Phase 1 Trial of Everolimus and Gefitinib in Patients With Advanced Nonsmall-Cell Lung Cancer

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BACKGROUND. Preclinical studies have demonstrated that the inhibition of the PI3K/Akt/mTOR pathway restores gefitinib sensitivity in resistant cancer cell lines. A phase 1 study was conducted of the combination of everolimus, an mTOR inhibitor, and gefitinib to determine a daily dose of everolimus with gefitinib in patients with advanced nonsmall-cell lung cancer (NSCLC).

METHODS. Oral everolimus and gefitinib were both administered daily to patients with progressive NSCLC. Patients were enrolled in 3-patient cohorts at everolimus dose levels of 5 and 10 mg daily. All patients received gefitinib 250 mg daily.

RESULTS. Ten patients were enrolled. The maximum tolerated dose of everolimus was 5 mg when administered daily with gefitinib 250 mg. Two patients who were treated at the 10 mg dose level of everolimus experienced dose-limiting toxicity, including grade 5 hypotension and grade 3 stomatitis. Pharmacokinetic studies demonstrated no consistent, significant interaction on the t_{max}, C_{max}, and $AUC_{0\text{-}8h}$ of either agent. Two partial radiographic responses were identified among the 8 response-evaluable patients.

CONCLUSIONS. For further study, everolimus at a dose of 5 mg daily in combination with daily gefitinib 250 mg is recommended. The 2 radiographic responses identified are encouraging. A phase 2 trial in patients with NSCLC is under way. ***Cancer* 2007;110:599–605.** © *2007 American Cancer Society.*

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The epidermal growth factor receptor (EGFR) is an important therapeutic target in the treatment of nonsmall-cell lung cancer (NSCLC). Gefitinib (Iressa, AstraZeneca Pharmaceuticals, Wilmington, Del) and erlotinib (Tarceva, Genentech, South San Francisco, Calif) selectively inhibit the tyrosine kinase located within the intracytoplasmic domain of EGFR and have demonstrated objective responses in individuals with advanced NSCLC.[1–3] Clinical predictors of response to these agents include: smoking history (never-smokers), pathology (adenocarcinoma, especially with bronchioloalveolar features), and enrollment in trials in East Asian countries.[4] More recently, somatic mutations within the *EGFR* tyrosine kinase domain have been associated with sensitivity to the EGFR tyrosine kinase inhibitors (EGFR-TKI) gefitinib and erlotinib.[5–7] Tumors from patients with clinical predictors of response demonstrate a higher incidence of *EGFR* mutations in exons 19 and 21.[8] Tumor-specific features, such as *EGFR* or *HER2* gene amplification and EGFR protein expression, may play a role as well.[9–12]

Despite insights into the basis of EGFR-TKI-sensitivity in NSCLC, many patients treated with these agents fail to experience benefit. *KRAS* mutations occur in 15% to 30% of patients with NSCLC and are associated with primary resistance to these agents.[13–15] For the

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majority of patients with NSCLC whose tumors have neither *EGFR* nor *KRAS* mutations, the mechanisms for primary resistance to EGFR-TKIs are not understood. Dysregulation of downstream apoptotic pathways, such as the phosphoinositide 3-kinase (PI3K)/Akt/phosphatase and tensin homolog (PTEN) axis is a potential explanation for resistance in these patients.[16,17] *PI3K* mutations, *PTEN* deletions, *PTEN* silencing through promoter hypermethylation, and Akt overexpression have been identified in 2% to 3%, 16%, 35%, and 60% to 70% of NSCLCs, respectively.[18–21] Further supporting this hypothesis is the observation that inhibition of the PI3K/Akt pathway restores gefitinib sensitivity in resistant cell lines.[22]

The mammalian target-of-rapamycin (mTOR) is a serine-threonine kinase located downstream of Akt and serves as a critical regulator of cellular growth and proliferation. mTOR-dependent activity may be essential for oncogenic transformation induced by PI3K and Akt.[23,24] Furthermore, mTOR inhibitors such as rapamycin have been shown to cause G1 cell cycle arrest in cancer cell lines and inhibit growth and metastatic progression in NSCLC animal models.[25] Everolimus (RAD001, Novartis Pharmaceuticals, East Hanover, NJ) is an oral mTOR inhibitor that has undergone extensive clinical testing in the organ-transplant setting.[26,27] A recent phase 1 study of everolimus in patients with cancer identified 10 mg as a biologically active dose, defined as the dose required to inhibit its downstream effectors such as $p70^{s6}$ kinase when administered on a daily schedule.[28] Principle toxicities associated with everolimus include rash, stomatitis, fatigue, leukopenia, and hypertriglyceridemia.

