part 1: Dose Escalation and PD*

No DLT was seen during part 1. PBMC-derived S6K1 activity was measured in four patients in each cohort. Two patients were replaced; one did not complete the first 5-week study period because of rapidly progressive disease, and the other had inadequate PBMC collection. PBMC-derived S6K1 activity was clearly suppressed 24 hours after the fourth dose at all dose levels; duration of suppression lengthened with increasing dosage (Fig 1) to persist over the complete dosing interval in four of four patients at 20 mg and in three of four patients at 30 mg; one patient had minimal evidence of recovery at day 6. Consequently, 20 mg was presumed to be the minimum dose ensuring target inhibition over 7 days.

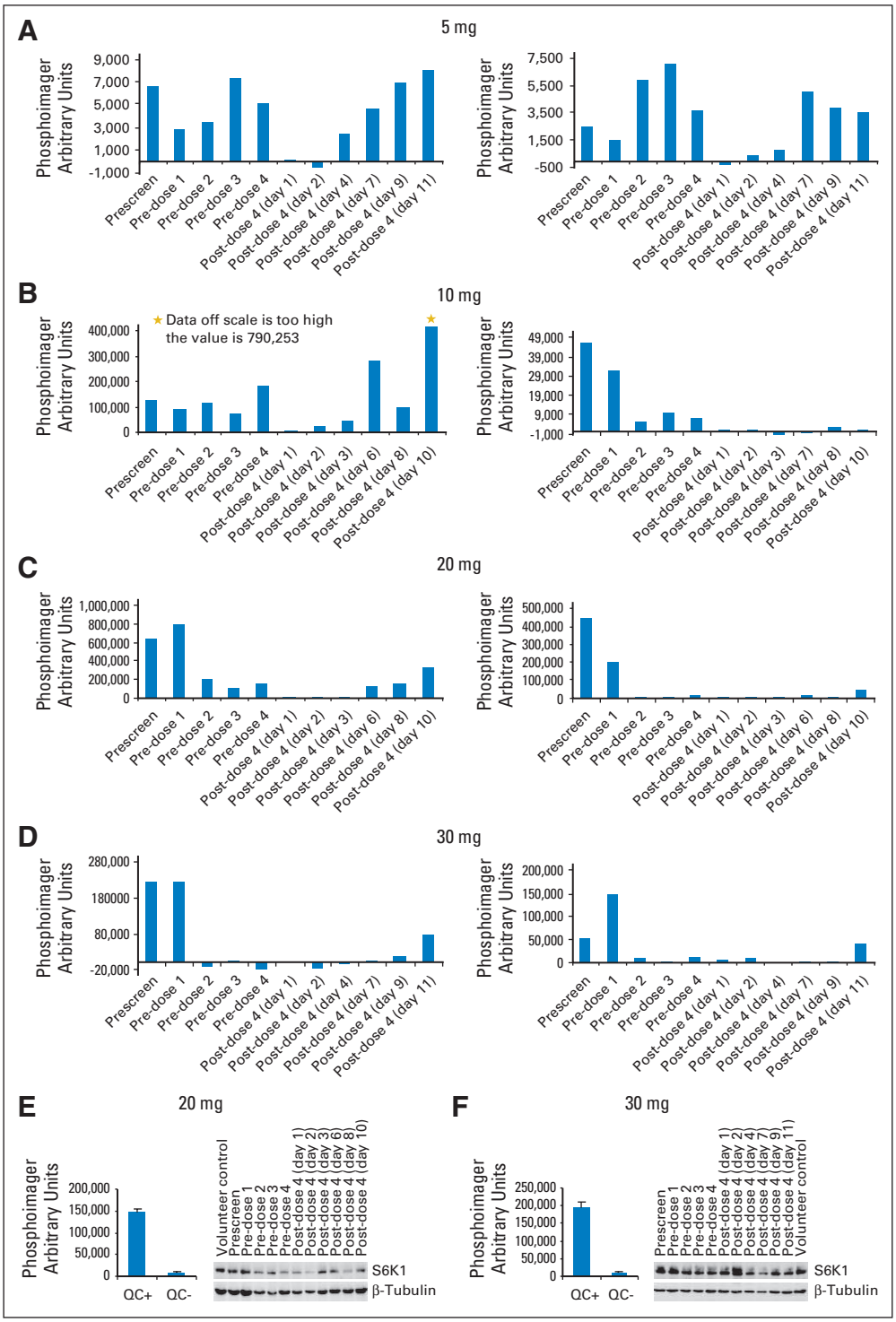


**Fig 1.** (A) Quantification of S6 kinase 1 (S6K1) activity in peripheral-blood mononuclear cell (PBMC) extracts. Four patients were included per cohort; representative data from two patients are shown. In the 10-mg cohort, two patients presented changes as illustrated in the left panel, and two presented changes as illustrated in the right panel. (B and C) Examples of quantification of S6K1 activity in positive quality controls (QC+) and negative quality controls (QC–) and Western blot analysis of S6K1 and $\beta$-tubulin protein levels (control) in the PBMC extracts.

PK-PD modeling showed an anticipated decline in tumor S6K1 inhibition during weekly dosing, contrasting with more sustained inhibition with daily dosing for the same cumulative dose.[7]

### *Part 2: Dose Escalation*

On the weekly regimen, DLT occurred in one of six patients at 50 mg (grade 3 stomatitis and fatigue) but none of four patients at 70 mg. On the daily regimen, DLT occurred in none of four patients at 5 mg and in one of six patients at 10 mg (grade 3 hyperglycemia). Consequently, the higher dosage cohorts were expanded for each regimen.

### *Safety Findings*

At data cutoff, only six patients remained on therapy. Treatment exposure was comparable in each treatment group, averaging approximately 3 months.

  

**Table 1.** Patient Demographic and Disease Characteristics

| Characteristic | No. of Patients (N = 92) |
