Published in final edited form as:
J Thorac Oncol. 2010 October ; 5(10): 1623–1629. doi:10.1097/JTO.0b013e3181ec1531.

Phase II Trial of Gefitinib and Everolimus in Advanced Non-small Cell Lung Cancer

Katharine A. Price, MD[*], **Christopher G. Azzoli, MD**[*], **Lee M. Krug, MD**[*], **Maria C. Pietanza, MD**[*], **Naiyer A. Rizvi, MD**[*], **William Pao, MD, PhD**[*], **Mark G. Kris, MD**[*], **Gregory J. Riely, MD, PhD**[*], **Robert T. Heelan, MD**[†], **Maria E. Arcila, MD**[‡], and **Vincent A. Miller, MD**[*]

[*]Department of Medicine, Thoracic Oncology Service, Division of Solid Tumor Oncology, Memorial Sloan-Kettering Cancer Center and the Weill Medical College of Cornell University, New York City, New York

[†]Departments of Radiology, Memorial Sloan-Kettering Cancer Center and the Weill Medical College of Cornell University, New York City, New York

[‡]Departments of Pathology, Memorial Sloan-Kettering Cancer Center and the Weill Medical College of Cornell University, New York City, New York

Abstract

Introduction—Concurrent signal transduction inhibition with the epidermal growth factor receptor (EGFR) inhibitor gefitinib and the mammalian target-of-rapamycin inhibitor everolimus has been hypothesized to result in enhanced antitumor activity in patients with non-small cell lung cancer (NSCLC). This phase II trial assessed the efficacy of the combination of gefitinib and everolimus in patients with advanced NSCLC.

Methods—Two cohorts of 31 patients with measurable stage IIIB/IV NSCLC were enrolled: (1) no prior chemotherapy and (2) previously treated with cisplatin or carboplatin and docetaxel or pemetrexed. All patients received daily everolimus 5 mg and gefitinib 250 mg. Response was assessed after 1 month and then every 2 months. Pretreatment tumor specimens were collected for mutation testing.

Results—Sixty-two patients were enrolled (median age: 66 years, 50% women, 98% stage IV, all current/former smokers, and 85% adenocarcinoma). Partial responses were seen in 8 of 62 patients (response rate: 13%; 95% confidence interval: 5–21%); five responders had received no prior chemotherapy. Three partial responders had an *EGFR* mutation. Both patients with a *KRAS* (G12F) mutation responded. The median time to progression was 4 months. Median overall

Address for correspondence: Dr. Vincent A. Miller, Memorial Sloan-Kettering Cancer Center, Room 1012-H Building, 1275 York Avenue, New York City, NY 10021. millerv@mskcc.org.

Disclosure: Lee M. Krug, MD, received a grant. William Poa, MD, PhD, consulted with MolecularMD and AstraZeneca. Mark G. Kris, MD, served as a consultant to Novartis and AstraZeneca. Gregory J. Riely, MD, PhD, received honorarium for consulting with AstraZeneca. Vincent A. Miller, MD, received payment for a contract with Novartis for the conduct of this trial.

Dr. Katharine A. Price is currently at Division of Medical Oncology, Mayo Clinic, 200 First Street SW, Rochester, Minnesota.

Dr. William Pao is currently at Department of Medicine, Hematology/Oncology, Vanderbilt-Ingram Cancer Center, Nashville, Tennessee.

Presented in part at the 43rd American Society of Clinical Oncology (ASCO) Annual Meeting, June 1–5, 2007, Chicago, IL.

survival was 12 months, 27 months for no prior chemotherapy patients, and 11 months for patients previously treated with chemotherapy.

Conclusions—The 13% partial response rate observed did not meet the prespecified response threshold to pursue further study of the combination of gefitinib and everolimus. The response rate in patients with non-*EGFR* mutant tumors was 8%, likely reflecting activity of everolimus. Further investigation of mammalian target-of-rapamycin inhibitors in patients with NSCLC with *KRAS* G12F-mutated tumors is warranted.

Keywords

Non-small cell lung cancer; Gefitinib; Everolimus.

The epidermal growth factor receptor (EGFR) pathway is critical to some lung adenocarcinoma cells. The EGFR-tyrosine kinase inhibitors (TKIs), gefitinib (Iressa, AstraZeneca, USA) and erlotinib (Tarceva, Genentech, South San Francisco, CA), have emerged as valuable treatments for some patients with non-small cell lung cancer (NSCLC). Sensitivity to these agents is largely conferred by activating mutations in the EGFR tyrosine kinase domain[1–3] with partial responses seen in 58 to 90% in patients with EGFR-mutant tumors.[4,5]

Unfortunately, the clinical benefit of the EGFR-TKIs is limited both by primary and acquired resistance. Patients who initially respond to EGFR TKIs develop acquired resistance after a median time of approximately 12 months.[6]*KRAS* mutations occur in 15 to 30% of patients with NSCLC and are associated with primary resistance to EGFR-TKIs.[7–9] Evidence also supports dysregulation of downstream apoptotic pathways, such as the phosphoinositide 3-kinase (PI3K)/Akt/phosphatase and tensin homolog (PTEN) pathway, as a possible mechanism for primary resistance.[10,11]

The mammalian target-of-rapamycin (mTOR) is a serinethreonine kinase that is a downstream effector of the PI3K/Akt/PTEN pathway and regulates cellular growth and proliferation. Several lines of preclinical data suggested a role for mTOR inhibitors in NSCLC.[12–14] In phase II clinical trials in advanced NSCLC, partial response rates to mTOR inhibitors range from 3 to 8%.[15,16] To date, no biomarker predicting efficacy of mTOR inhibitors in NSCLC has been validated.

