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Combining Erlotinib and Cetuximab Is Associated with Activity in Patients with Non–Small Cell Lung Cancer (Including Squamous Cell Carcinomas) and Wild-Type *EGFR* or Resistant Mutations

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Abstract

Preclinical data suggest that combined EGF receptor (EGFR) targeting with an EGFR tyrosine kinase inhibitor and an anti-EGFR monoclonal antibody may be superior over single-agent targeting. Therefore, as part of a phase I study, we analyzed the outcome of 20 patients with non–small cell lung cancer treated with the combination of erlotinib and cetuximab. *EGFR* mutation status was ascertained in a Clinical Laboratory Improvement Amendment–approved laboratory. There were 10 men; median number of prior therapies was five. Overall, two of 20 patients (10%) achieved partial response (PR), one of whom had a TKI-resistant *EGFR* insertion in exon 20, time to treatment failure (TTF) = 24+ months, and the other patient had squamous cell histology (*EGFR* wild-type), TTF = 7.4 months. In addition, three of 20 patients (15%) achieved stable disease (SD) ≥6 six months (one of whom had wild-type *EGFR* and squamous cell histology, and two patients had an *EGFR* TKI-sensitive mutation, one of whom had failed prior erlotinib therapy). Combination therapy with ertotinib plus cetuximab was well tolerated. The most common toxicities were rash, diarrhea, and hypomagnesemia. The recommended phase II dose was erlotinib 150 mg oral daily and cetuximab 250 mg/m^2 i.v. weekly. In summary, erlotinib and cetuximab treatment was associated with SD ≥ six months/PR in five of 20 patients with non–small cell lung cancer (25%), including individuals with squamous histology, TKI-resistant *EGFR* mutations, and wild-type *EGFR*, and those who had progressed on prior erlotinib after an initial response. This combination warrants further study in select populations of non–small cell lung cancer. *Mol Cancer Ther; 12(10); 2167–75. ©2013 AACR.*

Introduction

Lung cancer is the leading cause of cancer-related death in the United States (1). Recent progress in understanding the biology of this tumor has led to the development of targeted agents that show improved response rates in patients with non–small cell lung cancer (NSCLC; refs. 2, 3).

There is a broad literature on the efficacy of EGFR inhibitors in NSCLC (4–7). Currently, two distinct classes of drugs are used to target *EGFR* (8). EGFR tyrosine kinase inhibitors (TKI), erlotinib and gefitinib, bind to the intracellular tyrosine kinase domain and block the enzymatic function of the receptor. Cetuximab, a monoclonal antibody, binds to the extracellular ligand-binding domain of EGFR, suppressing EGFR-dependent signaling through inhibition of ligand-dependent activation and receptor dimerization, and induction of antibody-dependent cell-mediated cytotoxicity (9).

Resistance to EGFR therapy represents a major clinical problem. Primary resistance to EGFR inhibitors can be mediated by certain insertion mutations in exon 20 and other concomitant mutations such as those in the *KRAS* gene (10). Although many *EGFR* mutation-positive patients show tumor regression initially with EGFR TKI treatment, most will relapse within 1 year due to acquired resistance (10–13). Approximately 50% of erlotinib-resistant cases of NSCLC show the emergence of a second TKI-resistant mutation (T790M) in exon 20 (11, 13, 14).

Although preclinical studies have shown that combination therapy with two different classes of EGFR antagonists can be synergistic (15, 16), clinical trials have, to date, shown minimal activity (17, 18). We conducted a phase I study to evaluate the combination of EGFR TKI erlotinib with anti-EGFR monoclonal antibody cetuximab

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in patients with advanced cancer (19). Here, we report the results of the subset of 20 patients with NSCLC who were treated in this study.