|---|---|
| Age, years | |
| Median | 61 |
| Range | 28-83 |
| Sex | |
| Male | 51 |
| Female | 41 |
| Race | |
| White | 91 |
| Black | 1 |
| WHO performance status | |
| 0 | 23 |
| 1 | 68 |
| 2 | 1 |
| Primary tumor | |
| Colorectal | 18 |
| Lung, non–small-cell | 12 |
| Melanoma | 12 |
| Renal | 10 |
| Sarcoma | 5 |
| Breast | 4 |
| Prostate | 4 |
| Pancreas | 3 |
| Ovarian | 3 |
| Adenoid cystic carcinoma | 2 |
| Bladder | 2 |
| Hepatoma | 2 |
| Lower esophagus | 2 |
| Mesothelioma | 2 |
| Peritoneal | 2 |
| Lung, small-cell | 2 |
| Others* | 7 |
| Prior therapy for advanced cancer | |
| Cytotoxics | 72 |
| ≥ 2 regimens | 59 |
| Hormone therapy | 9 |
| Immunotherapy | 6 |
| Other | 16 |

*Other tumors, in one patient each: adrenal, cervical (squamous cell), cholangiocarcinoma, head and neck (squamous cell), neuroendocrine (lung), unknown primary, and vulva.

### Suspected Drug-Related Adverse Events and Discontinuations

Tables 2 and 3 list suspected drug-related adverse events. Fatigue was observed in 31 patients (34%). Grade 3 fatigue was observed in only two patients, in association with stomatitis in one patient and depression in the other; both patients discontinued therapy. Rash in 44 patients (48%) appeared within the first month in 32 patients, was principally acneiform (34%) or erythematous (18%), and occurred most commonly over the upper body and head. It was severe (grade 3) in one patient (10 mg/d), with severity decreasing to grade 1 after interruption and dose reduction to 5 mg/d. GI toxicities reported in 61 patients (66%) included stomatitis, nausea, vomiting, anorexia, constipation, and abdominal pain or distension. Although GI toxicity was usually mild, stomatitis (erythematous, ulcerative) was grade 3 in three patients, one of whom discontinued treatment. Hematologic abnormalities were reported in 17 patients. In general, a mostly moderate decrease in platelet and neutrophil counts occurred rapidly after introduction of treatment but remained constant thereafter. Hemoglobin showed a tendency to decline over time, possibly related to the underlying disease.

