A Phase I Trial to Determine the Safety, Tolerability, and Maximum Tolerated Dose of Deforolimus in Patients with Advanced Malignancies

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Abstract Purpose: This was a phase I trial to determine the maximum tolerated dose and toxicity of deforolimus (AP23573, MK-8669), an inhibitor of mammalian target of rapamycin (mTOR). The pharmacokinetics, pharmacodynamics, and antineoplastic effects were also studied. Experimental Design: Deforolimus was administered intravenously over 30 min every 7 days according to a flat dosing schedule. Dose was escalated according to an accelerated titration design. Patients remained on study until disease progression as long as they tolerated the drug without significant toxicities. Results: Forty-six patients were enrolled on the study. Common side effects included fatigue, anorexia, and mucositis. The maximum tolerated dose was 75 mg and mucositis was the dose-limiting toxicity. Similar to other mTOR inhibitors, deforolimus exhibited nonlinear pharmacokinetics and a prolonged half-life. Among 34 patients evaluable for response, 1 patient had a partial response, 21 patients had stable disease, and 12 had progressed. Percent change in tumor size was significantly associated with AUC (P = 0.015). A significant association was also detected for maximum change in cholesterol within the first two cycles of therapy and change in tumor size (r = -0.38; P = 0.029). Conclusions: Deforolimus was well tolerated on the schedule tested in this trial with toxicity and pharmacokinetic profiles that were similar to that of other mTOR inhibitors. Additional phase II studies are needed to determine if deforolimus is superior to other mTOR inhibitors in terms of efficacy. The change in serum cholesterol as a potential biomarker of activity should be studied further.

The mammalian target of rapamycin (mTOR) pathway is a validated therapeutic target in renal cell carcinoma and is being evaluated in other malignancies due to the antiproliferative, antiangiogenic, and proapoptotic consequences of blocking this pathway (1–12). Sirolimus (rapamycin), the first mTOR inhibitor, was initially discovered in the late 1970s (13) and has been developed primarily as an immunosuppressant. In recent years, several analogues of sirolimus have been developed including temsirolimus, everolimus, and deforolimus (AP23573, MK-8669). Deforolimus, the focus of the current study, is a derivative of sirolimus manufactured through a synthetic process that uses sirolimus as a substrate. In vitro studies done by Ariad Pharmaceuticals showed that deforolimus, like sirolimus, is primarily metabolized by CYP3A4 (and to a lesser extent by CYP3A5 and CYP2C8). Unlike temsirolimus, deforolimus is not a sirolimus prodrug, and a prior phase I study of deforolimus confirmed that sirolimus plasma concentrations were below the limit of quantification or <1% that of deforolimus (14).

Nonclinical studies showed that deforolimus inhibits proliferation of several human tumor cell lines and xenografts (15, 16). In two multiple-dose toxicity studies done in rats, neither a no effect dose level nor a severely toxic dose level was identified. In both studies, myelosuppression and increases in glucose and cholesterol levels were observed.

The current study was a phase I, open-label, dose escalation trial of deforolimus. The primary objective was to determine the safety, tolerability, and maximum tolerated dose (MTD) of deforolimus when administered intravenously once weekly. Secondary objectives included characterization of the pharmacokinetics of the drug, evaluation of potential pharmacodynamic biomarkers, and exploration of its antineoplastic characteristics.

Patients and Methods

Eligibility criteria. Patients were eligible for this study if they were ages ≥18 years and had a histologically or cytologically proven...
malignancy that was refractory to or not amenable to standard therapy or surgical intervention. Patients were required to have a Karnofsky performance status of ≥70 and those of child-bearing potential agreed to use an effective method of contraception. The following pre-study organ function criteria had to be met: hemoglobin ≥9.0 g/dL, absolute neutrophil count ≥1,500/μm³, platelet count ≥100,000/μm³, total bilirubin ≤1.5 times the upper limit of normal, AST or ALT <3 times the upper limit of normal, serum albumin ≥2.5 g/dL, serum cholesterol ≤350 mg/dL, triglycerides ≤400 mg/dL, creatinine ≤1.5 times the upper limit of normal, or a calculated creatinine clearance ≥50 mL/min/1.73 m². All patients had to have the ability to understand and give written informed consent.

