KRAS Mutation Is an Important Predictor of Resistance to Therapy with Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Non–Small Cell Lung Cancer

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Abstract

Purpose: *EGFR* gene mutations and increased *EGFR* copy number have been associated with favorable response to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (EGFR-TKI) in patients with non–small-cell lung cancer (NSCLC). In contrast, *KRAS* mutation has been shown to predict poor response to such therapy. We tested the utility of combinations of these three markers in predicting response and survival in patients with NSCLC treated with EGFR-TKIs.

Experimental Design: Patients with advanced NSCLC treated with EGFR-TKI with available archival tissue specimens were included. *EGFR* and *KRAS* mutations were analyzed using PCR-based sequencing. *EGFR* copy number was analyzed using fluorescence *in situ* hybridization.

Results: The study included 73 patients, 59 of whom had all three potential markers successfully analyzed. *EGFR* mutation was detected in 7 of 71 patients (9.8%), increased *EGFR* copy number in 32 of 59 (54.2%), and *KRAS* mutation in 16 of 70 (22.8%). *EGFR* mutation ($P < 0.0001$) but not increased *EGFR* copy number ($P = 0.48$) correlated with favorable response. No survival benefit was detected in patients with either of these features. *KRAS* mutation correlated with progressive disease ($P = 0.04$) and shorter median time to progression ($P = 0.0025$) but not with survival. Patients with both *EGFR* mutation and increased *EGFR* copy number had a >99.7% chance of objective response, whereas patients with *KRAS* mutation with or without increased *EGFR* copy number had a >96.5% chance of disease progression.

Conclusion: *KRAS* mutation should be included as indicator of resistance in the panel of markers used to predict response to EGFR-TKIs in NSCLC.

Lung cancer remains the leading cause of cancer death in the United States and is expected to cause 162,000 deaths in the United States in 2006 (1). Epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, is expressed in the majority of non–small-cell lung cancers (NSCLC). Gefitinib (ZD1839, Iressa; AstraZeneca) and erlotinib (Tarceva, OSI-774; OSI Pharmaceuticals), small-molecule inhibitors that target the tyrosine kinase domain of the EGFR, produce responses in ~10% of patients with NSCLC that has progressed with prior chemotherapy (2–6). In patients with NSCLC who benefit from gefitinib or erlotinib, the responses can be dramatic and may last for longer than a year (2–6).

Several markers have been identified that predict response to the EGFR-specific tyrosine kinase inhibitors (EGFR-TKI) in patients with NSCLC. Activating mutations in the *EGFR* tyrosine kinase domain (exons 18-21), increased *EGFR* copy number, and increased EGFR protein expression have been associated with favorable response to EGFR-TKIs (7–17). In contrast, *KRAS* gene mutation, which occurs in 20% to 30% of NSCLCs, mainly in adenocarcinomas (30%) and smokers (18), has been reported to be associated with poor response to EGFR-TKIs (19–23).

Studies have also investigated potential markers of survival in patients with NSCLC treated with EGFR-TKIs. Whether activating mutations in the *EGFR* tyrosine kinase domain are associated with a survival advantage from gefitinib or erlotinib, especially in Western populations with NSCLC, is controversial. Several retrospective studies showed prolonged survival in gefitinib-treated patients with tyrosine kinase activating mutations, mostly in Asian populations (8–14), whereas in the BR.21 study, the hazard ratio for death was almost identical in patients with mutated and wild-type *EGFR* (0.73 and 0.77, respectively; ref. 16). *EGFR* increased copy number has been shown to predict favorable survival outcomes after EGFR-TKI therapy (7, 13, 15, 17).