Given the possible role of dysregulation of the PI3K/Akt/PTEN/mTOR pathway in both primary and secondary resistance to the EGFR-TKIs, we hypothesized that concurrent signal transduction inhibition with the EGFR-TKI, gefitinib, and the mTOR inhibitor, everolimus (Afinitor, Novartis, Switzerland), would result in improved antitumor activity in patients with NSCLC. Enhanced antitumor activity of gefitinib would be of particular use in patients less likely to benefit from EGFR TKIs such as smokers or patients with KRAS-mutant tumors. The phase I portion of our phase I/II clinical trial of gefitinib and everolimus was reported previously.[17] The results of the phase II portion of this trial are reported in this study. The objective of the phase II portion of the study was to determine the major objective response rate of the combination of daily gefitinib and everolimus in patients with advanced NSCLC.

PATIENTS AND METHODS

Patient Eligibility

All patients had pathologically confirmed NSCLC and stage IIIB (with malignant pleural or pericardial effusion), stage IV, or recurrent disease. Eligibility requirements included Karnofsky performance status ≥70% and measurable disease. Unstained slides or a tissue block were also required for molecular correlative studies. Patients were enrolled in two cohorts: no prior chemotherapy for advanced NSCLC and previously treated with one or more prior chemotherapy regimens that had included (1) cisplatin or carboplatin and (2) docetaxel or pemetrexed. Laboratory parameters included white blood cell count ≥3000/μl; hemoglobin ≥9 g/dl; platelet count ≥100,000/μl; total bilirubin ≤1.5 × the upper limit of normal (ULN); aspar-tate aminotransferase ≤1.5 × ULN; and creatinine ≤1.5 × ULN or creatinine clearance ≥60 ml/min. Patients were excluded if they had unstable brain metastases, other active cancer, or prior treatment with EGFR TKIs. This trial was reviewed and approved by the Institutional Review Board of the Memorial Sloan-Kettering Cancer Center.

Treatment

After obtaining informed consent, patients were treated with gefitinib 250 mg daily and everolimus 5 mg daily as determined in our earlier phase I study.[17] Dose reduction of everolimus to 2.5 mg daily was allowed for toxicity not managed by optimal supportive care. Dose reduction of gefitinib to 250 mg every other day was allowed for side effects attributable to gefitinib. Dose interruption of both everolimus and gefitinib for grade 3 or 4 toxicities was allowed until resolution of the toxicity (≤ grade 1). For grade 3 or 4 skin toxicity, dose interruption of gefitinib only was allowed with continuation of everolimus unless the toxicity did not resolve within 1 week. For grade 3 or 4 dyslipidemia, dose interruption of everolimus only was permitted. Patients with grade 3 or 4 toxicities that did not resolve in 2 weeks were removed from the study.

Evaluation/Assessment

During the first month of therapy, patients were assessed weekly with a history, physical examination, performance status evaluation, and toxicity assessment. A complete blood count and comprehensive metabolic panel were performed during the second and third week of treatment. After the first month, patients were assessed, and blood work was obtained on a monthly basis. All toxicities were graded using National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. Patients had baseline computed tomography scanning with reassessment in the fourth and eighth week of therapy, then every 8 weeks thereafter. Tumor response was determined using the Response Evaluation Criteria in Solid Tumors.[18]

Biostatistics

A Simon two-stage design was used to test the null hypothesis of a 10% response rate against the alternative of 25% response rate. Sixty-two patients were enrolled in two 31-patient cohorts. Each cohort was considered separately. The first stage of each cohort

enrolled 16 patients. If the number of responses was fewer than two, the drug would be considered inactive in that cohort and accrual would stop. If two or more of 16 patients had an objective response, the cohort was to be expanded to 31 patients. The combination of gefitinib and everolimus would be considered worthy of further evaluation if six or more of 31 patients (19%) in either cohort had objective responses. This design has a 10% type I error rate and 80% power. Time to progression was defined as the time from the first dose of study drug to the first objective documentation of tumor progression. Survival time was defined as the time from study enrollment to the time of death. Overall survival was estimated using the Kaplan-Meier method.

Molecular Studies

Tumor samples were analyzed for mutations within *KRAS* exon 2 and *EGFR* exons 19 and 21 using previously described standard methods.[3]

RESULTS

Baseline Characteristics

Between May 2004 and April 2005, 10 patients were enrolled in the phase I study. Two previously treated patients from the phase I trial who met all requirements for this phase II study and had received the identical doses and schedule of gefitinib and everolimus were included. From July 2005 to August 2008, 65 patients were enrolled. Only current or former smokers enrolled in the study because of competing clinical trials that preferentially enrolled never smokers. Three patients never received treatment and were replaced. Thirty-one patients had no prior chemotherapy for advanced NSCLC, and thirty-one patients had received one or more prior chemotherapies. The majority of patients had stage IV disease and adenocarcinoma histology. Patient characteristics are outlined in Table 1.