### Serum Biochemistry

Hyperglycemia, which was considered drug related, was reported in seven patients at the higher dosage levels and was grade 3 in three patients. As mentioned earlier, one of these patients fulfilled the criteria for DLT. Hyperlipidemia (hypercholesterolemia and/or hypertriglyceridemia), which was again considered drug related, was reported in seven patients and was grade 3 (hypertriglyceridemia) in two patients, both of whom improved with statin therapy.

### Infections

Infections were reported in 41 patients. A relationship to study drug was suspected in only 12 patients, whose infections included cutaneous herpes simplex (five patients), oral candidiasis complicating stomatitis (four patients), pneumonia (two patients), rhinitis (two patients), conjunctivitis (one patient), influenza-like illness (one patient), and a combination of upper respiratory and urinary tract infections (one patient).

### Suspected Serious Toxicity

Five patients had severe toxicity that was suspected to be drug related, four of whom required hospitalization. Hemorrhagic gastritis occurred after 6 days of therapy (10 mg/d) in an 82-year-old man with a history of peptic ulcer and without thrombocytopenia, who recovered after drug discontinuation. Recurrent epistaxis occurred in a patient (10 mg/d) with moderate thrombocytopenia (platelet count, $97 \times 10^9$/L). Bronchiolitis obliterans organizing pneumonia was confirmed histologically in one patient (70 mg/wk) who developed cough and dyspnea after 4 to 6 weeks of therapy, which worsened to grade 3 severity by month 3 but resolved completely after drug discontinuation and glucocorticoid therapy. A patient (10 mg/d) with lung metastases and grade 2 lymphopenia, without neutropenia, developed grade 3 pneumonia that resolved with antibiotic therapy. Finally, grade 3 fatigue and stomatitis developed in another patient after three doses (50 mg/wk). There were no suspected drug-associated fatalities.

### PK

Samples were assessable for full 24-hour PK profiles in 26 of the 31 patients who received the weekly regimen and in 10 of the daily regimen patients (Table 4).

*Weekly regimen.* Stable predose serum trough concentration levels from weeks 2 to 5 indicated minimal accumulation at all weekly dose levels, with steady-state achieved by week 2 of treatment. Dose normalization (maximum serum steady-state concentration [$C_{max}^{ss}$]/dose in Table 3) showed that mean $C_{max}^{ss}$ increased in a roughly dose-proportional manner from 5 to 20 mg per week but increased less than proportionally at higher doses. The increase in area under the curve at steady state ($AUC^{ss}$) was dose proportional over the full dose range, with a regression slope of 0.963 (95% CI, 0.814 to 1.114). The corresponding linear regression equation was area under the curve (AUC) = 51 × dose + 93 ($r^2 = 0.709$, $P < .001$). Elimination half-life averaged 30 ± 8 hours across all patients and was similar to that in healthy controls.[17]

*Daily regimen.* Steady-state was reached within a week. Trough levels were stable thereafter, averaging 5.4 ng/mL (5 mg/d dosing) and 13.2 ng/mL (10 mg/d dosing). Peak concentrations were achieved