Patients and Methods

Eligibility criteria

To be eligible for this study, patients must have had pathologically confirmed advanced or metastatic cancer, refractory to standard therapy, with an Eastern Cooperative Oncology Group (ECOG) performance status score of $\leq$2 (20). Other key inclusion criteria were absolute neutrophil count $\geq$1,000/mL, platelets $\geq$50,000/mL, serum creatinine $\leq$2 times the upper limit of normal, total bilirubin $\leq$2 mg/dL, and alanine amino transferase (ALT) $\leq$3 times the upper limit of normal. In the presence of liver metastases, total bilirubin can be $\leq$3 and ALT $\leq$5 times the upper limit of normal. In the dose-escalation cohorts, neither the presence of *EGFR* mutation nor prior EGFR inhibitor therapy was required. Patients who were pregnant or unwilling to use contraception, a history of cerebrovascular accidents or myocardial infarction within 6 months, or known hypersensitivity to any component of the drugs tested were excluded from the study. The study and all treatments were conducted in accordance with the guidelines of the MD Anderson Institutional Review Board and written informed consent was obtained from all the patients before study-related procedures were started.

Study design

Patients were enrolled in a phase I, open-label, dose-escalation study with a standard 3 + 3 design conducted by the Department of Investigational Cancer Therapeutics at the MD Anderson Cancer Center (MDACC, Houston, TX) beginning May, 2009. Erlotinib was given orally daily with cetuximab given intravenously on days 1, 8, 15, and 22 of a 28-day cycle. Patients were treated on one of the two dose levels in 28-day cycles (Table 1). Patients remained on the study until disease progression, unacceptable toxicity, death, or withdrawal of consent. Primary endpoints were to establish the maximum-tolerated dose (MTD) and to characterize toxicity profiles. Secondary endpoints included a preliminary assessment of biologic activity.

Table 1. Dose-escalation schedule for erlotinib and cetuximab

		Cetuximab i.v. on days 1, 8, 15, and 22 (mg/m^2)	
Dose level	Erlotinib *per os* daily (mg)	Loading dose	Maintenance dose
Level −2	50	200	125
Level −1	75	200	125
Level 1	100	200	125
Level 2	150	400	250

Dose-limiting toxicity and MTD

Dose-limiting toxicity (DLT) was defined as any grade 3 or 4 nonhematologic toxicity as defined in the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version 3.0 (21), any grade 4 hematologic toxicity lasting 2 weeks or longer (as defined by the NCI-CTCAE) despite supportive care, grade 4 nausea or vomiting >5 days despite maximum antinausea regimens, or any severe/life-threatening complication not defined in the NCI-CTCAE that was attributable to the therapy during the first treatment cycle. Correctable electrolyte imbalances and alopecia were not considered DLTs.

Dose levels were escalated in cohorts of 3 patients as long as no DLT was observed. If a DLT was observed in one patient at a particular dose level, 3 more patients were treated at this dose level. If no additional patients in the expanded cohort of 6 patients experienced a DLT, dose escalation resumed. If a second patient enrolled at the same dose level experienced a DLT, the MTD was considered to have been exceeded. The next lower dose level was considered the MTD, and an additional 3 patients were treated at the MTD level unless 6 patients were already treated at that dose level. The MTD was the highest dose at which no more than one of every 6 patients had a DLT. Dose escalation was not permitted for individual patients.

Toxicity evaluation

Adverse events were recorded from day 1 of each cycle, and up to 30 days after the last dose on study. Severity of the events was assessed using the NCI-CTCAE v3.0 (21). MTD was defined by DLTs that occurred during only the first cycle of therapy.

Assessment of antitumor efficacy

Treatment efficacy was evaluated by computed tomography (CT) scans and/or MRI studies according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.0 (22) criteria at baseline before treatment initiation and every 3 cycles (8–12 weeks) thereafter and were reported as best response. All radiographs were read in the Department of Radiology at MDACC and reviewed in the Department of Investigational Cancer Therapeutics tumor measurement clinic. Responses were categorized per RECIST 1.0 criteria. In brief, complete response was defined as the disappearance of all measurable and nonmeasurable disease; partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameter of measurable target lesions; progressive disease (PD) was defined as at least a 20% increase in the sum of the longest diameter of measurable target lesions, or unequivocal progression of a nontarget lesion, or the appearance of a new lesion; and stable disease (SD) was defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD. A waterfall plot was used to illustrate antitumor efficacy, as previously described (23).