Several studies have shown that *EGFR* mutation and *KRAS* mutation are mutually exclusive (19, 24, 25), and EGFR

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Table 1. *EGFR* mutation status, *EGFR* gene copy number status, and *KRAS* mutation status by patients' characteristics

Characteristic	*EGFR* mutation status (tested, 71 of 73)			*EGFR* gene copy number status (tested, 59 of 73)			*KRAS* mutation status (tested, 70 of 73)		
	Wild type (*n* = 64), *n* (%)	Mutated (*n* = 7), *n* (%)	*P**	Not increased (*n* = 27), *n* (%)	Increased (*n* = 32), *n* (%)	*P**	Wild type (*n* = 54), *n* (%)	Mutated (*n* = 16), *n* (%)	*P**
Age, median, y	60	51	0.2	64	58	0.19	57.5	65	0.05
Gender									
Female	36 (87.8)	5 (12.2)	0.69	18 (54.5)	15 (45.5)	0.19	30 (73.2)	11 (26.8)	0.4
Male	28 (93.3)	2 (6.7)		9 (34.6)	17 (65.4)		24 (82.8)	5 (17.2)	
Race									
Asian	4 (57.1)	3 (42.9)	0.03	4 (80.0)	1 (20)	0.34	7 (100)	0 (0)	0.41
Caucasian	52 (92.9)	4 (7.1)		21 (42.9)	28 (57.1)		41 (74.5)	14 (25.5)	
Other	8 (100)	0 (0)		2 (40)	3 (60)		6 (75)	2 (25)	
Smoking history									
Current smoker	23 (95.8)	1 (4.2)	0.15	10 (50)	10 (50)	0.28	18 (78.3)	5 (21.7)	0.1
Former smoker	28 (93.3)	2 (6.7)		9 (34.6)	17 (65.4)		20 (66.7)	10 (33.3)	
Never smoker	13 (76.5)	4 (23.5)		8 (61.5)	5 (38.5)		16 (94.1)	1 (5.9)	
Histology									
Adenocarcinoma	40 (85.1)	7 (14.9)	0.22	17 (43.6)	22 (56.4)	0.93	34 (72.3)	13 (27.7)	0.13
NSCLC	13 (100)	0		5 (55.6)	4 (44.4)		9 (75)	3 (25)	
Squamous cell carcinoma	11 (100)	0		5 (45.5)	6 (54.5)		11 (100)	0	
PS (ECOG) †									
0-1	32 (82.1)	7 (17.9)	0.01	14 (43.8)	18 (56.3)	0.8	31 (81.6)	7 (18.4)	0.4
2-3	32 (100)	0		13 (48.1)	14 (51.9)		23 (71.9)	9 (28.1)	
Stage †									
IIIB	5 (100)	0	1.0	3 (75)	1 (25)	0.32	5 (100)	0	0.58
IV	59 (89.4)	7 (10.6)		24 (43.6)	31 (56.4)		49 (75.4)	16 (24.6)	
Previous chemotherapy regimens †									
0	10 (90.9)	1 (9.1)	1.0	6 (54.5)	5 (45.5)	0.34	7 (63.6)	4 (36.4)	0.35
1	23 (92)	2 (8)		11 (55)	9 (45)		21 (84)	4 (16)	
≥2	31 (88.6)	4 (11.4)		10 (35.7)	18 (64.3)		26 (76.5)	8 (23.5)	
Pleural effusion †									
No	37 (88.1)	5 (11.9)	0.69	15 (40.5)	22 (59.5)	0.42	30 (73.2)	11 (26.8)	0.4
Yes	27 (93.1)	2 (6.9)		12 (54.5)	10 (45.5)		24 (82.8)	5 (17.2)	
Brain metastasis †									
No	42 (91.3)	4 (8.7)	0.69	21 (58.3)	15 (41.7)	0.02	34 (74)	12 (26)	0.55
Yes	22 (88)	3 (12)		6 (26.1)	17 (73.9)		20 (83.3)	4 (16.7)	

Abbreviations: PS, performance status; ECOG, Eastern Cooperative Oncology Group.
*χ^2 test or Fisher's exact test.
†Data retrieved at start of gefitinib or erlotinib treatment.

mutation and genomic gain are associated (7), but the relationship between increased *EGFR* copy number and *KRAS* mutation and the effect of this combination on response to EGFR-TKI therapy have not yet been investigated. The purpose of this retrospective study was to investigate the concomitant presence of increased *EGFR* copy number and *KRAS* mutation in tumor specimens from patients with NSCLC and to clarify the predictive value of combinations of *EGFR* mutation status, *EGFR* copy number status, and *KRAS* mutation status in predicting response and survival in patients with NSCLC treated with EGFR-TKIs.