Treatment

All 62 patients were treated with gefitinib 250 mg daily and everolimus 5 mg daily. Patients remained on treatment from 7 to 787 days (median 107 ± 139 days).

Efficacy

All patients who received even a single dose of study drug were included. Eight patients (8 of 62) had a confirmed partial response for an overall response rate of 13% (95% confidence interval [CI]: 5–21%). The median duration of response for patients with a partial response was 10 months.

In the no prior chemotherapy cohort, five patients had a partial response (Figure 1). Nineteen of 31 patients (61%) achieved disease stability lasting a median of 4 months. Seven of 31 (23%) had disease progression as the best response. The median follow-up time was 16 months (range: 10–37 months).

In the cohort previously treated with chemotherapy, three patients had a partial response (Figure 2). All three partial responders had received cisplatin or carboplatin and docetaxel. Seventeen of 31 (55%) achieved disease stability lasting a median of 3 months. Eleven of 31

(35%) had disease progression as the best response. The median follow-up time was 7 months (range: 1–28 months). The distribution of tumor responses is shown in waterfall format in Figures 1 and 2.

The median time to progression for all patients was 4 months (range: 1–26). Median overall survival was 12 months for all patients. By cohort, the median overall survival was 27 months for patients who had received no prior chemotherapy and 11 months for patients previously treated with chemotherapy. The 1-year survival was 59% for the no prior chemotherapy patients and 39% for the previously treated cohort (Figure 3).

Correlative Studies

EGFR (exons 19 and 21) mutation testing was performed on 59 of 62 enrolled patients. *KRAS* mutation testing was performed on 55 of 62 enrolled patients. One patient had squamous histology, so mutation testing for *EGFR* and *KRAS* was not performed. Tissue was not available for *EGFR* mutation testing in two patients. Two patients were first found to have an *EGFR* mutation, so *KRAS* testing was not performed. Tissue was not available for *KRAS* testing in three patients; one patient had tissue sent, but DNA could not be extracted. A total of three patients (two with no prior chemotherapy and one previously treated with chemotherapy) had an *EGFR*-activating mutation. A total of 16 patients (nine with no prior chemotherapy and seven previously treated) had a *KRAS* mutation for a *KRAS* mutation rate of 29% (16 of 55 tested). The *KRAS* mutation subtypes and distribution are shown in Table 1. Of the eight partial responders, three patients had an *EGFR*-activating mutation (exon 19 deletion). One patient with an *EGFR* mutation who initially responded developed acquired resistance and disease progression and on rebiopsy was found to have a T790M mutation. Five of eight partial responders did not have an *EGFR* mutation for a response rate of 8% (5 of 59; 95% CI: 1–15%) in the non-*EGFR* mutated population. Of the eight responders, two patients had a *KRAS* mutation (both subtype G12F); both were in the cohort previously untreated with chemotherapy. The response rate in *KRAS* mutated patients was 13% (2 of 16; 95% CI: 4–36%).

Toxicity

Table 2 lists the treatment-related adverse effects by grade. Adverse effects are reported as the highest grades experienced by individual patients at any time on the trial. The most frequent toxicities were rash, diarrhea, oral mucosal ulcerations, and fatigue. The most common hematologic toxicity was lymphopenia. Most toxicities were grades 1 to 2 and easily managed. Thirteen patients experienced nonhematologic treatment-related toxicities ≥ grade 3. One patient experienced grade 3 dyslipidemia. Seven patients (11%) required a dose reduction for toxicity. Nineteen patients (31%) required a treatment interrup- tion for toxicity. Of the seven patients removed from the protocol because of drug-related toxicity, two patients were removed for possible drug-related pulmonary toxicity manifesting as lung infiltrates, cough, and dyspnea. In one patient, the pulmonary toxicity was felt to be secondary to everolimus; in the second patient, the pulmonary toxicity was attributed to either everolimus or gefitinib. Two patients were removed for rash, two with anorexia and fatigue, and one patient due to dry skin and extremity pain.

There were 30 serious adverse events reported for 21 patients. Only one hospitalization was definitely related to the study drugs (grade 3 diarrhea). Three patients died while on study. One patient was hospitalized with new dyspnea, cough, and fever. A computed tomography scan was suspicious for pneumonia, but no specific infectious etiology was identified. Although the most likely diagnosis was pneumonia, pulmonary toxicity related to study drug(s) remained a possibility. The patient was treated aggressively with broad- spectrum antibiotics and steroids but died 5 days later. One patient was hospitalized for dyspnea and was found to have a new left pleural effusion, new bilateral pulmonary emboli, and disease progression. Study medications were stopped 15 days before death. At the last follow-up, he complained of increasing pain, dyspnea, and weakness, and died 5 days later. One patient was removed from study for disease pro gression 26 days before death. At the last follow-up, he had grade 3 dyspnea and fatigue. He was enrolled in hospice care and died 20 days later.