Molecular assays

All histologies were centrally reviewed at the MDACC. Mutation testing was conducted in the Clinical Laboratory Improvement Amendment (CLIA)-certified Molecular Diagnostic Laboratory at the MDACC. PCR-based DNA sequencing analysis was done on DNA extracted from paraffin-embedded tissue or tissue from fine-needle aspiration/surgical biopsies. Analysis was conducted on exons 18 to 21 of the kinase domain of the *EGFR* gene, the sites of the most common mutations observed in lung adenocarcinomas. The lower limit of sensitivity of detection was approximately one mutated cell per five total cells in sample (20%). Whenever possible, in addition to *EGFR*, we tested for other mutations such as *PIK3CA* (codons 532–554 in exon 9 and codons 1011–1062 in exon 20), *KRAS*/*NRAS* (codons 12, 13, and 61), *TP53* (exons 4 to 9), and *AKT1* (exon 4 and 7 of the *AKT* gene). PTEN expression was assessed, if tissue was available, using immunohistochemistry and the DAKO antibody (24).

Statistical analysis

Descriptive statistics were used to summarize patient characteristics and adverse events. Fisher exact test was used to assess the association between categorical variables. Time to treatment failure (TTF) was defined as the time interval between the start of therapy and the date of disease progression or death or removal from study for any reason, whichever occurred first. Patients who were alive and on study were censored at the time of their last follow-up.

Results

Patient characteristics

As part of a dose-escalation study (19), 20 patients with NSCLC were enrolled on the study. Two patients were enrolled on dose level 1 (erlotinib 100 mg oral daily and cetuximab 125 mg/m^2 i.v. on days 1, 8, 15, and 22 after a loading dose of 200 mg/m^2 i.v.) and 18 patients on dose level 2 (erlotinib 150 mg oral daily and cetuximab 250 mg/m^2 i.v. on days 1, 8, 15, and 22 after a loading dose of 400 mg/m^2 i.v.). Demographics and baseline characteristics of the 20 patients with NSCLC are summarized in Table 2.

Table 2. Demographics and pretreatment characteristics of 20 patients with NSCLC

Characteristics	No. of patients (%)
Age, y	
Median	66
Range	32–82
≤60 y	8 (40)
>60 y	12 (60)
Sex	
Female	10 (50)
Male	10 (50)
Race	
White	13 (65)
Asian	4 (20)
Black	3 (15)
Histology	
Adenocarcinoma	15 (75)
Squamous cell	4 (20)
Adenosquamous	1 (5)
EGFR mutation	
Mutation in exon 19 only	5 (25)
Mutation in exon 20 only	1 (5)
Mutation in exon 21 only	2 (10)
Mutations in exon 19 and 20	1 (5)
Wild-type	8 (40)
Unknown	3 (15)
KRAS mutation	
Present	2 (10)
Wild-type	11 (55)
Unknown	7 (35)
PIK3CA mutation	
Present	0 (0)
Wild-type	10 (50)
Unknown	10 (50)
Smoking history	
No	10 (50)
Yes	10 (50)
Prior therapies	
Median	4.5
Range	2–9
<3	5 (25)
≥3	15 (75)
Prior EGFR therapy	
No	5 (25)
Yes	15 (75)
ECOG PS	
0	3 (15)
1	12 (60)
2	5 (25)

Abbreviations: *KRAS*, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; PS, performance status; *PIK3CA*, phosphatidylinositol 3-kinase, catalytic, α polypeptide.

EGFR mutations

Of 20 patients with NSCLC, *EGFR* mutations were assessed in 17 patients. Ten *EGFR* mutations were seen in 9 patients (Table 3). More specifically, known *EGFR* TKI-sensitive mutations were observed in 8 patients, including 6 patients with deletions in exon 19 (cases #3, 5, 6, 8, 16 and 19; Table 3) and 2 patients (cases #17 and 18; Table 3) with point mutations in exon 21 (L858R). One of these 8 patients had a coexisting TKI-resistant mutation, T790M in exon 20 (case #5; Table 3). One other patient (case #2; Table 3) had an *EGFR* TKI-resistant insertion, D770>GY in exon 20. The only significant association that was noted between patient characteristics and *EGFR* mutation status was that of nonsmokers and *EGFR* mutation-positive status ($P = 0.015$).

