



Published in final edited form as:

*Cancer*. 2010 August 15; 116(16): 3903–3909. doi:10.1002/cncr.25264.

## Phase I and pharmacokinetic study of everolimus, an mTOR inhibitor, in combination with docetaxel for recurrent/refractory non-small cell lung cancer

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### Abstract

**Purpose**—Everolimus is a novel inhibitor of the mammalian target of rapamycin (mTOR) pathway, which is aberrantly activated in non-small cell lung cancer (NSCLC). We conducted a phase I and pharmacokinetic study of everolimus and docetaxel for recurrent NSCLC.

**Methods**—Patients with advanced stage NSCLC and progression following prior platinum-based chemotherapy were eligible. Sequential cohorts were treated with escalating doses of docetaxel (day 1) and everolimus (PO daily, days 1–19), every 3 weeks. Pharmacokinetic (PK) sampling of everolimus and docetaxel were done in cycle 1. The primary endpoint was determination of the recommended phase II doses (RP2D) of the combination.

**Results**—Twenty-four patients were enrolled. Median age, 62 yrs; Females, 11; number of prior regimens, 1 (n=13), 2 (n=6), ≥3 (n=5) ECOG PS 0 (n=6), 1 (n=17). The dose-limiting toxicities (DLT) were fever with grade 3/4 neutropenia, grade 3 fatigue and grade 3 mucositis. None of the 7 patients treated at the RP2D (docetaxel 60 mg/m<sup>2</sup> and everolimus 5 mg daily) experienced DLT. Everolimus area under the concentration time curve (AUC) was not different with 60 or 75 mg/m<sup>2</sup> docetaxel. Mean ±SD AUC-based accumulation factors (R) for everolimus on days 8 and 15 were 1.16 ± 0.37 and 1.42 ± 0.42, respectively. Docetaxel day 1 half-life was 9.4 ± 3.4 hours. Among 21 patients evaluable, 1 had a partial response, and 10 had disease stabilization.

**Conclusions**—The RP2D of docetaxel and everolimus for combination therapy are 60 mg/m<sup>2</sup> and 5 mg PO daily, respectively. Promising anti-cancer activity has been noted.

### Keywords

Everolimus; docetaxel; phase I; pharmacokinetics; non-small cell lung cancer

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Presented, in part, at the 45<sup>th</sup> Annual Meeting of the American Society of Clinical Oncology, Orlando, FL, June 2009.

## Introduction

The mammalian target of rapamycin (mTOR) pathway plays a central role in regulating cell growth, proliferation and survival [1]. It is activated in response to cellular stress or nutrient deprivation and consequently, induces translation of a number of cell survival proteins. mTOR is an important part of the PI3K/Akt cell signaling axis, which is a major cell survival pathway [2]. Activation of the PI3K/Akt axis is associated with malignant transformation and resistance to apoptosis [3]. Therefore, inhibitors of the mTOR pathway are under evaluation for the treatment of cancer. Temsirolimus and everolimus are novel inhibitors of the mTOR pathway that have received approval by the Food and Drug Administration for the treatment of renal cell carcinoma. Their approval was based on randomized clinical trials that each drug improved overall survival and progression-free survival [4,5]. Everolimus binds with high affinity to FKB12, the intracellular receptor for mTOR; forms an intracellular complex that interacts with mTOR; and inhibits downstream signaling [6]. It is administered orally and can be given either on a daily or a weekly schedule [7]. Everolimus has demonstrated anti-cancer effects in a variety of pre-clinical models [8,9].

The mTOR pathway is aberrantly activated in non-small cell lung cancer (NSCLC). Approximately 70% of NSCLCs have activated mTOR signaling [10]. In addition, PI3K/Akt signaling is dysregulated in a majority of NSCLCs [11,12]. A phase II study by Soria et al. demonstrated a response rate of 5% when everolimus was used as monotherapy in patients with recurrent or refractory NSCLC [13]. In addition, several patients had disease stabilization. These interesting observations form the basis for evaluating mTOR inhibitors in combination with agents that have proven activity in the treatment of NSCLC.