Our overall hypothesis is that concurrent signal transduction inhibition within this pathway with gefitinib and everolimus would result in additive or synergistic antitumor activity in patients with advanced NSCLC. As a first step to test this hypothesis we designed and conducted this phase 1 trial.

MATERIALS AND METHODS

Patient Eligibility

All patients had pathologically confirmed NSCLC, either stage IIIB (with malignant effusion) or IV, or recurrent disease. Eligibility requirements included: progressive disease despite prior treatment with at least 1 chemotherapy regimen including docetaxel and either carboplatin or cisplatin; completion of any prior treatment $\geq$4 weeks previous to study entry; measurable or evaluable disease in a nonirradiated field; and a Karnofsky performance status of $\geq$70%. Laboratory parameters included: white blood cell count (WBC) $\geq$3000/μL; hemoglobin $\geq$9 g/dL; platelet count $\geq$100,000/μL; total bilirubin $\leq$1.5 $\times$ the upper limit of normal (ULN); aspartate aminotransferase (AST) $\leq$1.5 $\times$ ULN; and creatinine $\leq$1.5 $\times$ ULN or creatinine clearance $\geq$60 mL/min. Patients were excluded if they had brain metastases that required escalating doses of corticosteroids; other active cancer; prior treatment with any EGFR-directed therapy, such as an EGFR-TKI or antibody directed against EGFR; or were currently pregnant or lactating. Fertile men or women not using effective contraception were also ineligible. All patients provided written informed consent. This trial was reviewed and approved by the Institutional Review Board of the Memorial Sloan-Kettering Cancer Center.

Objectives and Definitions

The primary endpoint was to determine the maximum tolerated dose (MTD) or to establish the safety and tolerability of the final predefined dose level. Patients who received $\geq$4 weeks of concurrent treatment with everolimus and gefitinib or those who completed <4 weeks of concurrent treatment due to drug toxicity were included in the analysis of drug tolerability. Patients who developed progressive disease before completing 4 weeks of concurrent everolimus and gefitinib were replaced.

Treatment Plan

Two dose levels of everolimus were tested, 5 and 10 mg given orally once daily. All patients received oral gefitinib 250 mg daily. Patients were enrolled in 3-patient cohorts, with no intrapatient escalation. The final dose level was predefined to include 6 patients who completed 4 weeks of concomitant therapy. Based on the half-life of everolimus and gefitinib, the study was designed such that patients received a single dose of everolimus on Day 1, followed by initiation of daily gefitinib on Day 8 (Fig. 1). Patients then began concurrent daily everolimus on Day 22.

Dose escalation was based on the toxicities encountered through Day 28 of therapy. Toxicities were graded using NIH Common Toxicity Criteria for Adverse Events, v. 3.0. Dose-limiting toxicity (DLT) was defined as any of the following: grade 3 diarrhea lasting longer than 48 hours (despite loperamide); grade 4 diarrhea; grade 3 rash; grade 3 fatigue lasting more than 1 week; grade 4 hematologic toxicity; grade 3 hypertriglyceridemia; any toxicity necessitating a 2-week treatment interruption; or any other nonhematologic grade 3 or 4 toxicity. If DLT occurred in 1 of the first 3 patients at a given dose level, 3 additional patients were treated at that dose level.