Patients with a primary central nervous system malignancy or any form of leukemia were excluded. Pregnant or lactating women and patients with known hypersensitivity to macrolide antibiotics or drugs formulated with polysorbate 80 were also excluded. Patients with significant underlying medical problems, including patients with a history of congestive heart failure requiring therapy, a need for antiarrhythmic medication for the treatment of a ventricular arrhythmia, a severe conduction disturbance, angina pectoris requiring therapy, uncontrolled hypertension, and a myocardial infarction in the last 6 months, or those with class III or IV cardiovascular disease according to the New York Heart Association's functional criteria were excluded. Patients with active central nervous system metastases or leptomeningeal disease were also excluded. However, patients with treated brain metastases were eligible if they were on a stable dose of corticosteroids or were without change in brain disease status for at least 4 weeks. Patients with known HIV infection or any active infection were excluded. Patients who had not adequately recovered from any prior surgical procedure or had a major surgical procedure within 2 weeks were not eligible.

Patients were not eligible if they had received any prior therapy within 4 weeks (6 weeks for mitomycin C and nitrosoureas), with the exception of any agents for which the half-life is known to be ≤24 h (3 weeks). Exceptions included stable dose maintenance steroids for central nervous system metastases, low-dose maintenance steroid therapy for other conditions, and stable luteinizing hormone-releasing hormone agonist therapy for prostate cancer. Patients could not take any herbal preparations or related over-the-counter preparations containing herbal ingredients within 2 weeks. Patients were not permitted to have received prior therapy with a mTOR inhibitor and were not permitted to take any other experimental therapies while on study.

**Treatment.** Deforolimus was administered intravenously over 30 min every 7 days in a total volume of 250 mL. The drug was manufactured by Ariad Pharmaceuticals and supplied by Fisher Clinical Services. A flat dosing schedule was used, with a starting dose of 6.25 mg and a maximum planned dose of 225 mg. This starting dose was approximately 1/30th the maximum dose tested in rats (based on body surface area) and 1/36th the maximum dose tested in nonhuman primates. Patients were treated weekly, and cycles were defined as consisting of 4 weeks of therapy. Patients were initially treated for 8 weeks and allowed to continue treatment for additional cycles if they tolerated the drug well and there was no evidence of disease progression. All patients were treated at the University of Chicago. Approval by the University of Chicago Institutional Review Board was obtained before enrollment of any subjects.

Patients were treated in the outpatient clinic. After the first infusion of drug (cycle 1, day 1), each patient was observed for at least 6 h. Patients were not premedicated before the first infusion. On cycle 1, day 8, each patient was observed for at least 4 h after the infusion, and after all other infusions in cycle 1, the patients were observed for at least 2 h. Thereafter, patients were observed for at least 1 h after each infusion. Patients who experienced grade 1 or 2 hypersensitivity had the infusion interrupted. Such patients were allowed to resume drug with the following cycle but were premedicated in advance of all subsequent infusions with a H1 and/or H2 receptor antagonist. Patients who experienced a grade 3 or 4 hypersensitivity reaction were discontinued from the study.

Patients were evaluated for tumor response using the appropriate diagnostic imaging technique after the first two cycles of therapy and then after every two additional cycles. Patients with documented progressive disease or who experienced unacceptable toxicity were removed from the study. Patients were also taken off study if they developed an intercurrent illness that would prevent completion of study-related procedures, were noncompliant, or withdrew consent. Patients with stable disease or an objective partial or complete response could continue on therapy at the same dose level. All patients were followed for 6 months after the end of the last dose cycle and safety data were collected for this duration.