Docetaxel is a semi-synthetic taxane that is approved for the treatment of advanced NSCLC [14]. It can be used in the front-line therapy setting in combination with a platinum compound or as monotherapy in the second-line treatment of advanced stage NSCLC. In addition to its activity in NSCLC, docetaxel is also used in routine care for several other solid organ malignancies including breast, prostate, ovarian, head and neck and gastric cancers [15]. Docetaxel exerts anti-cancer effects by inducing polymerization of tubulin, which leads to mitotic arrest. Activation of the PI3K/Akt/mTOR axis is a known mechanism of resistance to taxane therapy [16]. Up-regulation of p-Akt has been noted in response to exposure to tubulin-interactive agents, such as taxanes and vincristine, and is postulated as contributing to resistance to the anti-cancer effects of these agents. Inhibition of mTOR in cancer cells with upregulated p-AKT is associated with sensitization to tubulin-interactive agents [17]. Pre-clinical studies using a variety of cell lines have demonstrated synergistic interactions between docetaxel and everolimus [17–21]. Based on these observations, we hypothesized that co-administration of an mTOR inhibitor with docetaxel would be associated with enhanced anti-cancer effects in patients with advanced NSCLC. Both docetaxel and everolimus are extensively metabolized by CYP3A4 and exhibit dose-proportional increases in peak concentrations and exposure. Everolimus was associated with a 33% inter-patient variability in area under the concentration versus time curve (AUC) as a single agent and warrants pharmacokinetic (PK) evaluation when given in combination with other CYP3A4 substrates [7]. We conducted a phase I study to determine the optimal doses of everolimus and docetaxel that can be administered as a combination to patients with advanced stage NSCLC and to evaluate the feasibility of this approach.

## Patients and methods

The primary objective of this study was to determine the recommended doses of docetaxel and everolimus that can be administered as a combination. The secondary objectives were:

to define the dose-limiting and non-dose-limiting toxicities of the regimen; to characterize the PK disposition of everolimus and docetaxel when given in combination; and to obtain preliminary information regarding the efficacy of the combination in patients with advanced stage NSCLC.

### Patient eligibility

Eligible patients had histological or cytological confirmation of NSCLC that was advanced stage and progressed following at least one prior chemotherapy regimen. Other pertinent eligibility criteria were: an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–2; age > 18 years; and life expectancy > 12 weeks. Qualifying laboratory criteria were: leukocytes  $\geq 3,000/\mu\text{L}$ ; absolute neutrophil count (ANC)  $\geq 1,500/\mu\text{L}$ ; platelet count  $\geq 100,000/\mu\text{L}$ ; serum total bilirubin  $\leq 1.5$  X institutional upper limit of normal (ULN); serum transaminases  $\leq 2.5$  X ULN; and serum creatinine  $\leq 1.5$  X ULN. Patients had to have recovered from toxicities related to prior therapies to < grade 2, and at least 3 weeks should have elapsed since prior chemotherapy or radiotherapy. Patients with untreated or symptomatic brain metastasis were excluded. Patients who had received prior therapy with a known mTOR inhibitor, were on full dose anticoagulation therapy with warfarin, had known HIV seropositivity, or were unable to take oral medications on a regular basis were excluded. Pregnant or lactating women were not allowed on the study due to the potential for teratogenic effects with the combination regimen. Patients with a serious co-morbid illness, known hypersensitivity to docetaxel, or other active malignancies were also excluded. Men and women of reproductive age were required to use appropriate contraception. All patients provided written informed consent before entering the study. The study protocol and consent form were approved by the Institutional Review Board of Emory University.

### Treatment plan

Docetaxel was administered as a 60 min i.v. infusion on day 1 of each cycle. Everolimus was given orally on days 1 to 19 of each 3-week treatment cycle. All treatments were administered in the outpatient setting. Dexamethasone was administered as a pre-medication at a dose of 8 mg twice a day for 3 days beginning the day before docetaxel infusion. The dose escalation scheme is outlined in table 1. Intra-patient dose escalation was not permitted. Treatment cycles were continued until progression of disease, unacceptable toxicity, or withdrawal of informed consent. After 6 cycles of combination therapy, patients without disease progression were continued on single-agent everolimus at the same dose and schedule as when given with docetaxel.

The use of appropriate supportive care measures was allowed for adverse events associated with therapy. Prophylactic colony stimulating factors were not allowed for cycle 1 of therapy. For patients who experienced severe toxicity, dose modifications were done for subsequent cycles. The dose of everolimus was reduced by 2.5 mg/day decrements, and the dose of docetaxel was reduced by 15 mg/m<sup>2</sup> decrements. For patients who experienced grade 3/4 neutropenia with fever, grade 4 neutropenia lasting > 7 days, grade 4 thrombocytopenia, or grade  $\geq 3$  non-hematological toxicity, the doses of both agents were reduced. For grade  $\geq 2$  peripheral neuropathy or renal toxicity, the dose of docetaxel was reduced. Docetaxel was permanently discontinued in the event of a grade 4 hypersensitivity reaction. All dose reductions were permanent. With the exception of alopecia, neuropathy and unrelated laboratory values, toxicities had to resolve to grade  $\leq 1$  before initiation of the subsequent cycle of therapy. Initiation of a new cycle could be delayed up to 2 weeks to allow for recovery from toxicity, failing which the patient was removed from the study.