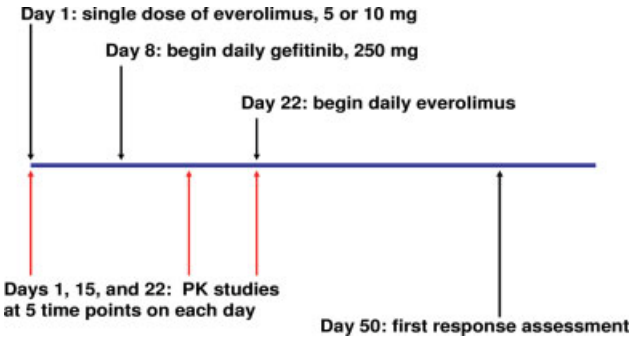

FIGURE 1. Study schema. Patients received a single dose of RAD001 on Day 1, began daily gefitinib on Day 8, and then began concurrent RAD001 and gefitinib on Day 22. Serum samples for Karnofsky performance studies were obtained on Days 1, 15, and 22, as depicted. Patients underwent initial response assessment with computed tomography (CT) scan on Day 50, 4 weeks after starting concomitant therapy.

If no other patient experienced unacceptable toxicity, dose escalation continued. If DLT occurred in 2 of 6 patients, dose escalation was stopped. The MTD was defined as 1 dose level lower than the dose level at which at least 2 of 6 patients experienced DLT.

Management of Toxicity, Dose Reductions, and Interruptions

Patients were educated on the appropriate use of loperamide for diarrhea.[29] Management of grade 2 or troublesome grade 1 rash included oral minocycline, topical clindamycin, topical silver sulfadiazine, oral diphenhydramine, or prednisone (short course).

Reduction or interruption of everolimus dosing for adverse events was allowed at any time. The dose of everolimus was reduced for any nondose-limiting toxicity not controlled by optimal supportive measures. Patients were allowed a single dose reduction on study. Those in the 5-mg dose cohort had everolimus discontinued; those in the 10-mg dose cohort had dose reduction to 5 mg daily. Re-escalation was not permitted. Dose interruptions for a maximum of 2 weeks were allowed if clinically indicated. Within 2 weeks after a dose interruption or reduction, study drug-related toxicity must have improved by at least 1 grade and be grade $\leq$1.

Pretreatment and Follow-up Evaluations

At baseline all patients provided a history and underwent a physical examination, and laboratory evaluation including complete blood count, serum chemistry (including total bilirubin, AST, alkaline phosphatase, sodium, potassium, chloride, bicarbonate, creatinine, and blood urea nitrogen), and electrocardiogram within 2 weeks of study entry; serum pregnancy test for women of childbearing potential; and baseline medical imaging that included computed tomography (CT) scanning of all relevant disease sites within 2 weeks of study entry. Patients then provided an interval history and physical exam weekly for the first 6 weeks and then every 4 weeks starting in the 8th week. Laboratory evaluation, including complete blood count and serum chemistry, was obtained in the 5th and 6th weeks and then every 4 weeks starting in the 8th week. Imaging with CT scans was obtained in the 8th and 12th weeks, and every 8th week thereafter.

Pharmacokinetic Studies

Blood samples were obtained at 5 timepoints (immediately before, 1 hour after, 2 hours after, 5 hours after, and 8 hours after treatment) on Days 1, 15, and 22 (Fig. 1). Blood was separated and stored at -20° C until analysis. Everolimus samples were sent frozen to Novartis Pharmaceuticals SAS (Rueil-Malmaison, France); gefitinib samples were sent frozen to Avantix Laboratories (New Castle, Del). After liquid–liquid extraction of the blood samples and evaporation of the extracts, samples were analyzed by high-performance liquid chromatography tandem mass spectrometry. Pharmacokinetic analyses were performed using WinNonlin 4.0 software (Pharsight, Mountain View, Calif). Standard noncompartmental methods were used to calculate the blood concentration vs time curve within the dosing interval ($AUC_{0\text{-}8h}$), peak serum concentration (C_{max}), and the time at which C_{max} occurred (t_{max}).

Evaluation of Clinical Activity

All imaging studies were reviewed by a single reference radiologist (R.T.H.) who graded responses using Response Evaluation Criteria in Solid Tumors (RECIST).[30] All responses were confirmed with a follow-up scan $\geq$4 weeks later.

Gene Sequencing

Pretreatment tumor samples from patients with partial responses were analyzed for mutations within *KRAS* exon 2 and *EGFR* exons 19 and 21 using methods previously described.[7]

RESULTS

Patient Characteristics

A total of 10 patients were enrolled between May 2004 and April 2005. The patient characteristics are outlined in Table 1.