Materials and Methods

Patients and data collection. Tumor specimens were obtained from patients with advanced NSCLC treated with gefitinib or erlotinib at The University of Texas M. D. Anderson Cancer Center between May 1999 and December 2004. Patients either received gefitinib as part of an extended-access protocol approved by the institutional review board or received gefitinib or erlotinib after the drugs were approved by the U.S. Food and Drug Administration. Both drugs were administered orally once daily: gefitinib at 250 mg and erlotinib at 150 mg. Only patients with at least four formalin-fixed, paraffin-embedded tissue sections with at least 1,000 tumor cells per section (necessary for DNA extraction and mutation analyses) were eligible.

This study was approved by the M. D. Anderson Cancer Center. All specimens were histologically classified according to the WHO classification for lung cancer by an experienced thoracic pathologist (I.I.W.; ref. 26). Imaging studies were assessed by a medical oncologist (E.M.), who graded responses according to the Response Evaluation Criteria in Solid Tumors (27). In case of stable disease, measurements had to meet the stable disease criteria at least once after the first evaluation at a minimum interval of 6 to 8 weeks. All the investigators were blinded to patient outcomes.

EGFR *and* KRAS *mutation analysis.* Exons 18 through 21 of *EGFR* and exon 1 of *KRAS* were PCR amplified using intron-based primers as previously described (25, 28, 29). From microdissected formalin-fixed, paraffin-embedded cells, ~200 cells were used for each PCR

amplification, as previously described (29). All PCR products were directly sequenced using the Applied Biosystems PRISM dye terminator cycle sequencing method. All sequence variants were confirmed by independent PCR amplifications from at least two independent microdissections and sequenced in both directions, as previously reported (29).

EGFR *copy number analysis.* *EGFR* copy number per cell was investigated using fluorescence *in situ* hybridization (FISH) done with the LSI EGFR SpectrumOrange/CEP 7 SpectrumGreen probe (Vysis) according to a published protocol (7, 30). Serial 5-μm-thick tissue sections were incubated at 56°C overnight, deparaffinized, and dehydrated in 100% ethanol. After incubation in 2× saline sodium citrate buffer (2× SSC, pH 7.0) at 75°C for 15 to 25 min, sections were digested with proteinase K (0.25 mg/mL in 2× SSC, pH 7.0) at 37°C for 15 to 25 min, rinsed in 2× SSC (pH 7.0) at room temperature for 5 min, and dehydrated using ethanol in increasing concentrations (70%, 85%, and 100%). The EGFR/CEP 7 probe set was applied per the manufacturer's instructions to an area of the slide containing tumor foci, and the hybridization area was covered with a glass coverslip and sealed with rubber cement. The slides were incubated at 80 °C for 8 to 10 min to permit co-denaturation of chromosomal and probe DNA and were then placed in a humidified chamber at 37°C and left for 20 to 24 h to allow hybridization. Post-hybridization washes were done in 1.5 mol/L urea and 0.1× SSC (pH 7.0-7.5) at 45°C for 30 min and in 2× SSC for 2 min at room temperature. After the samples were dehydrated in ethanol, 4′,6-diamidino-2-phenylindole (DAPI; 0.3 mg/mL in Vectashield mounting medium, Vector Laboratories) was applied for chromatin counterstaining.

FISH assessment was done independently by two authors (M.V-G. and A.C.X.) who were blinded to the patients' clinical characteristics and all other molecular variables. Patients were classified into six FISH strata, as previously described (7, 30). High polysomy and gene amplification categories were considered to have increased *EGFR* copy number, and the categories disomy to low polysomy were considered not to have increased gene copy number.

Statistical analysis. Data were summarized using standard descriptive statistics and frequency tabulation. Associations between categorical variables were assessed using cross-tabulation, the χ^2 test, and Fisher's exact test. The Kruskal-Wallis test and Wilcoxon rank-sum test were done to assess differences in continuous variables between clinical-pathologic groups. Logistic regression analysis was applied to estimate the effect of covariates on response (complete response + partial response versus other). Time to disease progression (TTP) and overall survival (OS) were measured for each patient from the first day of treatment with gefitinib or erlotinib. Survival curves were estimated using the Kaplan-Meier method. Univariate and multivariate Cox proportional hazards models were applied to assess the effect of covariates on TTP and OS from the first day of TKI therapy.