## Definition of dose-limiting toxicity (DLT)

DLT was defined as the occurrence of one or more of the following events during cycle 1 of therapy: grade 3/4 neutropenia associated with fever; grade 4 neutropenia lasting > 7 days; grade 4 thrombocytopenia; grade  $\geq 3$  non-hematological toxicity; or a toxicity-related delay of > 2 weeks to initiate cycle 2. An 'up and down' dose escalation scheme, with cohorts of 3 patients, was utilized. The first cohort was treated at dose level 1. If 1 of the 3 patients in a cohort experienced DLT, 3 more patients were added at the same level. If 0 of 3 initial patients or 1 of 6 patients in an expanded cohort experienced DLT, the dose for the next cohort was escalated by one level. Escalation stopped, and de-escalation by 1 dose level began as soon as 2 patients at a dose level experienced DLT. If only 3 patients had been treated at the previous dose level, up to 3 additional patients were to be entered at that dose level. Dose de-escalation continued until  $\leq 1/6$  patients at a dose level experienced DLT, and that level was defined as the recommended phase II dose.

## Patient evaluation

Pretreatment evaluations included: history and physical examination (H&P); assessment of PS; complete blood count (CBC); and hepatic and renal function tests. Serum triglycerides and a full lipid profile were obtained at baseline. Women of reproductive age underwent a serum pregnancy test. Patients were evaluated weekly with a CBC, hepatic and renal function tests. Serum chemistry tests were done on day 1 of each cycle from cycle 2 onwards. Radiological studies to assess response were performed after every 2 cycles of therapy. All patients underwent a positron emission tomogram (PET) scan at baseline and after cycle 2. H & P and assessment of PS were done before initiation of each cycle. Responses were assessed with RECIST criteria [22].

## Pharmacokinetic assessments

Pharmacokinetic studies were conducted during cycle 1 of therapy. On days 1, 8 and 15, peripheral blood was obtained 30 min prior to administration of everolimus and 1, 2, 5, 8 and 24 hours after dosing. Docetaxel sampling occurred on day 1 only. Blood samples (2 mL) were obtained from a peripheral vein into potassium-EDTA tubes (everolimus) and sodium heparin tubes (docetaxel). Each potassium-EDTA tube was inverted several times to mix the contents and then stored at  $-20^{\circ}\text{C}$  within 60 min. Everolimus concentrations were measured by a validated method including LC/MS analysis following a high throughput liquid/liquid extraction as previously described [23]. The lower limit of quantitation was 0.3 ng/mL. Plasma was prepared from sodium heparin tubes which were centrifuged at  $4^{\circ}\text{C}$  at  $3000 \times g$  for 10 min, and the resulting plasma was stored at  $-80^{\circ}\text{C}$  until analysis. Docetaxel concentrations in plasma were measured by a validated LC-MS/MS assay [24]. WinNonlin Professional software version 5.2 (Pharsight Corporation) and standard noncompartmental methods were used to calculate area under the concentration versus time curve (AUC), maximum observed concentration ( $C_{\text{max}}$ ), and elimination half-life ( $t_{1/2}$ ).

## Results

### Patient characteristics

Twenty-four patients were enrolled between October 2007 and December 2008. One patient who signed informed consent did not start treatment due to rapid progression of disease. The median age was 62 years, and majority of the patients had an ECOG performance status of 0 or 1. Eleven patients were females. NSCLC 'not otherwise specified (NOS)' was the most common histological subtype (42%) followed by adenocarcinoma (38%) and squamous cell carcinoma (13%). Approximately half the patients had received at least two lines of prior therapy for advanced stage NSCLC.