**Table 2.** Most Frequently Reported Suspected Drug-Related Toxicities (≥ five patients) Including All NCI-CTC Grades

| AE | Weekly Regimen Dose (No. of patients) | | | | | | Daily Regimen Dose (No. of patients) | | Total Patients (N = 92) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 mg (n = 4) | 10 mg (n = 4) | 20 mg (n = 5) | 30 mg (n = 5) | 50 mg (n = 6) | 70 mg (n = 31) | 5 mg (n = 4) | 10 mg (n = 33) | No. | % |
| Total drug-related AEs | 2 | 3 | 2 | 5 | 6 | 31 | 3 | 31 | 83 | 90 |
| Rash and erythema | — | 1 | 1 | 2 | 3 | 15 | 2 | 20 | 44 | 48 |
| Stomatitis/mucositis | — | 1 | — | 3 | 3 | 12 | 2 | 17 | 38 | 41 |
| Fatigue | 1 | — | — | 4 | 3 | 11 | — | 12 | 31 | 34 |
| Nausea | 2 | 1 | — | 1 | 2 | 8 | 1 | 14 | 29 | 32 |
| Anorexia | — | — | — | — | 2 | 9 | 1 | 11 | 23 | 25 |
| Diarrhea | — | — | — | 1 | 4 | 6 | — | 7 | 18 | 20 |
| Headache | — | — | — | 3 | 1 | 3 | — | 7 | 14 | 15 |
| Vomiting | 2 | — | — | 1 | 1 | 5 | — | 5 | 14 | 15 |
| Constipation | — | — | — | — | — | 2 | 1 | 7 | 10 | 11 |
| Abdominal distension | — | — | — | — | 1 | 2 | — | 6 | 9 | 10 |
| Pruritus | — | — | — | 1 | — | 5 | 1 | 2 | 9 | 10 |
| Thrombocytopenia | — | — | 1 | — | — | 4 | — | 4 | 9 | 10 |
| Hyperglycemia | — | — | — | — | — | 4 | — | 3 | 7 | 8 |
| Lethargy | — | — | — | — | — | 3 | — | 4 | 7 | 8 |
| Anemia | — | — | — | — | — | 2 | 1 | 3 | 6 | 7 |
| Dysgeusia | — | — | — | — | — | 2 | — | 4 | 6 | 7 |
| Abdominal pain | 1 | — | — | — | — | 2 | — | 2 | 5 | 5 |
| Dry mouth | — | — | — | — | 1 | 3 | — | 1 | 5 | 5 |
| Herpes simplex infection | — | — | — | — | 1 | — | 1 | 3 | 5 | 5 |

Abbreviations: AE, adverse event; NCI-CTC, National Cancer Institute Common Toxicity Criteria version 2.0.

within 1 hour of daily dosing with one exception (6 hours). Both maximum serum concentration and AUC increased in a dose-proportional manner (Table 4). Steady-state trough levels were highly predictive of AUC, with a coefficient of determination ($r^2$) of 0.96, as has been reported in renal transplantation patients (Fig 2).[18] Plasma concentrations and levels of sustained S6K1 inhibition observed at ≥ 20 mg everolimus weekly and ≥ 5 mg daily correlate with those seen in preclinical models resulting in antitumor activity.[7,8]

### *Tumor Assessments*

Table 5 lists clinical benefit in progression-free patients for 6 months or more. Partial response (PR) was observed in four patients;

**Table 3.** Suspected Drug-Related Severe (NCI-CTC grade 3 to 4) Adverse Events Reported in Any Patient

| Adverse Event | Weekly Regimen Dose (No. of patients) | | | Daily Regimen Dose (No. of patients) | | Total No. of Patients (N = 92) |
|---|---|---|---|---|---|---|
| | 5-30 mg (n = 18) | 50 mg (n = 6) | 70 mg (n = 31) | 5 mg (n = 4) | 10 mg (n = 33) | |
| Total, any event | — | 1 | 6 | — | 12 | 19 |
| Stomatitis/mucositis | — | 1 | — | — | 2 | 3 |
| Hyperglycemia | — | — | 1 | — | 2 | 3 |
| Fatigue | — | 1 | 1 | — | — | 2 |
| GI hemorrhage | — | — | — | — | 1 | 1 |
| Dyspnea | — | — | — | — | 1 | 1 |
| Pneumonia | — | — | — | — | 1* | 1* |
| Neutropenia | — | — | 1 | — | — | 1 |
| Thrombocytopenia | — | — | 1* | — | 2 | 3 |
| Hypertriglyceridemia | — | — | 1 | — | 1 | 2 |
| Appetite loss/anorexia | — | — | — | — | 2 | 2 |
| Pneumonitis | — | — | 1 | — | — | 1 |
| Rash, erythematous | — | — | — | — | 1 | 1 |
| Nausea | — | — | — | — | 1 | 1 |
| Melena | — | — | 1 | — | — | 1 |
| Epistaxis | — | — | — | — | 1 | 1 |

Abbreviation: NCI-CTC, National Cancer Institute Common Toxicity Criteria version 2.0.
*Grade 4.