**Dose escalation.** An accelerated titration design was used for dose escalation (17). Initially, the dose was escalated 100% between cohorts and at least one patient was enrolled at each dose level. The dose was escalated when at least one patient completed cycle 1 and was fully evaluable. Once any grade ≥2 toxicity determined to be probably or definitely related to deforolimus or any grade ≥3 toxicity defined as possibly, probably, or definitely related to deforolimus (except alopecia and nausea and/or vomiting, unless receiving optimal medical therapy) occurred during cycle 1, the dose escalation was switched to 50% escalation between cohorts and at least 3 patients were enrolled in each cohort. Once a dose-limiting toxicity (DLT) was observed, the inter-cohort escalation was reduced to 25%, with a minimum of 6 patients per cohort. If ≥2 patients had a DLT during cycle 1, dose escalation terminated unless the DLTs were inconsistent with other data gathered up to that point. In this instance, a maximum of 12 patients could enroll at that dose level. If less than one-third of patients experienced, a DLT in cycle 1 dose escalation continued. DLT. Toxicities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). A DLT was defined as any of the following toxicities thought to be at least possibly due to deforolimus: grade 3 nonhematologic toxicity lasting >3 days despite optimal supportive care, with the exception of self-limiting or medically controllable toxicities such as fever without neutropenia, nausea, vomiting, fatigue, or hypersensitivity reactions; any grade 4 nonhematologic toxicity; neutropenia that was grade 4 and
sustained (absolute neutrophil count <500/mm³, >5 days' duration); grade 3 or 4 neutropenia associated with fever (<1,000/mm³; oral temperature >38.5°C); thrombocytopenia <25,000/mm²; inability, due to any toxicity thought to be associated with deforolimus, to complete a total of one dosing cycle (four doses); or treatment delays, due to any toxicity thought to be associated with deforolimus, >2 weeks from the scheduled next dose. Patients who experienced a DLT were allowed to continue on study at a reduced dose.

Pretreatment and follow-up studies. Pretreatment studies were done within 14 days before the first dose of deforolimus. These studies included medical and surgical history, medication history, performance status evaluation, and a complete physical examination. Because lenticular degeneration was noted in preclinical studies in rats, an ophthalmologic examination that included slit lamp evaluation and evaluation for evidence of lens opacification was required within 1 month of the first dose. Performance status evaluation and physical examination were done before each cycle, at the end of the last cycle, and 1 month after the end of the last cycle. The ophthalmologic examination was repeated at the end of cycle 6 and every 6 months thereafter if additional cycles were administered. Infusion site assessment was done before each administration of drug, every 15 min during the infusion, at the end of infusion, and 30 and 60 min after the end of the infusion.

Laboratory studies at screening included a complete blood count with differential, fasting serum chemistries, including cholesterol and glucose, and urinalysis. These were also repeated before each cycle, at the end of study, and 1 month after the end of the last cycle. All premenopausal, fertile females were required to have a serum pregnancy test within 5 days of the first dose and at the end of the last cycle of therapy. Fasting triglycerides were also measured during screening. Amylase, lipase, coagulation variables, fibrinogen, serum magnesium, and serum uric acid were measured on day 1 of cycle 1. In addition, a 12-lead electrocardiogram was required during screening, before each cycle, and at the end of the last cycle.

Assessment of disease was done during screening, within 1 month before beginning the study. Histologic or cytologic confirmation of the primary diagnosis was required. Diagnostic imaging and measurement of lesions was done, as appropriate based on tumor type and following Response Evaluation Criteria in Solid Tumors guidelines, during screening, at the end of each even-numbered cycle, and at the end of the last cycle with the same method of assessment and same technique used throughout the study.

Pharmacokinetics and pharmacodynamics. Blood samples for pharmacokinetic analysis were collected during cycle 1 on day 1 immediately before the first dose; 15 min after the start of the infusion; at the end of the infusion; and 5, 15, 30, and 60 min and 2, 4, 6, 24, and 48 h after the end of the infusion. Blood was also collected immediately before the second dose of cycle 1 (day 8), 60 min after the end of this infusion, and immediately before the first dose of cycle 2. Blood was collected into EDTA-containing tubes. Samples were kept refrigerated until the time they were shipped, on ice, to Ariad Pharmaceuticals where the pharmacodynamic analyses were done. Peripheral blood mononuclear cells were isolated from whole blood samples. Protein extracts were prepared and analyzed by Western blot using antibodies specific for 4E-BP1 phosphorylated at Ser65/Thr70. Phosphorylated 4E-BP1 levels were normalized against total levels of 4E-BP1 to assess the effects of mTOR inhibition by deforolimus.

Statistical analysis. Summary descriptive statistics of patient demographics and other baseline features were generated for each dose level and for the entire cohort of patients overall. These analyses included all patients who received at least one dose of study drug. The mean, median, SD, and minimum and maximum values were computed for continuous variables, whereas counts and percentages were calculated for discrete variables. Pharmacokinetic data were analyzed as described above.