TABLE 1
Patient Characteristics

Characteristic	Patients, N = 10
Age, median	64
Age, range	50–77
Women/men	4/6
Never-smokers	1
Karnofsky performance status	
90%	2
80%	7
70%	1
Histology	
Adenocarcinoma	4
Squamous	2
Other*	4
≥2 prior regimens of chemotherapy	7

* Includes nonsmall-cell lung cancer not otherwise specified on cytology.

Toxicity

None of the 3 patients who were initially treated with ≥4 weeks of concurrent therapy in the everolimus 5 mg dose level experienced DLT. After 2 of 3 patients in the everolimus 10 mg dose level experienced DLT, the initial 5 mg dose level was reexpanded. Selected toxicities are presented in Table 2.

Everolimus 5 mg and Gefitinib 250 mg

Seven patients were enrolled at this dose level. One patient developed symptomatic brain metastases within the first week on study and was replaced. The remaining 6 patients completed ≥4 weeks of concurrent everolimus and gefitinib.

No patient treated in this cohort required dose interruption, dose reduction, or discontinuation of treatment due to toxicity. Among these 6 patients, treatment-related toxicities included grade 2 rash (n = 1), grade 1 rash (n = 2), grade 2 stomatitis experienced as discrete oral mucosal ulcerations (n = 2), grade 1 stomatitis (n = 1), grade 2 fatigue (n = 3), grade 1 fatigue (n = 1), grade 1 diarrhea (n = 5), grade 1 nausea and/or emesis (n = 2), and grade 2 pneumonia without neutropenia (n = 1). Two patients experienced grade 1 fever immediately after the first dose of everolimus. No laboratory abnormalities thought to be possibly or probably related to treatment were associated with symptoms or clinically relevant findings. These toxicities included grade 3 lymphopenia, grade 2 lymphopenia, grade 1 thrombocytopenia, grade 1 anemia (all with n = 1), and grade 1 hypertriglyceridemia (n = 3).

Everolimus 10 mg and Gefitinib 250 mg

Three patients were treated. Treatment-related toxicities included grade 5 hypotension with grade 3 creatinine and grade 4 acidosis and grade 3 hypokalemia (n = 1), grade 3 stomatitis with grade 2 weight loss (n = 1), grade 2 diarrhea (n = 2), grade 2 hypertriglyceridemia (n = 1), grade 1 rash (n = 2), grade 1 fatigue (n = 1), and grade 1 anemia (n = 1). The grade 5 hypotension occurred in the third patient enrolled at this dose level. This patient had metastatic NSCLC with a large adrenal metastasis and a history of prior pulmonary embolus for which she was receiving daily enoxaparin. Fourteen days after starting concurrent everolimus and gefitinib, the patient reported grade 2 diarrhea with minimal use of the prescribed loperamide. Three days later the patient presented to a local hospital with severe back pain, lethargy, anorexia, and continued mild/moderate diarrhea. The patient was found to be tachypneic, hypoxic, and experiencing new back pain of 10/10 in severity. Studies identified prerenal azotemia and a new right upper lobe infiltrate. Within an hour of presentation she became hypotensive despite intravenous fluids. She elected to not be resuscitated and died within 7 hours of presentation. At the time of her death the differential diagnosis included pulmonary embolism, aortic dissection, bowel ischemia, tumor progression causing adrenal hemorrhage, postobstructive pneumonia, and drug-related death. Although her death appeared to be more plausibly related to these other potential events, we could not exclude the possibility that her death was a drug-related event. Review of this patient's pharmacokinetic data demonstrated no significant differences. This episode and another patient's grade 3 stomatitis defined 2 separate DLTs within this dose level, prompting this dose level's closure.

Pharmacokinetics

Eight patients had complete pharmacokinetic studies of everolimus and 9 gefitinib. One patient developed symptomatic brain metastases within the first week of treatment, was removed from the study, and as a result had no Day 8 or 21 samples drawn. A separate patient's everolimus samples were unavailable for study.