One of our interests in this research was in addressing the following question: what is the probability that the response rate in the ith group is greater than the response rates in all other groups? Bayesian methods provide a natural framework to address the above question. Bayesian methods, unlike classic methods, treat the probability of response as a quantity about which the investigator has some degree of uncertainty. This uncertainty is quantified directly via probability. We assume that the response data for each group follow a binomial distribution. The probability of response in the ith group is denoted by p_i. We also assume that the non-informative prior distribution for p_i follows a non-informative $\beta(0.5,0.5)$ distribution (for all i). Given these assumptions, we now calculate the posterior probability

$$\Pr(p_i > \max(p_1, \ldots p_{i-1}, p_{i+1}, p_M)|\text{Data})$$

The multidimensional integration underlying the calculation of this probability statement was done via Monte Carlo simulation (50,000 interactions).

All computations were carried out in SAS or S-plus 2000.

Results

Patient population. Seventy-three patients with advanced NSCLC were treated at M.D. Anderson with gefitinib (n = 72) or erlotinib (n = 1) during the study period and had sufficient tissue sections available for DNA extraction and *EGFR* and *KRAS* mutation analyses. However, of those 73 patients, only 59 had enough tumor cells (at least 200) in the remaining tissue sections for FISH analysis. Specimens were obtained before the start of TKI therapy in 62 patients and within a median time of 5 months (range, 2-19 months) after the start of the TKI therapy in 11 patients.

Correlation of* EGFR *and* KRAS *abnormalities with patients' clinical and pathologic features. Seventy-one patients were successfully tested for *EGFR* mutation, 59 for *EGFR* gene copy number, and 70 for *KRAS* mutation. Clinical and pathologic characteristics and their correlation with genetic abnormalities are shown in Table 1.

EGFR mutation was identified in 7 of the 71 tested patients (9.8%). *EGFR* mutations were significantly more frequent in Asian patients (P = 0.03) and patients with better performance status at the beginning of TKI treatment (P = 0.01; Table 1). Five of the seven patients with *EGFR* mutations had a 15-bp deletion (E746-E750) in exon 19; one patient had a 18-bp deletion (E746-E751) in exon 19; and one patient had a point mutation in exon 18 (G719A; Table 2).

EGFR gene copy number analysis revealed 1 patient (1.7%) with disomy, 9 (15.2%) with low trisomy, 17 (28.8%) with low

Table 2. *EGFR* and *KRAS* mutations detected in lung cancer tissue specimens

Study case no.	*EGFR* gene	*KRAS* gene
1	Wild type	Codon 12, GGT to GTT
2	Wild type	Codon 12, GGT to TGT
3	Wild type	Codon 12, GGT to TGT
7	Wild type	Codon 12, GGT to TGT
10	Wild type	Codon 12, GGT to GCT
19	Wild type	Codon 13, GGC to TGC
21	Wild type	Codon 12, GGT to TGT
24	Exon 19, 15-bp deletion (746-750)	Wild type
26	Wild type	Codon 12, GGT to TGT
28	Wild type	Codon 12, GGT to GAT
31	Wild type	Codon 12, GGT to TGT
33	Wild type	Codon 13, GGC to TGC
38	Wild type	Codon 12, GGT to TGT
39	Wild type	Codon 12, GGT to TGT
44	Wild type	Codon 12, GGT to TGT
45	Wild type	Codon 12, GGT to GAT
55	Wild type	Codon 12, GGT to TGT
57	Exon 18, Codon 719, GGC to GCC	Wild type
59	Exon 19, 15-bp deletion (746-750)	Wild type
61	Exon 19, 15-bp deletion (746-750)	Wild type
62	Exon 19, 15-bp deletion (746-750)	Wild type
64	Exon 19, 18-bp deletion (746-751)	Wild type
65	Exon 19, 15-bp deletion (746-750)	Wild type