Patients who received at least one dose of deforolimus were included in the analyses of adverse events. Patients who received at least three of four doses of deforolimus in cycle 1 were considered evaluable for DLTs, along with patients who received less than three doses but experienced a DLT. Laboratory assessments were summarized by study day and included the change in each laboratory variable from baseline.

Patients who completed at least two cycles of treatment and had baseline and at least one subsequent tumor assessment were evaluable for assessment of the antitumor activity of deforolimus, as were patients who withdrew from study due to progressive disease before completing two cycles. The response, duration of response, and time to progression of disease were recorded for all patients on whom adequate data were available.

The effect of dose level on toxicity was assessed using the Cochran-Armitage test for a linear trend in proportions. The relationship between pharmacokinetic variables and the severity of toxicity (specifically, mucositis), graded from 0 to 4, was analyzed using the proportional odds model for ordinal data (18). Linear or power models that characterized the relationship between patient factors and pharmacokinetic variables were evaluated using backward elimination (α = 0.05). Linear regression analysis was done to analyze the effects of dose, AUC, biomarkers, Response Evaluation Criteria in Solid Tumors-based tumor measurements, and laboratory variables. In the case of 4E-BP1 phosphorylation, the data were transformed to the log scale to reduce skewness and minimize the effects of outliers.

Results

Patients. Between June 2003 and October 2004, 46 patients with a wide variety of solid tumor diagnoses were enrolled. Thirty-one were male and 15 were female; 40 were Caucasian, 5 were Black, and 1 was Hispanic. Median (range) age was 62 (24-79) years. Seventeen patients had a performance status of 0 on the Eastern Cooperative Oncology Group scale or 100% on the Karnofsky scale, whereas 29 had a performance of 1 on the Eastern Cooperative Oncology Group scale or <100% on the Karnofsky scale. Patients had a wide variety of solid tumor diagnoses, including colorectal, breast, and non-small-cell lung cancer.

Blood samples for pharmacodynamic studies were collected during screening, immediately before the first dose of cycle 1, and 60 min and 24 h after the end of the first infusion. Blood was also collected immediately before the second dose of cycle 1 (day 8), 60 min after the end of this infusion, and immediately before the first dose of cycle 2. Blood was collected into EDTA-containing tubes. Samples were kept refrigerated until the time they were shipped, on ice, to Ariad Pharmaceuticals where the pharmacodynamic analyses were done. Peripheral blood mononuclear cells were isolated from whole blood samples. Protein extracts were prepared and analyzed by Western blot using antibodies specific for 4E-BP1 phosphorylated at Ser65/Thr70. Phosphorylated 4E-BP1 levels were normalized against total levels of 4E-BP1 to assess the effects of mTOR inhibition by deforolimus.
diagnoses and were for the most part heavily pretreated, with over one-half of patients \((n = 29)\) having received three or four prior chemotherapy, hormonal therapy, or immunotherapy regimens before enrollment. All 46 enrolled patients received at least one dose of deforolimus and 31 patients received two or more complete cycles, with a complete cycle defined as receiving at least three of the four infusions for that cycle. Five patients received less than one complete cycle (3 received one dose and 2 received two doses) due to symptomatic deterioration (2 patients), hypersensitivity reactions (2 patients), or investigator decision (1 patient, who also experienced a DLT at the 100 mg dose level). The median (range) number of completed cycles for all 46 patients was 2.0 (1-9).

Forty-six patients were evaluable for adverse events and 42 for DLTs. Only patients who received three of four doses of drug during cycle 1 or experienced a DLT during cycle 1 regardless of the number of doses they received were considered evaluable for DLTs. Of the 4 patients not evaluable for DLTs, 2 experienced a hypersensitivity reaction (hypersensitivity was not considered a DLT) and 2 patients were removed from the study due to symptomatic deterioration before receiving three doses of deforolimus. Thirty-four were evaluable for antitumor response to deforolimus. Reasons for withdrawal from the study were progressive disease \((n = 26)\), investigator decision \((n = 9)\), withdrawal of consent \((n = 4)\), need for a treatment not allowed by the protocol \((n = 1)\), adverse events \((n = 5)\), or other \((n = 1)\).

**Dose escalation and MTD.** A total of 479 doses of deforolimus were administered to 46 patients. The dose levels, number of patients treated at each level, cumulative dose administered, and number of patients evaluable for DLTs at each dose level are listed in Table 1.