  

**Table 4.** Everolimus Pharmacokinetics for Weekly and Daily Dosing

| Measure | Weekly Regimen Dose | | | | | | Daily Regimen Dose | |
|---|---|---|---|---|---|---|---|---|
| | 5 mg (n = 4) | 10 mg (n = 4) | 20 mg (n = 5) | 30 mg (n = 5) | 50 mg (n = 6) | 70 mg (n = 7) | 5 mg (n = 4) | 10 mg (n = 6) |
| No. of patients with values | 4 | 4 | 2 | 5 | 5 | 6 | 4 | 6 |
| $t_{max}$, hours | | | | | | | | |
| Median | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 |
| Range | 1-2 | 1 | 1 | 1-2 | 1-2 | 1 | 1 | 1-6 |
| $C_0^{ss}$, ng/mL | | | | | | | | |
| Mean | — | — | — | — | — | — | 5.4 | 13.2 |
| SD | — | — | — | — | — | — | 1.8 | 7.9 |
| $C_{max}^{ss}$, ng/mL | | | | | | | | |
| Mean | 32 | 69 | 94 | 88 | 163 | 174 | 32 | 61 |
| SD | 15 | 8 | 0 | 20 | 63 | 49 | 9 | 17 |
| $C_{max}^{ss}$/dose, ng/mL* | | | | | | | | |
| Mean | 6.5 | 6.9 | 4.7 | 2.9 | 3.3 | 2.5 | — | — |
| SD | 3.1 | 0.8 | 0.0 | 0.7 | 1.2 | 0.7 | — | — |
| $C_{avge}^{ss}$, ng/mL | | | | | | | | |
| Mean | — | — | — | — | — | — | 9.9 | 21.4 |
| SD | — | — | — | — | — | — | 3.2 | 9.6 |
| $AUC^{ss}$, ng · h/mL | | | | | | | | |
| Mean | 283 | 573 | 1,001 | 1,798 | 2,621 | 3,615 | 238 | 514 |
| SD | 48 | 258 | 301 | 827 | 633 | 1,497 | 77 | 231 |
| $AUC^{ss}$/dose, ng · h/mL* | | | | | | | | |
| Mean | 57 | 57 | 50 | 60 | 52 | 52 | — | — |
| SD | 10 | 26 | 15 | 28 | 13 | 21 | — | — |
| $t_{1/2}$, hours | | | | | | | | |
| Mean | 26 | 38 | 31 | 37 | 27 | 26 | — | — |
| SD | 3 | 14 | 12 | 6 | 7 | 2 | — | — |

Abbreviations: $t_{max}$, time to maximum serum concentration; $C_0^{ss}$, predose concentration at steady state; SD, standard deviation; $C_{max}^{ss}$, maximum serum steady-state concentration; $C_{avge}^{ss}$, average serum steady-state concentration; $AUC^{ss}$, area under the curve at steady state; $t_{1/2}$, terminal half-life.
*Dose-normalized parameters are per milligram.

one patient (30 mg/wk) had squamous cell lung cancer but discontinued treatment for pneumothorax on day 189, one (5 mg/d) had cancer of the esophagogastric junction, one (10 mg/d) had rectal carcinoma, and a 71-year-old man (70 mg/wk) had renal cell carcinoma (RCC). In another patient with RCC (10 mg/d), adrenal and lung lesions had shrunk with PR evaluation at day 198, but this was unconfirmed because of discontinuation for adverse events (fatigue and depression). Five of the 10 RCC patients remained progression free at 6 months, including the patient with PR after prolonged stable disease mentioned earlier.

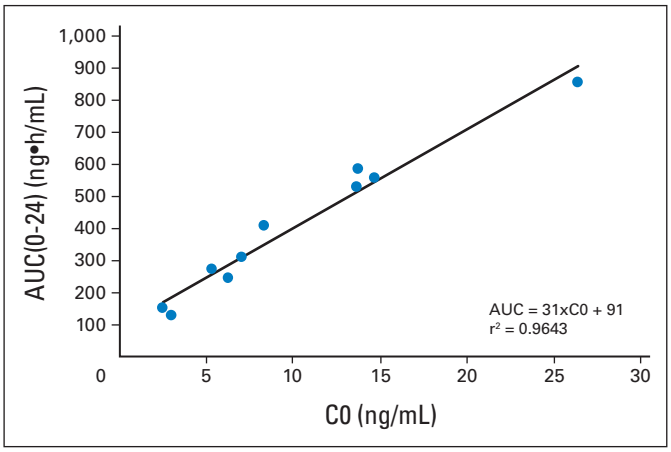


**Fig 2.** Linear regression analysis of steady-state trough levels ($C_{min}$) versus area under the curve (AUC) for patients treated with the daily regimen of everolimus.