Table 3. Genomic, proteomic, and histopathologic characterization and corresponding responses in 20 patients with NSCLC treated with erlotinib and cetuximab

Case no.	Tumor histology	*EGFR* mutation	Sensitive/ resistant to EGFR TKI	*KRAS* mutation	TTF-prior EGFR therapy (mo)	Erlotinib + cetuximab			
						Dose level	TTF (mo)[a]	Best response[b]	Recist%
1	Squamous cell carcinoma	Unknown	NA	Unknown	1.9	1	2.0	PD	+40
2	Adenocarcinoma	D770>GY insertion (exon 20)	Resistant	Wild-type	NA	2	24.2+	PR	−33
3	Adenocarcinoma	Deletion (exon 19)	Sensitive	Wild-type	24.7	2	0.9	PD*	+20
4	Adenocarcinoma	Unknown	NA	Unknown	22.8	2	1.2	PD*	+20
5	Adenocarcinoma	Deletion (exon 19) T790M (exon 20)	Sensitive; resistant	Wild-type	39.0	2	1.8	PD**	+20
6	Adenocarcinoma	Deletion (exon 19)	Sensitive	Unknown	16.3	2	2.1	PD*	+20
7	Adenocarcinoma	Wild-type	NA	Wild-type	NA	2	1.9	PD**	+20
8	Adenosquamous	Deletion (exon 19)	Sensitive	Unknown	3.2	2	1.1	PD	+29
9	Adenocarcinoma	Wild-type	NA	Wild-type	0.7	2	0.7	Withdrew	+20
10	Squamous cell carcinoma	Wild-type	NA	Unknown	NA	2	13.7+	SD	+0
11	Adenocarcinoma	Wild-type	NA	Wild-type	2.1	2	0.0	Taken off study	+20
12	Adenocarcinoma	Unknown	NA	Wild-type	6.5	2	4.3	SD	−14
13	Adenocarcinoma	Wild-type	NA	G12D	15.5	2	0.4	Withdrew	+20
14	Adenocarcinoma	Wild-type	NA	G12D	2.1	2	0.8	PD**	+20
15	Squamous cell carcinoma	Wild-type	NA	Wild-type	NA	2	7.4	PR	−38
16	Adenocarcinoma	Insertion/deletion (exon 19)	Sensitive	Unknown	7.5	2	1.9	PD	+27
17	Adenocarcinoma	L858R (exon 21)	Sensitive	Wild-type	6.1	1	7.7+	SD	−23
18	Adenocarcinoma	L858R (exon 21)	Sensitive	Unknown	NA	2	6.3+	SD	+0
19	Adenocarcinoma	Deletion (exon 19)	Sensitive	Wild-type	8.0	2	2.1	PD**	+20
20	Squamous cell carcinoma	Wild-type	NA	Wild-type	12.1	2	1.6	PD	+24

Abbreviations: *KRAS*, V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NA, not applicable.

[a]+, did not progress at the time of analysis.

[b]*, clinical progression; **, new metastasis.

Whenever possible, mutation testing was also conducted on other genes. Two of 13 patients assessed for *KRAS* had a G12D mutation in codon 12, and the only patient assessed for *P53* mutation had a V157F mutation. Three of 5 patients evaluated for expression of PTEN by immunohistochemistry had either partial or complete PTEN loss. Ten patients assessed for *NRAS* mutation, 10 for *PIK3CA* mutation, and 5 for *AKT1* mutation were all wild-type.

Toxicities

All 20 patients were evaluated for safety (Table 4). The most common toxicities considered at least possibly related to study drug were rash ($n = 9$, 45%), diarrhea ($n = 7$, 35%), hypomagnesemia ($n = 6$, 30%), fatigue ($n = 6$, 30%), nausea ($n = 4$, 20%), and anorexia ($n = 3$, 15%). Most of the toxicities (84%) were either grade 1 or 2 and, in most instances (41 of 46 grade 1 or 2 events), were reported in patients treated at dose level 2. Serious grade 3 toxicities that were at least possibly related to study drug are rash ($n = 5$), acute infusion reaction ($n = 2$), and hand–foot skin reaction ($n = 2$). All of these were reported at dose level 2, except for one patient with rash. There were no drug-related grade 4 toxicities or deaths reported.