Patients were enrolled sequentially in dose levels 1 to 5 (Table 1). After the 100 mg dose level was determined to be intolerable (all 4 patients developed mucositis, which resulted in dose interruption and/or dose reduction in 3 patients), the 50 mg dose level was expanded to further assess the safety of this dose. Subsequently, an intermediate dose level of 75 mg was also studied, which was eventually determined to be the MTD.

**Toxicity.** All 46 patients treated on this study experienced an adverse event and 70% of patients experienced an adverse event grade \( \geq 3 \). The most common adverse events were fatigue (76%), anorexia (67%), mucositis (63%), nausea (57%), and diarrhea (50%; Table 2). As the dose of deforolimus increased, the incidence of mucosal inflammation and rash of any grade also increased. The mucositis associated with deforolimus usually presented as ulcerations in the mouth occurring within the first cycle of treatment. Recurrence in subsequent cycles was unusual but was more common at the higher dose levels. In most instances, dose adjustment was not necessary and patients were treated with topical medications or required no intervention. The rash was typically maculopapular and pruritic. Six (13%) patients were discontinued from the study due to adverse events: 3 patients at the 50 mg dose level and 3 patients at the 75 mg dose level. These adverse events were dyspnea, cellulitis, hypersensitivity (2 patients), fatigue, and elevated creatinine phosphokinase.

Previously reported adverse events from other mTOR inhibitors also include metabolic disturbances, such as hyperglycemia, hypertriglyceridemia, and hypercholesterolemia (19, 20). These were experienced by 22%, 9%, and 7%, respectively, of patients. Hematologic toxicities were mild and included anemia (33%), thrombocytopenia (13%), neutropenia (9%), and leukopenia (7%).

Twenty-eight patients required interruption of treatment or dose reduction on at least one occasion, and in 17 instances, the associated adverse event was thought to be possibly, probably, or definitely related to deforolimus. Six patients had treatment discontinued (3 of which had previous dose interruptions), and in 4 patients, this was thought to be possibly (1 case of fatigue), probably (1 case each of hypersensitivity and elevated creatinine phosphokinase), or definitely (1 case of hypersensitivity) related to the study drug.

The main DLT in this study was mucositis. This was experienced by all 4 patients at the 100 mg dose level. Although the grade of toxicity was \( \geq 3 \) in only 1 of these patients, 3 of 4 patients required dosing delays and/or dose reduction secondary to mucositis. Twelve of the 17 patients at the 75 mg dose level also developed grade 1 to 2 mucositis.

Thirty-two serious adverse events occurred in 22 patients. Three of these serious adverse events were thought to be related to deforolimus and included mucositis, pulmonary embolism, and hypersensitivity. There were no deaths thought to be related to deforolimus.

**Pharmacokinetics.** Whole blood samples taken at the specified time points were analyzed for deforolimus and these levels were used to perform noncompartmental pharmacokinetic analyses. Of the 46 patients enrolled on the study, 43 were evaluable for pharmacokinetic analysis. Of these, 67% were male and 88% were Caucasian. The median (range) age of this group was 61 (25-79) years, the median (range) body weight was 82 (46-107) kg, and the median (range) body surface area was 1.95 (1.5-2.2) m\(^2\). The median (range) baseline RBC was 4.1 \( \times 10^{12} \) (3.4 \( \times 10^{12} - 5.2 \times 10^{12} \) cells/µL).

### Table 1. Dose levels

<table>
<thead>
<tr>
<th>Dose level (mg)</th>
<th>No. patients entered at dose level</th>
<th>No. doses administered</th>
<th>No. patients requiring dose reductions</th>
<th>No. patients requiring dose interruptions or reductions (no. at least possibly related to deforolimus)</th>
<th>Cumulative dose received (mg)</th>
<th>No. patients evaluable for DLTs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.25</td>
<td>2</td>
<td>16</td>
<td>0</td>
<td>100</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>3</td>
<td>33</td>
<td>0</td>
<td>413</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>4</td>
<td>22</td>
<td>0</td>
<td>550</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>16</td>
<td>167</td>
<td>3</td>
<td>7,283</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>4</td>
<td>56</td>
<td>2</td>
<td>4,000</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>75</td>
<td>17</td>
<td>185</td>
<td>2</td>
<td>13,045</td>
<td>15</td>
</tr>
</tbody>
</table>
were statistically significant predictors of CL (analyzed for an effect on the pharmacokinetics of weight, body surface area, gender, and baseline RBC were relatively constant at 45 to 52 h (Table 3).