Table 3. Objective responses by patients' characteristics

Covariate	Complete or partial response (7/73), *n* (%)	Stable disease (11/73), *n* (%)	Progressive disease (55/73), *n* (%)	*P**
Age, median, y	51	56	62	0.06
Gender				
Female	6 (14)	6 (14)	31 (72)	0.37
Male	1 (3.3)	5 (16.7)	24 (80)	
Race				
Other	0	3 (37.5)	5 (62.5)	0.12
Asian	2 (28.6)	0	5 (71.4)	
Caucasian	5 (8.6)	8 (13.8)	45 (77.6)	
Smoking history				
Current smoker	1 (4.2)	5 (20.8)	18 (75)	0.12
Former smoker	2 (6.7)	2 (6.7)	26 (86.6)	
Never smoker	4 (21.1)	4 (21.1)	11 (57.8)	
Histology				
Adenocarcinoma	7 (14.6)	6 (12.5)	35 (72.9)	0.44
NSCLC	0	3 (21.4)	11 (78.6)	
Squamous cell carcinoma	0	2 (18.2)	9 (81.8)	
PS (ECOG) †				
0-1	5 (12.2)	8 (19.5)	28 (68.3)	0.37
2-3	2 (6.2)	3 (9.4)	27 (84.4)	
Stage †				
IIIB	0	2 (33.3)	4 (66.7)	0.31
IV	7 (10.4)	9 (13.4)	51 (76.2)	
Previous chemotherapy regimens †				
0	1 (9.1)	1 (9.1)	9 (81.8)	0.67
1	3 (11.5)	6 (23.1)	17 (65.4)	
≥2	3 (8.3)	4 (11.1)	29 (80.6)	
Pleural effusion †				
No	4 (9.3)	6 (14)	33 (76.7)	0.92
Yes	3 (10)	5 (16.7)	22 (73.3)	
Brain metastasis †				
No	3 (6.4)	6 (12.8)	38 (80.8)	0.36
Yes	4 (16)	4 (16)	17 (68)	

*Fisher's exact test.
† Data retrieved at the start of gefitinib or erlotinib treatment.

polysomy, 21 (35.6%) with high polysomy, and 11 (18.7%) with gene amplification. Thus, 32 of the 59 patients tested (54.2%) had increased *EGFR* copy number. There was a statistically significant association between increased *EGFR* gene copy number and the presence of brain metastasis at the start of TKI therapy ($P = 0.02$; Table 1).

KRAS mutation was identified in 16 of the 70 tested patients (22.8%). Fourteen patients had a single-amino-acid substitution in codon 12, and two patients had a codon 13 mutation (Table 2).

None of the tumor samples analyzed harbored concomitant *KRAS* and *EGFR* mutation. In the cohort of cases ($n = 59$) analyzed for all three markers, increased *EGFR* copy number was detected in 5 of the 6 *EGFR* mutant cases (83%) and 8 of the 14 *KRAS* mutant cases (57%).

Correlation of* EGFR *and* KRAS *abnormalities with response to EGFR-TKIs. Seven patients (9.6%) had an objective response to TKI therapy (complete response in 1 and partial response in 6); 11 patients (15.1%) had stable disease; and 55 patients (75.3%) had progressive disease. No significant differences in demographic and clinical characteristics were observed between the different response groups (Table 3). The presence of *EGFR* mutation was significantly associated with objective response to TKI treatment ($P < 0.0001$; Table 4). Four (80%) of the five responders with available FISH data had increased *EGFR* copy number, but this finding was not statistically significant (Table 4). The presence of *KRAS* mutation was significantly associated with lack of response to TKI treatment ($P = 0.04$; Table 4).

Testing for *EGFR* mutation, *EGFR* copy number, and *KRAS* mutation was done in 59 patients, and the correlations between genetic alterations and response rate are shown in Table 5. The group of patients with both mutated *EGFR* and increased *EGFR* copy number had the highest response rate (80%), and the probability of having the highest response rate (calculated via Bayesian analysis described in the statistical considerations) was calculated as >92.6% (if the single patient *EGFR* mutation positive/*EGFR* gene copy number negative/*KRAS* mutation negative is removed, this probability increases to 99.7%). In contrast, the group of patients with mutated *KRAS*, independent of the *EGFR* copy number status, had the highest rate of progressive disease (100%), and this group's probability of having the highest progressive-disease rate was calculated as >96.5% (Table 5).