There were three DLTs, all at dose level 2. One patient (case #11; Table 3) had an anaphylactic reaction during the first infusion of cetuximab. Subsequently, the patient had a myocardial infarction with elevated troponins and was taken off study. A second patient (case #4; Table 3) had developed an acute hypersensitivity reaction during the first infusion of cetuximab and was subsequently continued on erlotinib alone. A third patient (case #7; Table 3) had a grade 3 rash that resolved with antibiotics. During the phase I study, dose level 2 was established as the MTD

Table 4. Adverse events[a] at least possibly related to study drug

Dose level		1 ($n = 2$)			2 ($n = 18$)		
Dose							
Erlotinib PO: daily (mg)		100			150		
Cetuximab i.v.: days 1, 8, 15, and 22							
Loading (mg/m^2)		200			400		
Maintenance (mg/m^2)		125			250		
Adverse event	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 1**	**Grade 2**	**Grade 3**	**Total ($n = 55$)**
Hematologic							
Anemia				1			1
Nonhematologic							
Rash			1	4		4 (1DLT)	9
Diarrhea	1			5	1		7
Hypomagnesemia				4	2		6
Fatigue	1	1		4			6
Nausea				4			4
Anorexia		1		2			3
Acute infusion reaction						2 (DLTs)	2
Hand–foot skin reaction						2	2
Constipation				2			2
Hyperbilirubinemia				1			1
Hyperkalemia					1		1
Dermatitis					1		1
Vomiting				1			1
Esophagitis				1			1
Folliculitis				1			1
Hypotension		1					1
Mucositis				1			1
Abdominal pain				1			1
Weight loss				1			1
Edema				1			1
Fever				1			1
Paronychia					1		1

Abbreviation: PO, *per os* (by mouth).

[a]Adverse events were considered by worst severity for each patient.

(erlotinib 150 mg oral daily and cetuximab 250 mg/m^2 i.v. on days 1, 8, 15, and 22 after a loading dose of 400 mg/m^2 i.v.; ref. 19). Therefore, the recommended phase II dose was erlotinib 150 mg oral daily and cetuximab 250 mg/m^2 i.v. on days 1, 8, 15, and 22 after a loading dose of 400 mg/m^2 i.v.

Antitumor activity

All 20 treated patients were included in the efficacy evaluation. Fourteen of the 20 patients had at least one posttreatment imaging evaluation, and 3 patients came off study before posttreatment imaging evaluation due to clinical progression. The remaining 3 patients were taken off study for the following reasons: withdrawal of consent ($n = 2$) and adverse event (acute infusion reaction, $n = 1$). These patients were considered as treatment failures.

The best overall responses ($n = 20$) are illustrated in Fig. 1. Of the 20 patients, 2 patients (10%) attained PR for 24.2+ and 7.4 months. In addition, 3 patients (15%) attained SD $\geq$6 months (13.7+, 7.7+, and 6.3+ months).

Responses in patients who had received prior EGFR inhibitors. Fifteen of the 20 patients (75%) had received prior EGFR inhibitors (Table 3). Of the 15 patients who had progressed previously on single-agent erlotinib, one patient (6.7%; case #17; Table 3) attained SD $\geq$6 months on this study. The duration of treatment was longer (7.7+ months) on this combination study with dual EGFR inhibitors than on prior single-agent erlotinib (6.1 months).

Responses in NSCLC patients with mutant EGFR. Of the 9 patients with *EGFR*-mutant NSCLC, one patient achieved PR and 2 patients attained SD $\geq$ 6months. One patient (case #2; Table 3; Fig. 2) had a known *EGFR* TKI-resistant mutation (insertion in exon 20, D770>GY) and achieved a PR (−33%; duration, 24.2+ months). This patient had previously received two lines of standard