DISCUSSION

We report the results of a phase II trial of gefitinib and everolimus in advanced NSCLC. The combination of gefitinib and everolimus had a partial response rate of 13%, which did not meet the predetermined criteria of a 19% response rate to declare the combination worthy of further study. The long median overall survival in the cohort of patients who had not received prior therapy underscores the feasibility of enrolling patients with advanced NSCLC in clinical trials as front-line therapy. The hypothesis of the study that concurrent signal transduction inhibition with gefitinib and everolimus would result in enhanced antitumor activity was based on preclinical data showing a synergistic antitumor effect on human lung cancer cells.[19,20] However, the efficacy of the combination of gefitinib and everolimus seems to be similar to that seen with treatment with either everolimus alone or gefitinib alone in unselected patients.[15,21] One possible explanation for the lack of efficacy with the combination of gefitinib and everolimus is activation of another pathway(s) that drives tumor growth. Recent data demonstrate that mTOR inhibition can lead to activation of the mitogen-activated protein kinase (MAPK) and Akt pathways and that targeting these pathways enhanced mTOR efficacy.[19,22–25] Another possible explanation is the presence of a coexisting mutation, such as a *PIK3CA* or *PTEN* mutation, which could interfere with the efficacy of gefitinib and everolimus. A *PIK3CA* mutation, which can occur concomitantly with *KRAS* mutations,[26–28] can lead to persistent activation of the PI3K/Akt pathway despite EGFR inhibition and has been shown to confer resistance in vitro in gefitinib-sensitive lung cancer cell lines.[29] In this study, *KRAS* mutations occurred in 29% of the samples tested, and the presence of a *PIK3CA* mutation could represent a targetable resistance pathway. PTEN-deficient cell lines have been shown to be associated with resistance to EGFR inhibitors[30,31] and a PTEN deficiency could lead to activation of the PI3K/Akt pathway and mTOR resistance by accumulation of phosphatidylinositol 3,4,5-triphosphate and persistent signaling through Akt/protein kinase B. In future studies, comprehensive assessment of *PI3K*, *AKT*, *MEK*, *BRAF*, and *LKB* should be performed but was not possible in this study because of limitations of available tissue.

One especially valuable aspect of this trial is the availability of *EGFR* and *KRAS* mutation testing in the majority of patients, which provides some insight into the mechanisms of

response. Excluding the three responders who had an *EGFR* mutation where responses to gefitinib are expected, the response rate was 8%. The results of a phase III randomized trial comparing gefitinib with carboplatin and paclitaxel in advanced NSCLC reported a response rate to gefitinib in *EGFR* mutation-negative patients of 1%,[32] suggesting that our 8% response rate in patients with wild-type tumors likely reflects the activity of everolimus or the combination of the agents, perhaps through the presence of an *EGFR* mutation undetectable by the study assay or an alternative molecular target. One responder with an *EGFR* mutation subsequently developed acquired resistance and was found to have a second mutation, T790M, indicating that, in that patient, the mutant EGFR was the therapeutic target.

Intriguingly, we observed 2 *KRAS* (G12F) mutations among the eight responders. Although *KRAS* mutations are common in NSCLC, the G12F mutation subtype represents <1% of the total *KRAS* mutations in lung cancer.[33] Because responses to gefitinib in tumors harboring *KRAS* mutations are mechanistically unlikely and less than 1% in the literature, responses in the individuals whose tumors harbored a G12F mutation are likely attributable to everolimus. We observed a response rate of 13% in patients with *KRAS*-mutated tumors. Whether response is more common in the G12F variant or because of other coexistent sensitizing or resistance mechanisms in *KRAS* mutant tumors is unclear but is an area of active investigation. The use of mTOR inhibitors in patients with a *KRAS* mutation, and perhaps specifically *KRAS* (G12F) mutation, may represent a targeted therapy for a subset of patients with lung adenocarcinomas. Preclinical studies investigating mTOR inhibition on *KRAS* (G12F)-mutated cell lines are being planned, and phase II clinical trials of other mTOR inhibitors in patients with *KRAS* mutant NSCLC are currently underway.

Pulmonary toxicity has been described for both gefitinib and the mTOR inhibitors. Gefitinib-induced pulmonary toxicity occurs in approximately 1% of patients and manifests as inter-stitial lung disease or diffuse alveolar damage. Gefitinib pulmonary toxicity typically presents as the acute onset of dyspnea with cough and possibly a low-grade fever. Approximately one third of cases are fatal.[34] Consequently, gefitinib should be discontinued permanently in any case of suspected pulmonary toxicity. The rate of pulmonary toxicity related to mTOR inhibitors has been reported to be as high as 25 to 36%, with typical radiographic findings including ground glass opacities and lung consolidation.[35,36] Unlike gefitinib pulmonary toxicity, patients with mTOR pulmonary toxicity can be asymptomatic or have mild symptoms, and treatment can often be continued with close monitoring. In our study, two patients discontinued study therapy due to concern for possible drug-related pulmonary toxicity. One patient was believed to have everolimus lung toxicity and the other to have gefitinib toxicity. Given the high risk of fatality with gefitinib pulmonary toxicity, it was appropriate to remove both patients from study as they had both been receiving gefitinib. The patient suspected to have gefitinib pulmonary toxicity died 5 days after discontinuation of therapy, underscoring the importance of maintaining a heightened clinical suspicion for gefitinib pulmonary toxicity. It is possible that the incidence of mTOR pulmonary toxicity in our study is underreported because the trial was not designed to capture radiographic findings other than response, and patients may have had minimal or no

symptoms that could have been reported (dyspnea 16% and cough 8%) but not recognized as drug toxicity.