The dose of deforolimus as well as patient factors of age, body weight, body surface area, gender, and baseline RBC were analyzed for an effect on the pharmacokinetics (Vss and CL) of the drug. Of these covariates, only dose was a statistically significant predictor of Vss (P < 0.0001), whereas dose and gender were statistically significant predictors of CL (P < 0.0001 and P = 0.001, respectively). Clearance estimated from the model was 1.49 L/h (SE, 0.41) greater in females. Using a backward elimination approach, it was determined that the significant patient factor, together with dose, accounted for ~66% of the variability in clearance and 70% of the variability in volume.

Pharmacodynamics. Because mucositis was the main toxicity, the relationship between deforolimus exposure and mucositis was studied. Increasing dose (P = 0.001), Cmax (P = 0.024), and AUC (P = 0.003) were associated with increased severity of mucositis based on univariate analyses using the proportional odds model. The odds of more severe mucositis increased by a factor of 1.58 (95% confidence interval, 1.21-2.07) per 10 mg increase in dose, by a factor of 2.82 (95% confidence interval, 1.15-6.91) per 0.5 μg/mL increase in Cmax, and by a factor of 1.32 (95% confidence interval, 1.10-1.58) per 1 μg/mL increase in AUC. There was no statistically significant effect of gender (adjusted for dose) on the severity of mucositis (P = 0.54) or significant interaction between gender and dose (P = 0.48).

Several laboratory variables were monitored throughout the course of this study. Those of most interest to mTOR inhibitors, based on previous studies, include metabolic variables such as glucose and cholesterol, and hemato logical variables. When considering patients who received at least three doses of deforolimus, there was a significant effect of dose on the nadir platelet count (P = 0.015) with a greater decrease in patients treated at higher doses, as expected, with an estimated slope of -12.3 × 10^{-3}/μL (95% confidence interval, -22.1 to -2.6) per 10 mg increase in dose. There was also a significant dose-response effect on the maximum change in cholesterol (P = 0.002) with an estimated absolute increase in cholesterol.

### Table 2. Summary of adverse events by cohort

<table>
<thead>
<tr>
<th>Event</th>
<th>6.25 mg (n = 2)</th>
<th>12.5 mg (n = 3)</th>
<th>25 mg (n = 4)</th>
<th>50 mg (n = 16)</th>
<th>75 mg (n = 17)</th>
<th>100 mg (n = 4)</th>
<th>Total (n = 46)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>11 (1)</td>
<td>14 (1)</td>
<td>3</td>
<td>35</td>
<td>0.83 (—)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>13</td>
<td>3</td>
<td>31</td>
<td>0.93 (—)</td>
</tr>
<tr>
<td>Mucosal inflammation</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>11</td>
<td>12</td>
<td>4</td>
<td>29</td>
<td>0.0046 (—)</td>
</tr>
<tr>
<td>Nausea</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>9</td>
<td>4</td>
<td>26</td>
<td>0.73 (—)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>9 (1)</td>
<td>2</td>
<td>23</td>
<td>0.94 (—)</td>
</tr>
<tr>
<td>Rash</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>8</td>
<td>10</td>
<td>3</td>
<td>22</td>
<td>0.018 (—)</td>
</tr>
<tr>
<td>Constipation</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>21</td>
<td>0.93 (—)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td>2</td>
<td>21</td>
<td>0.93 (—)</td>
</tr>
<tr>
<td>Anemia</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5 (1)</td>
<td>7 (3)</td>
<td>1</td>
<td>15</td>
<td>0.48 (0.33)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>2 (1)</td>
<td>0</td>
<td>6</td>
<td>5 (1)</td>
<td>1 (1)</td>
<td>14</td>
<td>0.99 (0.70)</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>13</td>
<td>0.66 (—)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>13</td>
<td>0.082 (—)</td>
</tr>
<tr>
<td>Dysgeusia</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>12</td>
<td>0.20 (—)</td>
</tr>
<tr>
<td>Hypophosphatemia</td>
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<td>0</td>
<td>4 (3)</td>
<td>6 (3)</td>
<td>1 (1)</td>
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<td>0.092 (0.22)</td>
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<tr>
<td>Hyperglycemia</td>
<td>0</td>
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<td>0</td>
<td>5 (2)</td>
<td>5 (4)</td>
<td>0</td>
<td>10</td>
<td>0.44 (0.31)</td>
</tr>
<tr>
<td>Cough</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>10</td>
<td>0.44 (—)</td>
</tr>
</tbody>
</table>

NOTE: Number in parentheses is the number of adverse events, that are grade 3 or 4.