Table 3 displays the t_{max}, C_{max}, and $AUC_{0\text{-}8h}$ of everolimus after a single dose (Day 1) of everolimus and after the addition of a single dose of everolimus after patients had reached gefitinib steady-state (Day 22). The maximum plasma concentration (C_{max}) at the 2 doses of everolimus averaged 20.45 ng/mL and 57.17 ng/mL, respectively. The everolimus $AUC_{0\text{-}8h}$'s obtained before gefitinib initiation were compared with everolimus $AUC_{0\text{-}8h}$'s obtained while patients were on combination therapy with gefitinib. This comparison demonstrated a mean decrease in evero-

TABLE 2
Selected Toxicities* Considered Possibly or Probably Related to Treatment With Everolimus and Gefitinib

	Everolimus 5 mg + Gefitinib 250 mg (n = 6)			Everolimus 10 mg + Gefitinib 250 mg (n = 3)				
Toxicity	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Anemia	1	0	0	1	0	0	0	0
Acidosis	0	0	0	0	0	1	0	0
Creatinine	0	0	0	0	0	0	1	0
Diarrhea	4	0	0	0	2	0	0	0
Fatigue	2	2	0	1	0	0	0	0
Hypertriglyceridemia	3	0	0	0	1	0	0	0
Lymphopenia	0	1	1	0	0	0	0	0
Nausea and/or emesis	2	0	0	0	0	0	0	0
Pneumonia/Pneumonitis	0	1	0	0	0	0	0	0
Rash	2	2	0	3	0	0	0	0
Stomatitis†	2	1	0	0	0	1	0	0
Weight loss	0	0	0	0	1	0	0	0
Hypotension	0	0	0	0	0	0	0	1

* Numbers indicate the highest grade of toxicity seen for any patient at that dose level at any time on study. The National Cancer Institute Common Toxicity Criteria for Adverse Events v. 3 was used for grading.
† Manifested as discrete oral mucosal ulcerations.

TABLE 3
RAD001 Pharmacokinetic Parameters (n = 8)

RAD001 dose	No.	Parameter	RAD001 alone, day 1	With Gefitinib, day 22	Ratio day 28 / day 1
5 mg	5	$t_{max,}$ h	1 (range 1–2)	1.50 (range 1–5)	
		$C_{max,}$ ng/mL	20.45 (SD, 19.90)	19.00 (SD, 17.44)	
		$AUC_{0\text{-}8h}$,ng/mL* h	81.30 (SD, 54.00)	77.12 (SD, 58.31)	0.94
10 mg	3	$t_{max,}$ h	1 (range 1–1)	1 (range 1–2)	
		C_{max}, ng/mL	57.16 (SD, 30.02)	44.70 (SD, 20.14)	
		$AUC_{0\text{-}8h}$, ng/mL* h	136.23 (SD, 56.38)	152.42 (SD, 81.05)	1.11

$AUC_{0\text{-}8h}$ and C_{max} data are presented as means; t_{max} data is presented as the median.

limus $AUC_{0\text{-}8h}$ by 6% when coadministered with gefitinib, although the variation among individuals' changes in $AUC_{0\text{-}8h}$ demonstrates significant heterogeneity between patients. Among the 5 patients in the everolimus 5 mg dose level, exposure to everolimus increased by 87% and 27% in 2 patients, and decreased by 53% in 1 patient in the presence of gefitinib. Gefitinib did not alter everolimus pharmacokinetics in the remaining 2 patients in this group. Among the 3 patients in the everolimus 10 mg dose level gefitinib did not alter everolimus pharmacokinetics in 2 out of 3 patients and increased the exposure to everolimus by 31% in 1 patient.

Neither dose of everolimus altered the steady-state pharmacokinetics of gefitinib in 6 of 9 patients, whereas 3 patients (2 in the everolimus 5 mg group and 1 in the everolimus 10 mg group) had increases in the gefitinib $AUC_{0\text{-}8h}$ of approximately 30% in the presence of everolimus. Table 4 demonstrates the mean gefitinib $AUC_{0\text{-}8h}$ for gefitinib alone and after a single dose of everolimus. No consistent, significant change in the mean gefitinib $AUC_{0\text{-}8h}$ after a single dose of everolimus was noted.