In the group of 11 cases collected after the EGFR-TKI start date, 2 patients (18%) obtained a partial response, 2 patients (18%) had stable disease, and 7 patients (64%) progressed. Of the 2 cases (18%) with *EGFR* mutation, one had partial response, and the other had progressive disease. No *KRAS* mutation was detected in the 11 patients. Increased *EGFR* copy number was detected in 6 of the 7 cases (86%) tested for the

Table 4. Response rate and survival data by *EGFR* mutation status, *EGFR* gene copy number status, and *KRAS* mutation status

	***EGFR* mutation status (tested, 71/73), *n* (%)**		***EGFR* gene copy number status (tested, 59/73), *n* (%)**		***KRAS* mutation status (tested, 70/73), *n* (%)**	
	Wild type (*n* = 64)	**Mutant (*n* = 7)**	**Not increased (*n* = 27)**	**Increased (*n* = 32)**	**Wild type (*n* = 54)**	**Mutant (*n* = 16)**
CR + PR	2 (28.6)	5 (71.4)	1 (20)	4 (80)	7 (100)	0
PD	53 (96.4)	2 (3.6)	23 (50)	23 (50)	38 (70.4)	16 (29.6)
SD	9 (100)	0	3 (37.5)	5 (62.5)	9 (100)	0
*P**	<0.0001		0.48		0.04	
Median OS, mo	7.8	21.9	8.2	9.3	9.4	5.0
*P**	0.08		0.68		0.62	
Median TTP, mo	2.1	9.3	2.1	2.8	2.9	1.7
*P**	0.15		0.44		0.0025	

Abbreviations: CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.
*Log-rank test.

combination of the three markers. Two cases showed both *EGFR* mutation and increased copy number. When statistical analyses included only the cohort of 62 tumors collected before the EGFR-TKI therapy start date, data previously shown with the larger data set ($n = 73$) were largely confirmed. In summary, *EGFR* mutation resulted to be a predictor of best response to *EGFR* TKI treatment ($P = 0.0001$), and increased gene copy number did not show a statistical association with response ($P = 0.68$). *KRAS* mutation was borderline associated with poor response ($P = 0.06$). In the cohort of 52 tumor cases collected before the EGFR-TKI therapy start date cases tested for the combination of the three markers, the group of tumors with mutated *EGFR* and increased *EGFR* copy number ($n = 3$) was confirmed to have the highest response rate (100%), and the probability of having the highest response rate was calculated as >99.8%. In contrast, the group of patients with mutated *KRAS*, with ($n = 8$) or without ($n = 6$) increased *EGFR* copy number, had the highest rate of progressive disease (100%), and this group's probability of having the highest progressive disease rate was calculated as >95%.

Correlation of* EGFR *and* KRAS *abnormalities with survival. With a median follow-up of 3.2 years, 57 patients had died, and 72 had experienced disease progression. A trend toward better OS was observed for the patients with mutant *EGFR*, but this did not reach statistical significance (log-rank test, $P = 0.08$; Table 4). In the multivariate analysis for OS, age [hazard ratio (HR), 1.02; $P = 0.07$], male gender (HR, 1.96; $P = 0.01$), and performance status ≥2 (HR, 1.68; $P = 0.07$) were important predictors of OS. Median TTP was significantly shorter in the patients with mutated *KRAS* (log-rank test, $P = 0.0025$, Table 4). Longer median TTP was also observed in patients with mutated *EGFR*, but this finding was not statistically significant (Table 4). The multivariable Cox model indicated that, adjusted for age (HR, 1.02; $P = 0.04$), *KRAS* mutation (HR, 2.14; $P = 0.01$) remained a statistically significant predictor of TTP.

The group of patients with both mutated *EGFR* and increased *EGFR* copy number had the longest TTP, whereas the group of patients with mutated *KRAS* had the shortest TTP (log-rank test, $P = 0.008$). Similar results were obtained when only patients whose tumor specimens were collected before the start of TKI treatment ($n = 62$) were included in the analysis, and at multivariable Cox model, *KRAS* mutation remained a strong predictor of poor TTP (HR, 2.45; $P = 0.004$).