* Cochrán-Armitage trend test: First P value corresponds to any grade toxicity. Second P value (in parentheses) corresponds to grade ≥3. Dash indicates too few grade ≥3 events for statistical analysis.

### Table 3. Summary statistics of pharmacokinetic variable estimates from noncompartmental analysis

<table>
<thead>
<tr>
<th>Dose, mg (n)</th>
<th>Cmax, ng/mL</th>
<th>Cmax %CV</th>
<th>AUCmax, μg h/mL</th>
<th>AUCmax %CV</th>
<th>t1/2, h</th>
<th>t1/2, h %CV</th>
<th>CL, L/h</th>
<th>CL, L/h %CV</th>
<th>Vss, L</th>
<th>Vss, L %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25 (1)</td>
<td>329</td>
<td>3.8</td>
<td>—</td>
<td>—</td>
<td>52.2</td>
<td>1.7</td>
<td>80.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>12.5 (3)</td>
<td>394</td>
<td>14.2</td>
<td>5.1</td>
<td>32.7</td>
<td>47.0</td>
<td>20.2</td>
<td>35.6</td>
<td>111 (14.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 (4)*</td>
<td>670</td>
<td>9.0</td>
<td>9.0*</td>
<td>21.1</td>
<td>46.2*</td>
<td>17.2</td>
<td>21.0</td>
<td>136 (14.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 (15)</td>
<td>982</td>
<td>19.7</td>
<td>10.7</td>
<td>22.9</td>
<td>44.9</td>
<td>10.0</td>
<td>24.3</td>
<td>235 (19.6)</td>
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<tr>
<td>75 (16)†</td>
<td>1,195</td>
<td>23.4</td>
<td>12.7†</td>
<td>28.3</td>
<td>47.3†</td>
<td>26.0</td>
<td>6.4†</td>
<td>28.2</td>
<td>323†</td>
<td>22.0</td>
</tr>
<tr>
<td>100 (4)</td>
<td>1,255</td>
<td>7.8</td>
<td>13.4</td>
<td>16.8</td>
<td>46.0</td>
<td>9.7</td>
<td>19.0</td>
<td>373 (11.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: The mean is listed for each variable.

* One patient in the 25 mg dose group did not have sufficient data to calculate the AUC, CL, t1/2, or Vss.

† One patient in the 75 mg dose group did not have sufficient data to calculate the AUC, CL, t1/2, or Vss.
of 7.4 mg/dL (95% confidence interval, 3.0-11.9) per 10 mg increase in dose. A statistically significant dose effect on change in glucose was not seen ($P = 0.68$).

Other pharmacodynamic measures evaluated in this study included the change in 4E-BP1 phosphorylation, relative to baseline, after treatment with deforolimus. This was successfully measured in peripheral blood mononuclear cells from 44 of the 46 treated patients. In all patients, the level of phosphorylated 4E-BP1 was rapidly reduced after treatment with deforolimus, with a median level of inhibition of 95% at 1 h after the end of the infusion. This inhibition was prolonged, with a median of >90% inhibition at 48 h after infusion and a median of >70% at 7 days after infusion. Across dose cohorts, there was no evidence of dose dependency of inhibition of 4E-BP1 phosphorylation at the early time points ($P = 0.068, 0.89,$ and $0.64$ at 24, 48, and 120-144 h, respectively, by linear regression analysis). However, at 7 days after deforolimus administration, the median inhibition was 65% to 89% in those who received ≥25 mg drug, whereas only 27% to 39% in those patients who received a lower dose. Here, the regression of 4E-BP1 phosphorylation 7 days post-initial deforolimus administration showed increased inhibition with increasing dose level, but the slope was not statistically significant ($P = 0.26$).