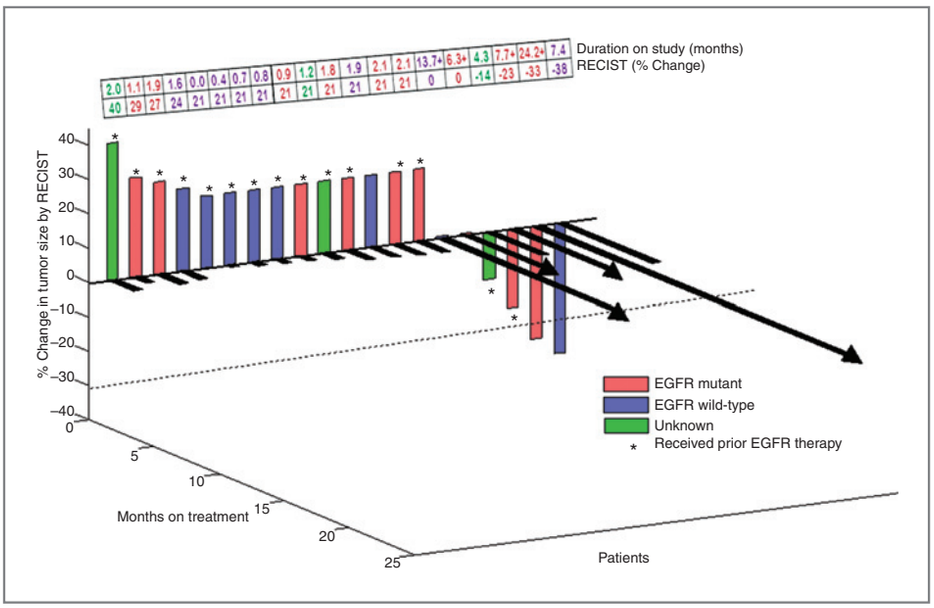

Figure 1. Three-dimensional waterfall plot. Best response by RECIST of 20 patients with NSCLC. Time to treatment failure in months is represented by solid black lines and the arrow indicates that the patient was still on study when the data was censored. Patients with clinical progression or with new metastasis and those who did not reach the first restaging scan for any reason were graphed as 20% progression.

chemotherapy but had not received prior EGFR inhibitor therapy. A second patient (case #17; Table 3) had a known *EGFR* TKI-sensitive mutation (L858R) in exon 21 and has ongoing SD $\geq$ 6 months (−23%; duration, 7.7+ months). This patient had received seven lines of prior therapy including single-agent erlotinib (TTF, 6.1 months). A third patient (case #18; Table 3) with a known *EGFR* TKI-sensitive mutation (L858R) in exon 21 has SD ongoing for 6.3+ months. This patient had received two lines of prior therapy (with TTF of 4.2 months on the chemotherapy before this phase I therapy), but had not received prior erlotinib.

Responses in NSCLC patients with EGFR wild-type disease. Of the eight NSCLC patients with *EGFR* wild-type disease, one patient had PR and one patient attained SD $\geq$ 6 months. Both of these patients (cases #15 and 10; Table 3) had squamous cell histology. A total of four of 20 patients treated had squamous cell histology. One patient (case #15; Table 3) attained a PR (−38%; duration, 7.4 months). This patient had two lines of prior standard therapy with TTF on therapy before this study of 0.7 months. A second patient (case #10; Table 3) with SD for 13.7+ months also had two lines of prior standard therapy with TTF of 8.1 months on the last therapy before this study.

Smoking status. Ten of the 20 patients had a history of smoking. These included 6 patients with adenocarcinoma histology versus 4 patients with squamous cell carcinoma. Mutation status was *EGFR* wild-type in 7 patients, EGFR-mutant in 2 patients (exon 19 deletion, $n = 1$; exon 20 insertion, $n = 1$), and unknown in one patient. Of these, 2 patients achieved PR (cases #2 and 15; Table 3) and 1 patient (case #10; Table 3) attained SD $\geq$ 6 months (*EGFR*-mutant adenocarcinoma, $n = 1$; *EGFR* wild-type squamous cell carcinoma, $n = 2$).

Discussion

Patients with known *EGFR* TKI-sensitive mutations in exon 19 and 21 respond well to matched therapy with EGFR inhibitors, but often quickly develop resistance. Preclinical studies suggest that dual agent molecular targeting of EGFR with a combination of a TKI (erlotinib/gefitinib) and an anti-EGFR antibody (cetuximab) may effectively overcome resistance (15, 16, 25). We