One shortcoming of the trial design is that both gefitinib and everolimus affect partially overlapping molecular pathways. It may be beneficial to design trials testing agents that block different molecular pathways such as combination therapy with an mTOR inhibitor and a MAPK inhibitor, MAPK/ERK kinase inhibitor, or insulin-like growth factor receptor inhibitor with or without EGFR inhibition. Another limitation of this study is that the tissue was tested only for the two primary *EGFR* mutations (exon 19 deletion, L858R) which could potentially miss less common, drug-sensitizing EGFR mutations in up to 10% of patients. Furthermore, additional biomarker information such as phospho-Akt, *PTEN*, or *PIK3CA* mutation status to provide insight into the in vivo effect of mTOR inhibition and possible mechanisms of mTOR resistance was not investigated. Because currently there is no validated biomarker predictive of response to mTOR inhibitors, additional molecular studies beyond *EGFR* and *KRAS* mutation status were not investigated. Identification of markers predictive of response to mTOR inhibition would help to identify patients likely to benefit from therapy and should be considered as part of future clinical trials investigating mTOR-targeted agents.

Acknowledgments

Supported, in part, by Novartis.

The authors acknowledge Dr. Trever Bivona for his thoughtful review of the manuscript and Sara Subzwari for her assistance in data collection.

REFERENCES

1. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med. 2004; 350:2129–2139. [PubMed: 15118073]
2. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004; 304:1497–1500. [PubMed: 15118125]
3. Pao W, Miller V, Zakowski M, et al. EGF receptor gene mutations are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci USA. 2004; 101:13306–13311. [PubMed: 15329413]
4. Paz-Ares L, Sanchez JM, Garcia-Velasco A, et al. A prospective phase II trial of erlotinib in advanced non-small cell lung cancer (NSCLC) patients (p) with mutations in the tyrosine kinase (TK) domain of the epidermal growth factor receptor (EGFR). J Clin Oncol. 2006; 24:7020.
5. Sequist LV, Martins RG, Spigel D, et al. iTARGET: a phase II trial to assess the response to gefitinib in epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer (NSCLC) tumors. J Clin Oncol. 2007; 25:7504.
6. Riely GJ, Pao W, Pham D, et al. Clinical course of patients with non-small cell lung cancer and epidermal growth factor receptor exon 19 and exon 21 mutations treated with gefitinib or erlotinib. Clin Cancer Res. 2006; 12:839–844. [PubMed: 16467097]
7. Rodenhuis S, van de Wetering ML, Mooi WJ, et al. Mutational activation of the K-ras oncogene. A possible pathogenetic factor in adenocarcinoma of the lung. N Engl J Med. 1987; 317:929–935. [PubMed: 3041218]
8. Suzuki Y, Orita M, Shiraishi M, et al. Detection of ras gene-mutations in human lung cancers by single-strand conformation polymorphism analysis of polymerase chain-reaction products. Oncogene. 1990; 5:1037–1043. [PubMed: 2197591]

9. Pao W, Wang TY, Riely GJ, et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. PLoS Med. 2005; 2:e17. [PubMed: 15696205]
10. Janmaat ML, Kruyt FA, Rodriguez JA, et al. Response to epidermal growth factor receptor inhibitors in non-small cell lung cancer cells: limited antiproliferative effects and absence of apoptosis associated with persistent activity of extracellular signal-regulated kinase or Akt kinase pathways. Clin Cancer Res. 2003; 9:2316–2326. [PubMed: 12796401]
11. Kokubo Y, Gemma A, Noro R, et al. Reduction of PTEN protein and loss of epidermal growth factor receptor gene mutation in lung cancer with natural resistance to gefitinib (IRESSA). Br J Cancer. 2005; 92:1711–1719. [PubMed: 15870831]
12. Balsara BR, Pei J, Mitsuuchi Y, et al. Frequent activation of AKT in non-small cell lung carcinomas and preneoplastic bronchial lesions. Carcinogenesis. 2004; 25:2053–2059. [PubMed: 15240509]
13. Han S, Khuri FR, Roman J. Fibronectin stimulates non-small cell lung carcinoma cell growth through activation of Akt/mammalian target of rapamycin/S6 kinase and inactivation of LKB1/AMP-activated protein kinase signal pathways. Cancer Res. 2006; 66:315–323. [PubMed: 16397245]
14. Wislez M, Spencer ML, Izzo JG, et al. Inhibition of mammalian target of rapamycin reverses alveolar epithelial neoplasia induced by oncogenic K-ras. Cancer Res. 2005; 65:3226–3235. [PubMed: 15833854]
15. Molina JR, Mandrekar SJ, Rowland K, et al. A phase II NCCTG "Window of Opportunity Front-line" study of the mTOR inhibitor, CCI-779 (Temsirolimus) given as a single agent in patients with advanced NSCLC:D7–07. J Thorac Oncol. 2007; 2:S413.
16. Papadimitrakopoulou V, Soria JC, Douillard JY, et al. A phase II study of RAD001 (R) (everolimus) monotherapy in patients (pts) with advanced non-small cell lung cancer (NSCLC) failing prior platinum-based chemotherapy (C) or prior C and EGFR inhibitors (EGFR-I). J Clin Oncol. 2007; 25:7589.
17. Milton DT, Riely GJ, Azzoli CG, et al. Phase 1 trial of everolimus and gefitinib in patients with advanced nonsmall-cell lung cancer. Cancer. 2007; 110:599–605. [PubMed: 17577220]
18. Therasse P, Arbuck SG, Eisenhauer EA, et al. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst. 2000; 92:205–216. [PubMed: 10655437]
19. Wang X, Hawk N, Yue P, et al. Overcoming mTOR inhibition-induced paradoxical activation of survival signaling pathways enhances mTOR inhibitors' anticancer efficacy. Cancer Biol Ther. 2008; 7:1952–1958. [PubMed: 18981735]
20. La Monica S, Galetti M, Alfieri RR, et al. Everolimus restores gefitinib sensitivity in resistant non-small cell lung cancer cell lines. Biochem Pharmacol. 2009; 78:460–468. [PubMed: 19427302]
21. Thatcher N, Chang A, Parikh P, et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). Lancet. 2005; 366:1527–1537. [PubMed: 16257339]
22. Carracedo A, Ma L, Teruya-Feldstein J, et al. Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J Clin Invest. 2008; 118:3065–3074. [PubMed: 18725988]
23. Sun SY, Rosenberg LM, Wang X, et al. Activation of Akt and eIF4E survival pathways by rapamycin-mediated mammalian target of rapamycin inhibition. Cancer Res. 2005; 65:7052–7058. [PubMed: 16103051]
24. Wang X, Yue P, Kim YA, et al. Enhancing mammalian target of rapamycin (mTOR)-targeted cancer therapy by preventing mTOR/raptor inhibition-initiated, mTOR/rictor-independent Akt activation. Cancer Res. 2008; 68:7409–7418. [PubMed: 18794129]
25. O'Reilly KE, Rojo F, She QB, et al. mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res. 2006; 66:1500–1508. [PubMed: 16452206]
26. Barault L, Veyrie N, Jooste V, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer. 2008; 122:2255–2259. [PubMed: 18224685]