Response

Eight patients underwent response assessments. Two confirmed partial radiographic responses were identified, both treated at the everolimus 5 mg dose level. These 2 patients were men with prior cigarette smoking histories of 25 and 45 pack-years, respectively. One patient had a squamous cancer and the other had an adenocarcinoma. In pretreatment tumor specimens no mutations that have previously been described in NSCLC specimens were found within *KRAS* exon 2 and *EGFR* exons 19 and 21. The radio-

TABLE 4
Gefitinib Pharmacokinetic Parameters (n = 9)

Parameter	Gefitinib alone, Day 15	With RAD001, Day 22	Ratio, Day 22 / Day 15
t_{max}, h	5	5	
C_{max}, ng/mL	443.88	523.54	
$AUC_{0\text{-}8h}$, ng/mL* h	3026.93	3355.03	1.00

$AUC_{0\text{-}8h}$ and C_{max} data are presented as means; t_{max} data is presented as the median.

graphic responses were maintained for 4 and 5 months, respectively.

DISCUSSION

The EGFR signaling pathway is an area of intense investigation in NSCLC. Inhibition of the EGFR signaling pathway with the EGFR-TKIs gefitinib and erlotinib has elicited impressive antitumor responses in patients. Recent discoveries suggest that the presence of mutations in exons 19 and 21 of *EGFR* confer a high likelihood of sensitivity to these agents. Unfortunately, these responses are confined to a minority of patients with advanced NSCLC. Targeting the PI3k/Akt pathway in addition is a rational approach to disrupt cancer cell growth beyond that achieved with EGFR-TKI inhibition alone. Everolimus inhibits mTOR, a downstream effector of Akt.

This trial demonstrates that oral daily everolimus 5 mg is tolerable when coadministered with oral daily gefitinib 250 mg. Among the 6 patients who received concurrent everolimus 5 mg and gefitinib 250 mg for $\geq$4 weeks, the adverse effects were mild/moderate. The only toxicity identified within this dose level that has not been reported previously is grade 1 fever, experienced in 2 patients the night after receiving a single dose of everolimus and before initiating gefitinib.

Everolimus 10 mg is thought to represent a biologically active dose with respect to inhibition of signaling through mTOR. When administered as a single agent this dose of everolimus is safe and tolerable.[28] Given their overlapping toxicities, it is not surprising that the 10 mg dose of everolimus was intolerable when combined with gefitinib 250 mg. Among patients treated in our study with everolimus 10 mg and gefitinib 250 mg, 2 experienced DLT.

The 2 radiographic responses in these patients with chemotherapy-refractory, advanced NSCLC are noteworthy. Neither of these 2 patients had clinical or molecular predictors characteristic of gefitinib-responsive NSCLC.[4,8] Both were male former smokers and 1 had a confirmed squamous carcinoma. Whereas these responses may have been the result of combined signal transduction inhibition with gefitinib and everolimus, it is also possible that they might have happened with either agent alone. Indeed, responses to single-agent everolimus in NSCLC have already been reported.[28]

Potential criticisms in the design of this study include the limited dose escalation scheme and the lack of correlative studies. We chose a priori to study the 5 and 10 mg everolimus dose levels without further dose escalation based on extensive prior studies that have suggested that everolimus 10 mg daily would result in maximal mTOR inhibition as gauged by p70S6 kinase and p-eLF4G inhibition.[28] Further everolimus escalation beyond this dose level risks added toxicity without enhancing mTOR inhibition.

Based on these results, the combination of gefitinib and everolimus for patients with advanced NSCLC is being assessed in a phase 2 trial at our institution. Enrollment in this trial requires submission of pretreatment tumor specimens. Correlative studies will include immunohistochemical assessment of PTEN, EGFR, HER2, p-Akt, and p70S6 kinase expression, as well as sequencing of *EGFR* exons 19 and 21 and *KRAS* exon 2. The larger number of patients and the inclusion of these correlative studies will allow better characterization of the molecular basis of responses to combined EGFR and mTOR inhibition and provide the opportunity to confirm the efficacy of the combination of everolimus and gefitinib observed in this trial.

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