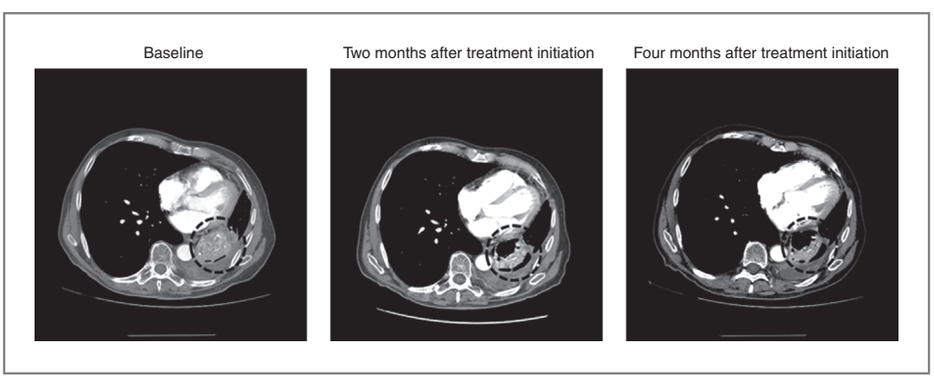

Figure 2. Computed tomography scans at baseline, 2 months, and 4 months after initiating treatment on this study, of a NSCLC patient with a TKI-resistant *EGFR* mutation in exon 20, who has a PR (−33%; duration = 24.2+ months).

conducted a phase I trial combining erlotinib and cetuximab in patients with advanced cancer (19). Here, we report that 5 of 20 patients with NSCLC treated on this study achieved PR ($n = 2$) or SD $\geq$ 6 months ($n = 3$).

The combination of erlotinib and cetuximab was well tolerated. The most frequently observed toxicities that were at least possibly related to study drug were rash ($n = 9$); diarrhea ($n = 7$); hypomagnesemia ($n = 6$), fatigue ($n = 6$), nausea ($n = 4$), and anorexia ($n = 3$; Table 4). The safety profile for the combination was consistent with the individual safety profile of each drug. These findings are similar to those reported in another phase I study of gefitinib and cetuximab in patients with refractory NSCLC, in which escalating doses of cetuximab were combined with fixed dose of gefitinib (17). We defined the recommended phase II dose of erlotinib 150 mg oral daily and cetuximab 250 mg/m^2 i.v. on days 1, 8, 15, and 22 after a loading dose of 400 mg/m^2 i.v. (dose level 2), with the main side effect being rash.

Among the 5 patients who showed antitumor activity (PR or SD $\geq$6 months), 2 had *EGFR* wild-type (of the 8 total with *EGFR* wild-type); both had squamous histology (of a total of 4 with this histology) and achieved SD for 13.7+ months and a PR for 7.4 months. The third patient had an *EGFR* TKI-resistant mutation in exon 20 (D770>GY insertion, of a total of 2 with *EGFR* TKI-resistant mutation). Contrary to the fact that insertions beyond the C-helix (beyond Tyr 764) of the EGFR kinase domain do not respond to usual doses of erlotinib or gefitinib (26, 27), this patient achieved a PR for 24.2+ months. Two other patients had an *EGFR* TKI-sensitive mutation (L858R) in exon 21 and showed SD for 7.7+ and 6.3+ months (the former had failed prior erlotinib after initial response and the latter had not received prior EGFR therapy). Three of 5 patients with PR/SD $\geq$ 6 months had adenocarcinoma and 2 patients had squamous cell carcinoma.

There are two prior clinical studies evaluating a combination of EGFR inhibitors in NSCLC (17, 18). Significant response was not noted in patients with acquired resistance to erlotinib. Although 11 of 13 patients had SD [median progression-free survival (PFS), 3 months], including patients with T790M mutation, prolonged stabilization of disease was not reported (18). In another study, SD was observed in 4 of 13 NSCLC patients with wild-type EGFR disease (17); however, no PRs were seen. The difference in efficacy observed between these studies and our study is not entirely clear, but it seems possibly due to the small number of patients enrolled on each study.