27. Parsons DW, Wang TL, Samuels Y, et al. Colorectal cancer: mutations in a signalling pathway. Nature. 2005; 436:792. [PubMed: 16094359]

28. Velho S, Oliveira C, Ferreira A, et al. The prevalence of PIK3CA mutations in gastric and colon cancer. Eur J Cancer. 2005; 41:1649–1654. [PubMed: 15994075]

29. Engelman JA, Mukohara T, Zejnullahu K, et al. Allelic dilution obscures detection of a biologically significant resistance mutation in EGFR-amplified lung cancer. J Clin Invest. 2006; 116:2695–2706. [PubMed: 16906227]

30. Bianco R, Shin I, Ritter CA, et al. Loss of PTEN/MMAC1/TEP in EGF receptor-expressing tumor cells counteracts the antitumor action of EGFR tyrosine kinase inhibitors. Oncogene. 2003; 22:2812–2822. [PubMed: 12743604]

31. Sos ML, Koker M, Weir BA, et al. PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. Cancer Res. 2009; 69:3256–3261. [PubMed: 19351834]

32. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or carboplatin-paclitaxel in pulmonary adenocarcinoma. N Engl J Med. 2009; 361:947–957. [PubMed: 19692680]

33. Catalogue of Somatic Mutations in Cancer (COSMIC) [database online]. Updated September. Vol. 18. Wellcome Trust Sanger Institute; Cambridge, UK: 2009. p. 2006

34. Cohen MH, Williams GA, Sridhara R, et al. FDA drug approval summary: gefitinib (ZD1839) (Iressa) tablets. Oncologist. 2003; 8:303–306. [PubMed: 12897327]

35. Soria JC, Shepherd FA, Douillard JY, et al. Efficacy of everolimus (RAD001) in patients with advanced NSCLC previously treated with chemotherapy alone or with chemotherapy and EGFR inhibitors. Ann Oncol. 2009; 20:1674–1681. [PubMed: 19549709]

36. Duran I, Siu LL, Oza AM, et al. Characterisation of the lung toxicity of the cell cycle inhibitor temsirolimus. Eur J Cancer. 2006; 42:1875–1880. [PubMed: 16806903]

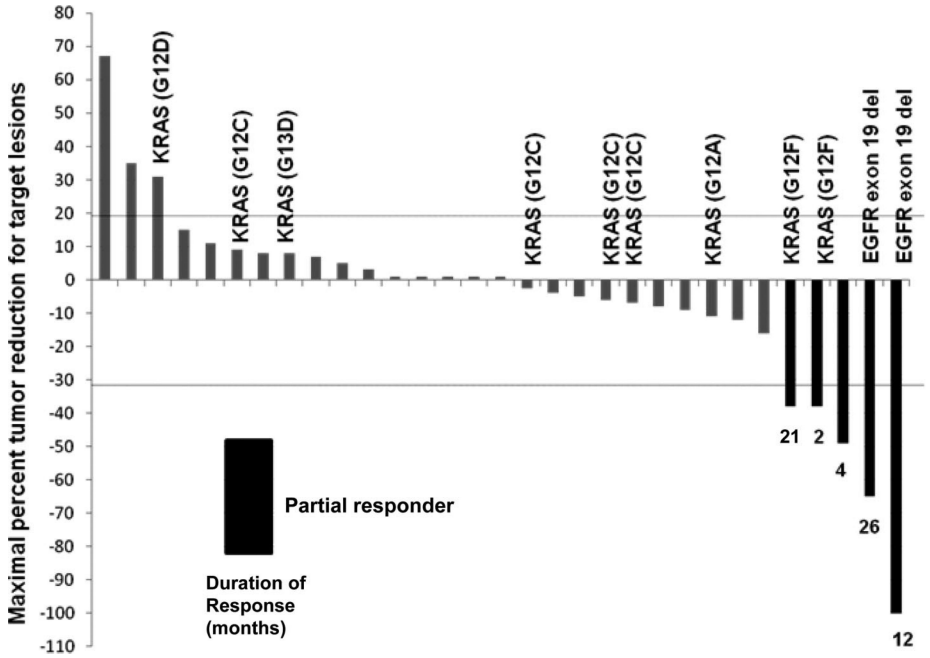

FIGURE 1.