## Toxicity

Three patients were initially entered onto dose level 1 (docetaxel 60 mg/m<sup>2</sup>, everolimus 5 mg PO daily), and no DLT was noted. Subsequently, 6 patients were entered to dose level 2 (docetaxel 75 mg/m<sup>2</sup>, everolimus 5 mg PO daily). One DLT (fever with grade 4 neutropenia) was observed. As specified in the protocol, dose escalation continued, and 3 patients were enrolled to dose level 3 (docetaxel 75 mg/m<sup>2</sup>, everolimus 7.5 mg PO daily). The second patient on dose level 3 developed grade 3 mucositis, which prompted enrollment 3 additional patients to dose level 3. The fourth patient enrolled at dose level 3 developed fever with grade 4 neutropenia. Based on the observation of 2 DLTs in 4 patients, dose level 3 was deemed undeliverable, and dose level 2 was expanded by an additional 6 patients to obtain additional safety data. However, 3 of those 6 additional patients developed DLT (fever with grade 3/4 neutropenia). One patient enrolled to dose level 2 only received 4 days of therapy and withdrew informed consent. Based on a telephonic conversation with the patient, there was no evidence of DLT, but the patient was replaced. Because there was a total of 4 DLTs in 13 patients, dose level 2 was also considered to be above the maximally tolerated dose for the combination. Therefore, dose level 1 was expanded to a total of 7 patients. Dose level 1 was tolerated well with only one episode of grade 3 neutropenia associated with fever. Therefore, dose level 1 (docetaxel 60 mg/m<sup>2</sup>, everolimus 5 mg QD) was defined as the recommended phase II dose (RP2D).

Overall, the treatment regimen was tolerated well at the RP2D. The median number of cycles of chemotherapy administered in the study was 2 (range 1–18). Eight patients completed all 6 cycles of combination therapy and were subsequently continued on monotherapy with everolimus. The common hematological and non-hematological toxicities associated with the regimen are outlined in tables 3 & 4.

## Pharmacokinetics

Everolimus and docetaxel concentrations and PK profiles were available in 19 and 5 patients, respectively, (table 5). Everolimus exposure as measured by mean AUC and C<sub>max</sub> increased proportionally with dose. Due to continuous dosing and the sampling schedule used, everolimus elimination half-lives could not be assessed accurately. Everolimus accumulation through cycle 1 based on ratios of day 8 or 15 AUC<sub>0-24</sub> to day 1 AUC<sub>0-∞</sub> measurements in 7 subjects receiving 5 mg daily is shown in figure 1. Although sample sizes were small, docetaxel half-lives were consistent with previously reported values [25].

## Anti-tumor activity

Sixteen patients received > 1 cycle of therapy. All patients who received at least one dose of therapy were considered evaluable for response. A 68 year-old female patient with adenocarcinoma of the lung that had failed 6 prior systemic therapy regimens experienced a partial response. There was regression in the primary tumor mass in the lung and multiple bilateral pulmonary nodules. She received a total of 6 cycles of combination therapy and 3 additional cycles of everolimus monotherapy. Ten patients achieved disease stabilization. Three of 6 patients treated at dose level 1 achieved disease stabilization and were given 18, 8 and 10 cycles of treatment respectively. One patient continues on active therapy.

## Discussion

Docetaxel is an agent used commonly as second-line therapy for advanced NSCLC. Several randomized studies that evaluated combination cytotoxic chemotherapy regimens for second-line therapy of advanced NSCLC failed to establish survival benefit, though there was higher toxicity [26]. Therefore, combinations of molecularly targeted agents with

standard chemotherapy are under investigation in an effort to overcome the therapeutic plateau that has been noted for second-line therapy of advanced NSCLC.

Inhibition of the mTOR pathway has emerged as a novel strategy for the treatment of cancer. Everolimus, an agent approved for the treatment of renal cell carcinoma, has demonstrated anti-cancer activity in NSCLC and exhibits pre-clinical synergy with docetaxel [13,17]. Our phase I study was conducted to determine the RP2D for the combination for docetaxel and everolimus. Overall, the regimen was tolerated well. Four of 13 patients experienced fever with grade 3/4 neutropenia when docetaxel was given at a dose of 75 mg/m<sup>2</sup> in combination with everolimus at 5 mg PO daily. In the pivotal studies of single-agent docetaxel for second line therapy of NSCLC, the incidence of fever with neutropenia was 2–12% [27–29]. The observed higher incidence in our study could be attributed to the degree of prior therapy in our patient population or to the addition of everolimus to docetaxel. We decided to evaluate the 60 mg/m<sup>2</sup> dose of docetaxel to improve the tolerability of the regimen. In randomized studies conducted in Japan, the 60 mg/m<sup>2</sup> dose of docetaxel has been shown to be efficacious in the treatment of NSCLC [30]. Notably, the 5 mg/day dose of everolimus has been documented to produce the desired pharmacodynamic effects [31,32].