## DISCUSSION

This was the first study of oral everolimus in patients with solid tumors. Everolimus was well tolerated, with toxicities (eg, rash and stomatitis) consistent with other mTOR inhibitors (eg, AP23573[19,20] and temsirolimus[12]). Two patients experienced DLT (stomatitis plus fatigue and hyperglycemia) at different dose levels, and an MTD was not defined. Stomatitis, although relatively frequent, may occur concurrently with oral *Candida* and herpes simplex infections. The incidence of infection was low, and no relationship was seen between infection and leukocytopenia. Rash, although common, was mild and generally well tolerated. Bone marrow suppression was mild and stabilized with continued dosing. One patient had interstitial lung disease, which has been observed in patients receiving sirolimus and temsirolimus. The condition invariably resolves on dose reduction, drug withdrawal, and glucocorticoid therapy.[21-24]

Everolimus is extensively metabolized by CYP3A4.[25] In the renal transplantation setting, a 35% interpatient variability in AUC has been observed.[26,27] In our study, PK characteristics are consistent with those observed both in normal volunteers and in patients in the transplantation

**Table 5.** Patients With Clinical Benefit by Primary Cancer

| Regimen and Dose | Progression Free for 4 Months to < 6 Months | Progression Free for ≥ 6 Months* | Response |
|---|---|---|---|
| Weekly, mg | | | |
| ≤ 30 | 4 patients: colon, HCC, NSCLC, fibrosarcoma | 1 patient: NSCLC (day 177)† | PR at day 115† |
| 50 | — | — | — |
| 70 | 2 patients: NSCLC, melanoma† | 6 patients: RCC (day 334‡), RCC (day 344‡), RCC (day 330), NSCLC (day 195), adenocystic carcinoma (day 177‡), melanoma (day 172) | PR at day 334 |
| Daily, mg | | | |
| 5 | — | 1 patient: esophagogastric carcinoma (day 278) | PR at day 57 |
| 10 | 3 patients: adenocystic carcinoma,† colon, melanoma | 4 patients: rectal (day 226), mesothelioma (day 169‡), RCC (day 169),† RCC (day 198)† | PR at day 58 |

Abbreviations: HCC, hepatocarcinoma; NSCLC, non–small-cell lung cancer; PR, partial response; RCC, renal cell carcinoma.
*Date in parentheses indicates last assessment showing absence of progression.
†Patient discontinued treatment for reason other than progressive disease.
‡Patient treatment is ongoing at time of cutoff for analysis (June 30, 2005).

setting,[28] with dose-proportional increases in AUC. The less than proportional increase in maximum serum concentration at doses greater than 20 mg seems unlikely to be of clinical relevance.

In part 1 of the study, a dose-response relationship between oral administration of everolimus and inhibition of S6K1 indicated sustained activity over 7 days at doses of ≥20 mg/wk. Modeling, as previously described,[7] based on PK-PD data from this study and from preclinical investigations was used to predict target inhibition in tumor for weekly and daily dosing. This led to the exploration in part 2 of 50 to 70 mg/wk and 5 to 10 mg/d without identification of MTD. PD effects in tumor need to be investigated as an essential next step in the selection of the optimal biologically effective dose. Although S6K1 inhibition in PBMCs is an accurate biomarker of mTOR inhibition, this may have limited ability to predict clinical activity. It has recently been demonstrated that inhibition of S6K1 silences a negative feedback loop to insulin receptor substrate 1 signaling. This has been suggested to attenuate the effects of mTOR inhibition on tumor cell proliferation, potentially circumventing the clinical efficacy of mTOR inhibition alone.[29] The clinical implications of this observation have yet to be assessed.