Maximal percentage of tumor reduction for target lesions by RECIST in patients receiving everolimus and gefitinib who had no prior treatment with chemotherapy. Bars without mutations represent patients whose tumors are wild type for epidermal growth factor receptor (*EGFR*) and *KRAS*.

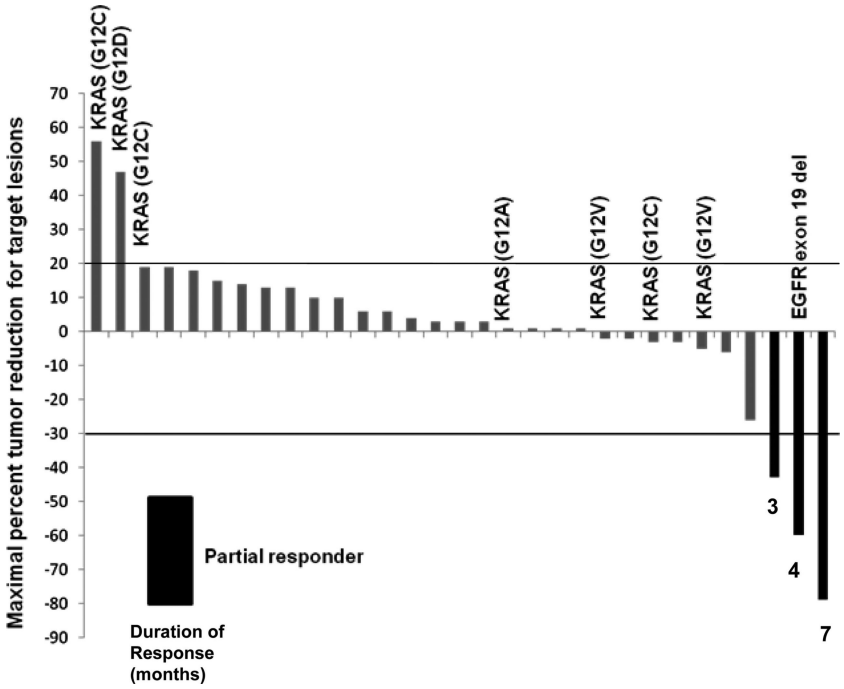

FIGURE 2.

Maximal percentage of tumor reduction for target lesions by RECIST in patients receiving everolimus and gefitinib who had been previously treated with chemotherapy. Bars without mutations represent patients whose tumors are wild type for epidermal growth factor receptor (*EGFR*) and *KRAS*.

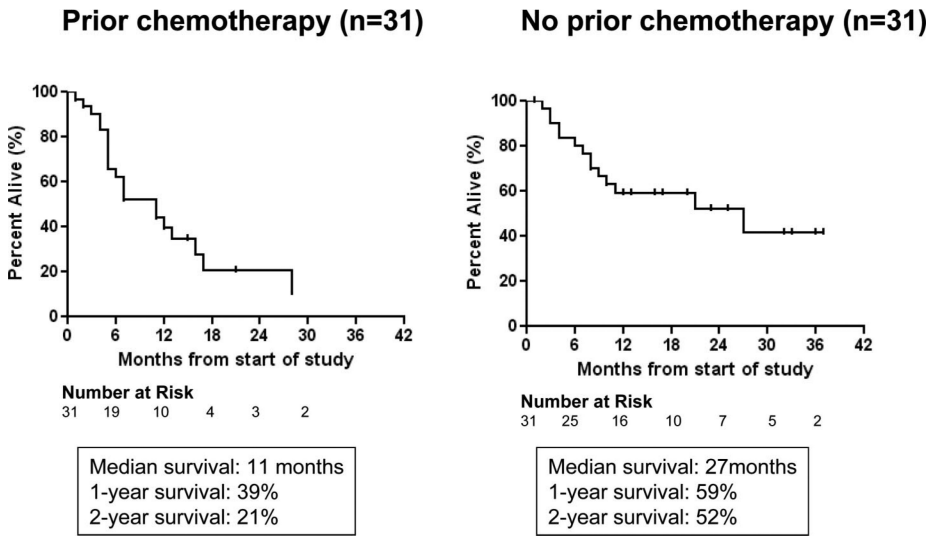

FIGURE 3.

Overall survival of patients with non-small cell lung cancer (NSCLC) receiving everolimus and gefitinib with and without prior chemotherapy.