The overall efficacy of the regimen used in our study was promising as disease control was achieved in approximately 50% of the patients. We feel that the promising efficacy of docetaxel and everolimus in light of multiple prior therapies in our patients justifies further evaluation of this combination. Therefore, we have initiated a phase II study of the combination in patients with advanced NSCLC that had progressed following 1 or 2 prior regimens. An important secondary objective of that phase II study will be to identify baseline molecular markers in tumor tissues that may predict for a favorable outcome in response to the combination. We intend to evaluate baseline status of Akt, which is upstream of mTOR, and of pS6k and 4E-BP1, which are downstream of mTOR.

Everolimus is a substrate for CYP3A4 and a mixed inhibitor of CYP2D6. However, at a dose of 5 mg/day, the steady-state concentration achieved is several-fold less than that required to alter the metabolism of substrates of CYP3A4 and CYP2D6. Because docetaxel is metabolized by CYP3A4, conducting full pharmacokinetic evaluations of docetaxel would have been instrumental in documenting the effect of everolimus on docetaxel disposition. However, the intervals at which samples were obtained in our study rendered it difficult to evaluate docetaxel pharmacokinetics completely. Similarly, the sampling interval used in our evaluation of continuous everolimus made detailed interpretation of half-life and elimination characteristics problematic. As a result, we intend to evaluate the PK of docetaxel in a selected subset of patients in our phase II study.

To our knowledge, this is the first report of the combination of a chemotherapeutic agent with a continuous daily dosing schedule of everolimus. Campone and colleagues conducted a phase I study using a weekly schedule of everolimus with weekly paclitaxel [33]. Their study included 16 patients with various solid organ malignancies and noted disease stabilization in 11 of the 16 patients. The DLT in their study was primarily myelosuppression. Everolimus has also been safely combined with inhibitors of the epidermal growth factor receptor (EGFR) pathway for the treatment of NSCLC [34,35]. A randomized phase II study of erlotinib alone or in combination with everolimus in patients with advanced NSCLC has recently completed accrual. Recently, phase I studies have been initiated to evaluate everolimus in combination with platinum-based, two-drug regimens for first-line therapy of advanced NSCLC. In that the molecular mechanisms behind resistance to common chemotherapeutic agents are not dissimilar across various tumor types, the everolimus-based combinations may also be applicable to other disease types.

In summary, our phase I study has demonstrated the feasibility of combining docetaxel with everolimus and also demonstrated promising efficacy of the combination in a refractory NSCLC patient population.

### Translational Significance

Agents that inhibit the mTOR pathway are now used as monotherapy for the treatment of renal cell carcinoma. In other solid organ malignancies, they are not effective as monotherapy. Preclinical studies have demonstrated favorable interactions when everolimus, an mTOR inhibitor, in combination with standard cytotoxic agents such as the taxanes, or with other molecularly targeted agents. In this study, we evaluated the combination of docetaxel and everolimus in patients with refractory non-small cell lung cancer. We report the optimal dose for the two agents for combination therapy and demonstrate the safety profile. The pharmacokinetic studies provide valuable information regarding potential drug-drug interactions. Furthermore, the promising anti-cancer activity noted with this regimen has prompted a phase II study in advanced non-small cell lung cancer. Since docetaxel is used for the treatment of a variety of solid organ malignancies, this combination is could be relevant across disease types.

### Acknowledgments

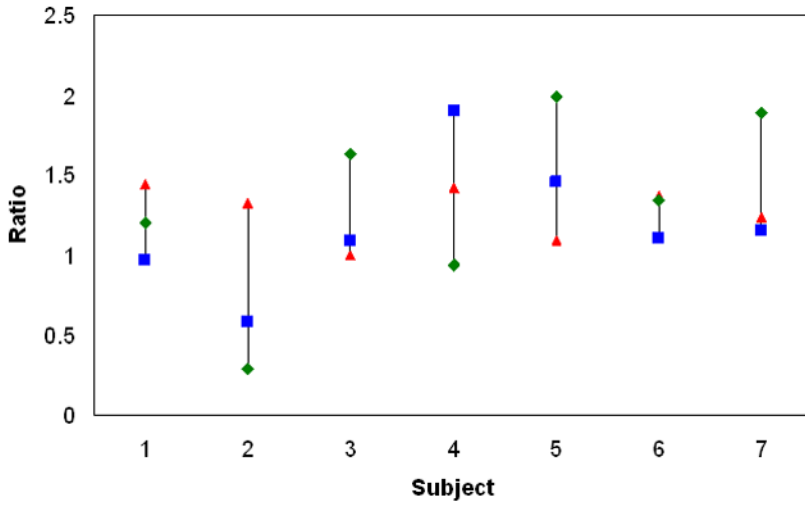
Supported by NCI 5 P01 CA116676-03and, in part, by Novartis pharmaceuticals.