**Tumor response.** Thirty-four patients were evaluable for response to deforolimus. After two cycles of treatment, a patient with transitional cell carcinoma of the bladder had a 34% shrinkage in target lesions, 21 patients had stable disease, and 12 patients had disease progression. The patient with transitional cell carcinoma was reimaged after three cycles, and at that point progression of the nontarget lesions was documented. There was no statistically significant relationship between dose and the percent change in size of target lesions after two cycles of therapy ($P = 0.28$; Fig. 1A). However, percent change in tumor size was significantly associated with change in cholesterol ($P = 0.015$; Fig. 1B). Given the observed relationships between dose and change in cholesterol and, to a lesser extent, dose and 4E-BP1 inhibition at 7 days, we explored the relationship between changes in these variables and tumor response. There was no correlation between 4E-BP1 inhibition and percent change in tumor size ($r = 0.09; P = 0.65$); however, a significant association was detected for maximum change in cholesterol within the first two cycles of therapy and tumor size change ($r = -0.38; P = 0.029$; Fig. 2). There was also a borderline statistically significant relationship between AUC and change in cholesterol ($r = 0.31; P = 0.055$).

**Discussion**

This phase I dose-escalation trial examined the safety, pharmacokinetics, pharmacodynamics, and antitumor response of deforolimus when administered intravenously on a once weekly schedule. A dose of 75 mg was safe and well-tolerated and was determined to be the MTD in this group of heavily pretreated patients. Dosing was limited by mucositis, but for the most part the adverse events experienced by patients in this study are similar to those reported for other inhibitors of the mTOR pathway (20, 21).

The pharmacokinetic analysis identified a nonlinear increase in the AUC, $C_{\text{max}}$, CL, and $V_{\text{ss}}$. This is similar to what has been described for temsirolimus (19). Also similar to other mTOR inhibitors is the long half-life of deforolimus (45-52 h), which allows for infrequent dosing. From this study, there do not appear to be any pharmacokinetic variables unique to deforolimus compared with those of other mTOR inhibitors.

A secondary objective of this study was to evaluate the antitumor effect of deforolimus when administered on this schedule. Although the majority of patients had progressive disease after two cycles, there was a statistically significant association between AUC and change in tumor size, although the percent of variation explained by AUC was just 18%.

We also explored pharmacodynamic biomarkers of deforolimus in peripheral blood mononuclear cells. The evaluation of changes in 4E-BP1 phosphorylation shows that the inhibition of phosphorylation occurs rapidly and persists for at least a week, providing evidence for the use of an intermittent treatment schedule.

The study of other pharmacodynamic effects of deforolimus showed a dose-dependent effect on platelet nadir and increase in cholesterol, which can be considered biomarkers. Of particular interest was our observation that the increase in cholesterol is significantly associated with change in tumor size, which suggests that the change in serum cholesterol after deforolimus treatment may be a predictive biomarker. This hypothesis could be tested in future studies of deforolimus and other mTOR inhibitors.
Two additional studies of deforolimus have been published (14, 22). In both studies, deforolimus was administered intravenously daily for 5 days every 2 weeks. The adverse effects of the drug when administered on this protracted schedule were similar to what was found in the current study, with mucositis being frequent. In the phase I study by Mita et al., metabolic effects included hyperglycemia, hypertriglyceridemia, and hypercholesterolemia and were more frequent than in the current study (28% versus 22%, 41% versus 9%, and 28% versus 7%, respectively). Deforolimus showed nonlinear pharmacokinetics and a prolonged half-life on the protracted schedule, similar to what was found in the current study. Tumor activity was shown in patients with both solid tumors and hematologic malignancies on the protracted schedule, with stable disease being the most common in both as was found in the current study. Based on these limited data, there does not seem to be an advantage to administering deforolimus on a protracted schedule.

Given the low response rate (by Response Evaluation Criteria in Solid Tumors) to mTOR inhibitors, phase II studies should incorporate randomized designs to detect both regression and disease stabilization as an antitumor effect (23–25). Such studies should also consider randomization across doses and/or schedules. In addition, these studies could explore further the use of serum cholesterol as a potential biomarker and should assess whether deforolimus is superior to other investigational and marketed mTOR inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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References

A Phase I Trial to Determine the Safety, Tolerability, and Maximum Tolerated Dose of Deforolimus in Patients with Advanced Malignancies


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