Interestingly, we observed responses in 2 of 4 patients (50%) with *EGFR* wild-type, squamous cell histology. Patients with squamous cell carcinoma of the lung have *EGFR* wild-type disease (28) and are, therefore, not generally treated with EGFR inhibitors. Currently, treatment options are limited for patients with squamous cell carcinoma of the lung. In a prior study of 121 patients with squamous cell carcinoma of the lung treated with single-agent erlotinib (29), PRs were seen in only approximately 7.5% of the 69 evaluable patients. In another study (30), 79 patients with advanced squamous cell carcinoma of the lung were treated with EGFR TKIs. Although the median PFS or OS was not statistically different between patients treated with erlotinib or gefitinib, *EGFR* mutation-positive patients had significantly improved disease-control rate, and prolonged median PFS and OS than patients with *EGFR* wild-type disease. A phase III study (FLEX; ref. 31) evaluating the survival benefit in advanced *EGFR* expressing NSCLC patients treated with cetuximab plus chemotherapy versus chemotherapy alone, included a significant number of patients with squamous cell histology ($n =$ 377; 34% of patients on study). A survival benefit of 10.2 versus 8.9 months (median survival) was seen with the addition of cetuximab in this subset of patients. However, no molecular profiling was conducted, and response rates were not correlated with histology. On the other hand, Fiala and colleagues (32) have concluded that the molecular profile of the tumor may not be predictive of the efficacy of the TKIs in patients with squamous cell carcinoma versus patients with adenocarcinoma. The median PFS and OS were not significantly different in 16 of the 179 patients with *EGFR*-mutant squamous cell NSCLC treated with EGFR TKIs versus 163 patients with wild-type disease. At present, response to EGFR inhibition is unclear in this subset of patients with NSCLC.

Importantly, our results suggest that dual EGFR therapy may help to overcome some cases of primary EGFR TKI resistance. Indeed, one patient (case #2; Table 3) with a known *EGFR* TKI-resistant mutation (insertion in exon 20, D770>GY), who had not received prior EGFR therapy, had an ongoing PR at 24.2+ months (Fig. 2). There is a lack of understanding of the molecular mechanisms that underlie the resistance patterns of these mutations (33). It has been reported that EGFR, through its kinase-independent activity, is able to maintain basal intracellular glucose levels that enhance the survival capacity of tumor cells even in the presence of EGFR TKIs (25). It is, therefore, conceivable that the effect of an antibody such as cetuximab may help to overcome this pathway of resistance. In preclinical models of *EGFR* TKI-resistant tumors (exon 20 insertions), exposure to dual EGFR inhibitors resulted in much more substantial levels of apoptosis than that seen with single types of EGFR inhibitors (15, 16, 34), suggesting synergy. This may possibly explain the response seen in some of our patients such as those with primary resistance to EGFR TKIs. In addition, we observed a response in a patient [case #17; Table 2; *EGFR* TKI-sensitive mutation (L858R) in codon 21] who had progressed on prior erlotinib (35). This patient now has SD for 7.7+ months (prior TTF = 6.1 months). Whether synergy with cetuximab or retreatment with erlotinib led to response is unclear (36, 37), but the fact that the TTF on the combination is longer than the prior TTF on single-agent erlotinib suggests that the cetuximab plays a role in the activity observed.

There are several clinical studies that are underway targeting other pathways of EGFR resistance including

HER2/ERBB2 amplifications or mutations, MET amplifications, and notch dysregulation in patients of NSCLC (38, 39). Encouraging clinical results have also been reported with use of irreversible EGFR tyrosine kinases in patients of NSCLC. Recently, Janjigian and colleagues had reported of confirmed objective response in 40% of the 60 evaluable *EGFR*-mutant patients of NSCLC with acquired resistance to erlotinib or gefitinib (including patients with T790M mutation) when treated on a combination with cetuximab and afatinib (40).

This study is not without limitations. The sample size is small (20 patients) and more so when we consider each specific subtype. In addition, patients were treated at two different dose levels. Furthermore, it is unclear if the antitumor activity (SD for 7.7+ months) seen in a patient who had progressed on prior treatment with erlotinib (case #17; Table 3) is due to the re-treatment effect that occurs with reintroduction of an EGFR TKI after a drug holiday (41).

In conclusion, this study showed that treatment with erlotinib plus cetuximab is feasible in patients of NSCLC. It is a safe combination with the main toxicity being rash. Although not conclusive due to the small sample size in this study, it is noteworthy that SD of six months or more/PR was observed in two of three patients (66%) with *EGFR* wild-type squamous cell carcinoma; one patient with an *EGFR* TKI-resistant mutation and two of eight patients with *EGFR* TKI-sensitive mutations, including one patient who had progressed on prior erlotinib therapy after initial response. The combination of erlotinib plus cetuximab, either alone or with chemotherapy, warrants further exploration in select populations of NSCLC.

Disclosure of Potential Conflicts of Interest

R. Kurzrock has commercial research grant from Eli Lilly. No potential conflicts of interest were disclosed by the other authors.

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