Discussion

In the current study, we found differences in response and outcome in patients with advanced NSCLC treated with EGFR-TKIs by *EGFR* mutation status, *EGFR* copy number, and *KRAS* mutation status. To our knowledge, this is the first study to analyze the combination of *EGFR* mutation, *EGFR* FISH copy number, and *KRAS* mutation in predicting response to EGFR-TKIs.

The frequencies of *EGFR* (9.8%) and *KRAS* (22.8%) mutation, the finding that these mutations were mutually

Table 5. Clinical responses by combination of *EGFR* mutation status, *EGFR* gene copy number status, and *KRAS* mutation status in the 59 NSCLC cases examined for all three markers

***EGFR* mutation status**	***EGFR* gene copy no. status**	***KRAS* mutation status**	**No. cases**	**CR + PR, *n* (%)**	**SD, *n* (%)**	**PD, *n* (%)**
Positive	Positive	Negative	5	4 (80)	0	1 (20)
Negative	Positive	Negative	19	0	5 (26)	14 (74)
Negative	Negative	Negative	20	1 (5)	3 (15)	16 (80)
Positive	Negative	Negative	1	0	0	1 (100)
Negative	Positive	Positive	8	0	0	8 (100)
Negative	Negative	Positive	6	0	0	6 (100)

exclusive, and the clinical-pathologic characteristics of the patients in our series are similar to previously published data (21). Our findings that *EGFR* mutations were detected only in adenocarcinomas and were common in Asian patients and never smokers whereas *KRAS* mutations were identified mostly in adenocarcinomas and were more common in smokers support the notion that there are at least two molecular pathways involved in the pathogenesis of lung adenocarcinoma: a nonsmoking EGFR signaling-associated pathway and a smoking KRAS signaling-associated pathway (31).

Whereas several recent studies have shown that increased *EGFR* copy number predicts favorable response to and outcome after treatment with EGFR-TKIs in patients with NSCLC (7, 13, 15, 17), *KRAS* mutation is known to predict poor outcome after treatment with EGFR-TKIs in patients with NSCLC (19–23). However, to our knowledge, our study is the first to show that activating *KRAS* mutation overcomes the potential favorable role of increased *EGFR* copy number in predicting response and survival of NSCLC patients after treatment with EGFR-TKIs. In fact, in our study, all 16 patients with *KRAS* mutations experienced progressive disease as the best response to treatment with EGFR-TKIs, including eight patients whose tumors had increased *EGFR* copy number. The strong association between the presence of *KRAS* mutation and poor response to treatment was also observed when the 11 patients whose specimens tested for the three markers were obtained after the start of TKI therapy (median time of 5 months; range, 2-19 months) were excluded.

Our findings that *EGFR* mutation was a significant predictor of favorable response to EGFR-TKIs ($P < 0.0001$) and that *EGFR* mutation was associated with a trend toward longer OS ($P = 0.08$; Table 4) are in line with the existing literature (7–17, 32). However, in contrast with previous studies, we did not find significant correlation between *EGFR* copy number and response or outcome in this cohort of patients (15, 17). Interestingly, eight tumors with increased *EGFR* copy number also carried *KRAS* mutation and reported poor response rate (Table 5) and TTP ($P = 0.008$). Conversely, the group of patients with concomitant *EGFR* mutation and increased *EGFR* copy number had the longest TTP interval ($P = 0.008$). This might be due to the association between *EGFR* mutation and increased *EGFR* gene copy number previously described by Cappuzzo et al. (7). In fact, among the five *EGFR* mutant patients who responded to TKI therapy, four also had increased *EGFR* copy number (two had high polysomy and two had gene amplification). Of the two *EGFR* mutant patients who did not respond to TKI therapy, one had high polysomy, and the other had low polysomy. Another interesting finding is the absence of *EGFR* mutations in tumors from patients who had stable disease (Table 4), three of whom had stable disease for more than 1 year. A similar observation was previously reported by other authors (7) and underscores the importance of defining selection criteria to identify which patients are most likely to benefit from EGFR-TKIs.

In summary, our findings indicate that in patients with NSCLC, *KRAS* mutation is an important predictor of poor response and outcome to EGFR-TKIs despite the concomitant presence of increased *EGFR* gene copy number and should be included in the panel of markers to be used to predict response to such therapy.

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