TABLE 1

Patient Characteristics

Characteristics	All Patients (*n* = 62)	No Prior Treatment (*n* = 31)	Prior Treatment (*n* = 31)
Median age (range)	66 (40–86)	68 (43–77)	64 (40–80)
Gender			
Female	31 (50%)	14 (45%)	17 (55%)
Karnofsky performance status			
90%	16 (26%)	6 (19%)	10 (32%)
80%	39 (63%)	22 (71%)	17 (55%)
70%	7 (11%)	3 (10%)	4 (13%)
Stage			
IIIB (malignant effusion)	1 (2%)	1 (3%)	—
IV	61 (98%)	30 (97%)	31 (100%)
Recurrent disease	26 (42%)	16 (52%)	10 (32%)
Histology			
Adenocarcinoma	53 (85%)	27 (87%)	26 (84%)
Squamous	3 (5%)	2 (6%)	1 (3%)
Non-small cell carcinoma	6 (10%)	2 (6%)	4 (13%)
Smoking history			
Former	59 (95%)	31 (100%)	28 (90%)
Current	3 (5%)	—	3 (10%)
Mutation status			
EGFR (*n* = 59)			
Exon 19 deletion	3 (5%)	2 (3%)	1 (2%)
L858R	—	—	—
KRAS (*n* = 55)	16 (29%)	9 (16%)	7 (13%)
G12A	2	1	1
G12V	2	—	2
G12D	2	1	1
G12F	2	2	—
G13D	1	1	—
G12C	7	4	3
Previous therapy (%)			
Cisplatin	—	—	41%
Carboplatin	—	—	61%
Docetaxel	—	—	61%
Paclitaxel	—	—	32%
Pemetrexed	—	—	51%
Gemcitabine	—	—	22%
Vinorelbine	—	—	16%
Bevacizumab	—	—	32%
≥3 previous lines	—	—	19%

Characteristics	All Patients (*n* = 62)	No Prior Treatment (*n* = 31)	Prior Treatment (*n* = 31)
Subsequent chemotherapy (%)			
Platinum	—	45%	6%
Taxanes	—	38%	19%
Pemetrexed	—	54%	19%
Gemcitabine	—	48%	38%
Vinorelbine	—	35%	38%
Mitomycin	—	19%	22%
Bevacizumab	—	29%	29%
Erlotinib	—	9%	9%
Experimental agent	—	9%	29%
Other	—	9%	—
No additional treatment		12%	12%

EGFR, epidermal growth factor receptor.

TABLE 2

Toxicities with Gefitinib and Everolimus Occurring in ≥5% of Patients

Toxicity (*n* = 62)	Any	Grade 1	Grade 2	Grade 3	Grade 4
Rash/desquamation	37 (60%)	17 (27%)	20 (32%)	0 (0%)	0 (0%)
Diarrhea	35 (56%)	28 (45%)	3 (5%)	4 (6%)	0 (0%)
Oral ulcerations	32 (52%)	11 (18%)	20 (32%)	1 (2%)	0 (0%)
Fatigue	28 (45%)	12 (19%)	12 (19%)	4 (6%)	0 (0%)
Nausea	19 (31%)	12 (19%)	6 (10%)	1 (2%)	0 (0%)
Epistaxis	18 (29%)	17 (27%)	1 (2%)	0 (0%)	0 (0%)
Lymphopenia	14 (23%)	0 (0%)	0 (0%)	13 (21%)	1 (2%)
Anorexia	11 (18%)	6 (10%)	4 (6%)	1 (2%)	0 (0%)
Dyspnea	10 (16%)	4 (6%)	4 (6%)	1 (2%)	1 (2%)
Neuropathy, sensory	8 (13%)	8 (13%)	0 (0%)	0 (0%)	0 (0%)
Thrombocytopenia	6 (10%)	2 (3%)	0 (0%)	4 (6%)	0 (0%)
Hyponatremia	6 (10%)	1 (2%)	0 (0%)	5 (8%)	0 (0%)
Vomiting	5 (8%)	5 (8%)	0 (0%)	0 (0%)	0 (0%)
Cough	5 (8%)	3 (5%)	2 (3%)	0 (0%)	0 (0%)
Anemia	5 (8%)	0 (0%)	1 (2%)	4 (6%)	0 (0%)
Elevated International Normalized Ratio	5 (8%)	0 (0%)	0 (0%)	5 (8%)	0 (0%)
Hypophosphatemia	4 (6%)	0 (0%)	0 (0%)	4 (6%)	0 (0%)
Constipation	4 (6%)	3 (5%)	1 (2%)	0 (0%)	0 (0%)
Lower extremity edema	4 (6%)	3 (5%)	1 (2%)	0 (0%)	0 (0%)
Fever, nonneutropenic	4 (6%)	3 (5%)	1 (2%)	0 (0%)	0 (0%)
Pruritus	3 (5%)	2 (3%)	1 (2%)	0 (0%)	0 (0%)
Dry skin	3 (5%)	3 (5%)	0 (0%)	0 (0%)	0 (0%)
Dry eye	3 (5%)	3 (5%)	0 (0%)	0 (0%)	0 (0%)
Dysgeusia	3 (5%)	3 (5%)	0 (0%)	0 (0%)	0 (0%)
Weight loss	3 (5%)	3 (5%)	0 (0%)	0 (0%)	0 (0%)
Headache	3 (5%)	3 (5%)	0 (0%)	0 (0%)	0 (0%)
Back pain	3 (5%)	3 (5%)	0 (0%)	0 (0%)	0 (0%)