Although response was not a primary outcome of our study, it was encouraging to note that a significant number of patients had sustained clinical benefit from treatment at all dose levels studied, including PR in patients with non–small-cell lung cancer, adenocarcinoma of the esophageal junction, rectal cancer, and RCC. Future phase II and III studies with everolimus should adopt response criteria and end points assessing both cytostatic and cytoreductive activity.

The activity seen in patients with clear cell RCC is particularly interesting because mTOR regulates hypoxia-inducible factor-1$\alpha$ and vascular endothelial growth factor and represents a rational therapeutic target in this disease as shown by improved survival with temsirolimus compared with interferon alfa in advanced RCC.[30] It remains to be seen which tumors will respond best to mTOR inhibition and whether response can be predicted by indications of mTOR and PI3-AKT pathway activation and the loss or inactivation of the tumor suppressors *PTEN* and *p53*.[31]

It is critical that future studies with mTOR inhibitors integrate tumor-based molecular biology. These data support the use of everolimus as a rationally designed molecularly targeted therapy. We acknowledge that these PD results were obtained in surrogate tissue and should be supported by confirmation using serial tumor biopsy. However, clinical surrogates such as sustained clinical benefit and/or tumor shrinkage and, possibly, functional imaging could also be used. From this phase I study, we recommend that such studies should be initiated with doses ≥ 20 mg/wk or ≥ 5 mg/d in an effort to derive the optimal effective dose.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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## REFERENCES


**1.** Corradetti MN, Guan KL: Upstream of the mammalian target of rapamycin: Do all roads pass through mTOR? Oncogene 25:6347-6360, 2006

**2.** Mamane Y, Petroulakis E, LeBacquer O, et al: MTOR, translation initiation and cancer. Oncogene 25:6416-6422, 2006

**3.** Faivre S, Kroemer G, Raymond E: Current development of mTOR inhibitors as anticancer agents. Nat Rev Drug Discov 5:671-688, 2006

**4.** Easton JB, Houghton PJ: MTOR and cancer therapy. Oncogene 25:6436-6446, 2006

**5.** Beuvink I, O'Reilly T, Zumstein S, et al: Antitumor activity of RAD001, an orally active rapamycin derivative. Proc Am Assoc Cancer Res 42:366, 2001 (suppl, abstr 1972)

**6.** O'Reilly T, Vaxelaire J, Muller M: In vivo activity of RAD001, an orally active rapamycin derivative, in experimental tumor models. Proc Am Assoc Cancer Res 43:71, 2002 (suppl, abstr 359)

**7.** Lane H, Tanaka C, Kovarik J, et al: Preclinical and clinical pharmacokinetic/ pharmacodynamic (PK/PD) modeling to help define an optimal biological dose for the oral mTOR inhibitor, RAD001, in oncology. Proc Am Soc Clin Oncol 22:237, 2003 (suppl, abstr 951)

**8.** Boulay A, Zumstein-Mecker S, Stephan C, et al: Antitumor efficacy of intermittent treatment schedules with the rapamycin derivative RAD001 correlates with prolonged inactivation of ribosomal protein S6 kinase 1 in peripheral blood mononuclear cells. Cancer Res 64:252-261, 2004

**9.** Boulay A, Rudloff J, Ye J, et al: Dual inhibition of mTOR and estrogen receptor signaling in vitro induces cell death in models of breast cancer. Clin Cancer Res 11:5319-5328, 2005

**10.** Beuvink I, Boulay A, Fumagalli S, et al: The mTOR inhibitor RAD001 sensitizes tumor cells to DNA-damaged induced apoptosis through inhibition of p21 translation. Cell 120:747-759, 2005

**11.** Goudar RK, Shi Q, Hjelmeland MD, et al: Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition. Mol Cancer Ther 4:101-112, 2005

**12.** Raymond E, Alexandre J, Faivre S, et al: Safety and pharmacokinetics of escalated doses of weekly intravenous infusion of CCI-779, a novel mTOR inhibitor, in patients with cancer. J Clin Oncol 22:2336-2347, 2004