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**Figure 1.**  
Everolimus accumulation ratios in 7 subjects receiving 5 mg daily

- ▲ = Predicted R
- = Day 8 R
- ◆ = Day 15 R

Predicted accumulation factor (R) =  $(1/1 - e^{-k_e\tau})$ , where  $k_e$  is the elimination rate constant and  $\tau$  is the dosing interval.

Actual accumulation =  $AUC_{Dx}/AUC_{D1}$  ( $AUC$  = area under the concentration-time curve).  
For day 1,  $AUC_{0-\infty}$ , days 8 and 15  $AUC_{0-24}$

**Table 1**

Dose escalation scheme

Dose level	Everolimus	Docetaxel	Number of patients
1	5 mg PO daily	60 mg/m <sup>2</sup>	7
2	5 mg PO daily	75 mg/m <sup>2</sup>	13
3	7.5 mg PO daily	75 mg/m <sup>2</sup>	4

**Table 2**

## Patient baseline characteristics

<b>Number of patients</b>	24
<b>Median age (range)</b>	62 (42–72)
<b>Female</b>	11
<b>ECOG PS</b>	
0	6
1	17
2	1
<b>Histology</b>	
Adenocarcinoma	9
Squamous cell carcinoma	3
NSCLC-NOS	10
Other	2
<b>Number of prior regimens</b>	
1	13
2	6
> 2	5

**Table 3**

## Hematological toxicity\*

Toxicity	Grade 3 n	Grade 4 n
Anemia	1	0
Thrombocytopenia	6	4
Neutropenia	0	6
Leukopenia	0	2

\* represents worst grade toxicity experienced per patient during the course of protocol treatment

**Table 4**

Non-hematological toxicity\*

Toxicity	Grade 2 n	Grade 3 n	Grade 4 n
Fever	3	0	0
Diarrhea	0	0	1
Vomiting	2	0	0
Rash	2	2	0
Fatigue	2	4	3
Weight loss	2	0	0
Insomnia	2	1	2
Pain	5	3	2
Headache	1	0	1
Hypercholesterolemia	2	0	0
Hypertriglyceridemia	1	1	0
Hyperglycemia	11	1	0

\* represents worst grade toxicity experienced per patient during the course of protocol treatment

**Table 5**

Mean (SD) everolimus and docetaxel pharmacokinetic parameters

Variable	Everolimus 5 mg/day, Docetaxel 60 mg/m <sup>2</sup>	Everolimus 5 mg/day, Docetaxel 75 mg/ m <sup>2</sup>	Everolimus 7.5 mg/day, Docetaxel 75 mg/m <sup>2</sup>
Everolimus	(n=3)	(Day 1 n=10, Days 8, 15 n=6)	(Day 1 n=4, Day 15 n=1)
<b>C<sub>max</sub> (ng/mL)</b>			
Day 1	37.7 (6.2)	22.4 (9.4)	44.5 (21.2)
Day 8	39.3 (16.4)	35.8 (11.4)	NA
Day 15	51 (23.6)	37.8 (15.2)	101.8
<b>AUC<sub>0-24</sub> (ng*hr/mL)</b>			
Day 1	244 (19)	330 (310)	379 (269)
Day 8	329 (98)	336 (208)	NA
Day 15	350 (185)	369 (124)	772
<b>Docetaxel</b>	<b>(n=1)</b>	<b>(n=3)</b>	<b>(n=1)</b>
<b>C<sub>max</sub> (nM)</b>	1013.7	1614.5 (1084.9)	3290.5
<b>AUC<sub>0-∞</sub> (hr*nM)</b>	2081	6952 (5862)	7298
<b>t<sub>1/2</sub> (hr)</b>	16.8	8.74 (0.8)	10.4

NA = data not available

C<sub>max</sub> = maximum concentration observedAUC<sub>0-24</sub> = area under the concentration-time curve to 24 hours post-doseAUC<sub>0-∞</sub> = area under the concentration-time curve to infinity