**13.** Pascual J, Boletis IN, Campistol JM: Everolimus (Cerican) in renal transplantation: A review of clinical trial data, current usage, and future directions. Transplant Rev 20:1-18, 2006

**14.** Eisen HJ, Tuzcu M, Dorent R, et al: Everolimus for the prevention of allograft rejection and vaculopathy in cardiac-transplant patients. N Engl J Med 349:847-858, 2003

**15.** Brignol N, McMahon LM, Luo S, et al: High-throughput semi-automatic 96-well liquid/liquid extraction and liquid chromatography/mass spectrometric analysis of everolimus (RAD001) and cyclosporine A (CsA) in whole blood. Rapid Commun Mass Spectrom 15:898-907, 2001

**16.** Therasse P, Arbuck SG, Eisenhauer EA, et al: New guidelines to evaluate the response to treatment in solid tumors: European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92:205-211, 2000

**17.** Kovarik JM, Hartmann S, Figueiredo J, et al: Effect of food on everolimus absorption: Quantification in healthy subjects and a confirmatory screening in patients with renal transplants. Pharmacotherapy 22: 154-159, 2002

**18.** Kovarik JM, Kahan BD, Kaplan B, et al: Longitudinal assessment of everolimus in de novo renal transplant recipients over the first post-transplant year: Pharmacokinetics, exposure-response relationships, and influence on cyclosporine. Clin Pharmacol Ther 69:48-56, 2001

**19.** Desai AA, Janisch L, Berk LR, et al: A phase I trial of a novel mTOR inhibitor AP23573 administered weekly (wkly) in patients (pts) with refractory or advanced malignancies: A pharmacokinetic (PK) and pharmacodynamic (PD) analysis. J Clin Oncol 22:232, 2004 (suppl, abstr 3150)

**20.** Mita MM, Rowinsky EK, Goldston ML, et al: Phase I, pharmacokinetic (PK), and pharmacodynamic (PD) study of AP23573, an mTOR inhibitor, administered IV daily x 5 every other week in patients (pts) with refractory or advanced malignancies. J Clin Oncol 22:214, 2004 (suppl, abstr 3076)

**21.** Duran I, Siu LL, Oza AM, et al: Characterisation of the lung toxicity of the cell cycle inhibitor temsirolimus. Eur J Cancer 42:1875-1880, 2006

**22.** Champion L, Stern M, Israël-Biet D, et al: Brief communication: Sirolimus-associated pneumonitis—24 cases in renal transplant recipients. Ann Intern Med 144:505-509, 2006

**23.** Pham PT, Pham PC, Danovitch GM, et al: Sirolimus-associated pulmonary toxicity. Transplantation 77:1215-1220, 2004

**24.** Garrean S, Massad MG, Tshibaka M, et al: Sirolimus-associated interstitial pneumonitis in solid organ transplant recipients. Clin Transplant 19:698-703, 2005

**25.** Nashan B: Review of the proliferation inhibitor everolimus. Expert Opin Investig Drugs 11:1845-1857, 2002

**26.** Kovarik JM, Eisen H, Dorent R, et al: Everolimus in de novo cardiac transplantation: Pharmacokinetics, therapeutic range, and influence on cyclosporine exposure. J Heart Lung Transplant 22:1117-1125, 2003

**27.** Budde K, Neumayer HH, Lehne G, et al: Tolerability and steady-state pharmacokinetics of everolimus in maintenance renal transplant patients. Nephrol Dial Transplant 19:2006-2614, 2004

**28.** Kovarik JM: Everolimus: A proliferation signal inhibitor targeting primary causes of allograft dysfunction. Drugs Today 40:101-109, 2004

**29.** Di Cosimo S, Scaltriti M, Val D, et al: The PI3-K/AKT/mTOR pathway as a target for breast cancer therapy. J Clin Oncol 25:140s, 2007 (suppl, abstr 3511)

**30.** Hudes G, Carducci M, Tomczak P, et al: Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N Engl J Med 356:2271-2281, 2007

**31.** O'Reilly KE, Rojo F, She Q-B, et al: MTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res 66:1500-1508, 2006



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