Prognostic and Therapeutic Implications of *EGFR* and *KRAS* Mutations in Resected Lung Adenocarcinoma

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Background: Somatic mutations in *EGFR* (exons 19 and 21) and *KRAS* (exon 2) are found in lung adenocarcinomas and have potential prognostic value in patients with advanced disease. These mutations also have therapeutic significance, as they predict for sensitivity and resistance, respectively, to EGFR tyrosine kinase inhibitor therapy. Whether *EGFR* and *KRAS* mutations also have an impact on survival in patients who undergo lung resection for curative intent in the absence of targeted therapy has not been established.

Methods: We analyzed the clinical characteristics and outcomes data for 296 patients who underwent resection at our institution for stage I–III lung adenocarcinoma. Tumors were assessed for both *EGFR* and *KRAS* mutations by established methods.

Results: *EGFR* and *KRAS* mutations were found in tumors from 40 (14%) and 50 (17%) patients, respectively. Patients with *EGFR* mutant tumors were more likely to be never smokers (48%), present with stage I disease (88%), and had a 90% (95% confidence interval [CI] 70–97%) 3-year overall survival, whereas patients with *KRAS* mutant tumors were more likely to be former/current smokers (92%), present with locally advanced disease (40%), and had a 66% (95% CI 48–79%) 3-year overall survival.

Conclusions: *EGFR* and *KRAS* mutations define distinct molecular subsets of resected lung adenocarcinoma. Because *EGFR* and *KRAS* mutations also predict whether tumors are sensitive or resistant, respectively, to EGFR tyrosine kinase inhibitors, they can readily be used in clinical trials to help guide the administration of specific types of adjuvant therapy.

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Adenocarcinoma of the lung is the leading cause of cancer-related death in the United States. The incidence of the adenocarcinoma subtype has been rising and now accounts for >50% of all cases of lung cancer.[1] The overwhelming majority of patients with lung cancer have smoked cigarettes. However, about 10% of lung cancers arise in "never smokers", i.e., patients who smoked less than 100 cigarettes in a lifetime.

The introduction of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) into the clinic has led to the identification of two tumor-specific somatic mutations that define clinically relevant molecular subsets of advanced lung adenocarcinoma.[2] Mutations in the kinase domain of *EGFR* are more often found in never smokers and East Asian populations. These mutations are exon 19 deletions that eliminate a common leucine-arginine-glutamic acid-alanine (LREA) motif and exon 21 point mutations that lead to substitution of arginine for leucine at position 858. Multiple prospective trials have demonstrated that patients whose tumors harbor these genetic changes have a collective response rate of nearly 75% to treatment with gefitinib or erlotinib.[3] Conversely, mutations in *KRAS,* which encodes a guanosine triphosphate hydrolase (GTPase) downstream of *EGFR*, are more often found in tumors from former/current smokers and North American/European populations and these tumors are resistant to therapy with EGFR TKI's.[4–6] These mutations lead to substitutions of amino acids for glycines at positions 12 and 13. Tumors rarely have mutations in both *EGFR* and *KRAS*. In North America, the prevalence of these mutations in lung adenocarcinoma is about 10% and 20%, respectively.

In addition to having predictive value for responses to EGFR TKIs, *EGFR* mutations also seem to have prognostic significance in the advanced or relapsed setting. For example, in a retrospective subgroup analysis of a clinical trial in which patients with untreated advanced non-small cell lung cancer (NSCLC) were randomized to treatment with chemotherapy plus placebo or chemotherapy plus erlotinib, patients with

EGFR mutations showed significantly better clinical outcomes than those with wild-type *EGFR* in response rate, time-to-progression, and overall survival, regardless of whether or not they received erlotinib.[7] By contrast, *KRAS* mutations did not have prognostic significance. In fact, although there was no difference in time to progression or overall survival among patients with *KRAS* mutant versus wild-type tumors who received chemotherapy alone, patients with *KRAS*-mutant tumors who received erlotinib plus chemotherapy showed poorer clinical outcomes.[7]

The prognostic value of *EGFR* and *KRAS* mutations in patients with resectable lung adenocarcinoma has not been established. Some recent studies have compared the survival of early stage patients with *EGFR* mutant tumors versus those with *EGFR* wild-type tumors and have not found a significant difference in their overall survival.[8–10] However in these studies, *EGFR* wild-type tumors were not further classified by *KRAS* status. Older studies have shown that patients harboring a *KRAS* mutation have a worse overall prognosis when compared with those with wild-type *KRAS*.[11] A recent meta-analysis of over 28 studies of *RAS* mutations in NSCLC concluded that patients with *KRAS* mutant tumors have a significantly worse prognosis, with a combined hazard ratio 1.35 (95% confidence interval [CI] 1.16–1.56).[12] However, *KRAS* wild-type tumors were not further classified by *EGFR* status, as *EGFR* mutations had not yet been discovered. Here, we sought to determine the prognostic significance of *EGFR* and *KRAS* mutations in nearly 300 patients who underwent resection for lung adenocarcinoma. None of the patients received neoadjuvant or adjuvant EGFR TKI therapy.

TABLE 1. Clinical Characteristics of 296 Patients Whose Tumors Were Genotyped

	EGFR Mutation *N* = 40	*KRAS* Mutation *N* = 50	*EGFR/KRAS* Wild-type *N* = 206	*p*
Age	70 (35–86)	70 (42–86)	68 (39–89)	
Gender				0.581
Men	14 (35%)	23 (46%)	83 (40%)	
Women	26 (65%)	27 (54%)	123 (60%)	
Stage				0.064
IA	21 (53%)	16 (32%)	103 (50%)	
IB	14 (35%)	14 (28%)	48 (23%)	
II	1 (2%)	7 (14%)	21 (10%)	
III	4 (10%)	13 (26%)	34 (17%)	
Cigarette smoking				<0.001
Never	18 (47%)	4 (8%)	14 (5%)	
Former	20 (53%)	37 (74%)	154 (83%)	
Current	0	9 (18%)	38 (12%)	
Chemotherapy				0.405
None	25 (62%)	26 (52%)	128 (62%)	
Adjuvant/ neoadjuvant	15 (38%)	24 (48%)	78 (38%)	
Mutation				
	L858R: 19	G12C: 22	N/A	
	Exon 19 del: 19	G12V: 14		
	Exon 20 ins: 1	G12A: 7		
	Exon 21 (H835L): 1	G12D: 6		
		G13C: 1		

Age listed is the mean age. Smoking history is defined as never smokers (<100 lifetime cigarettes), former smokers (quit ≥1 year before diagnosis), or current (quit <1 year before diagnosis). Note the *EGFR* exon 20 insertion and exon 21 H835L were not included in the survival analysis. All *p* values obtained using Fisher's exact test and represent comparisons between the 3 groups.

Del, deletion; ins, insertion.

PATIENTS AND METHODS

Tissue Procurement

Tumor specimens from patients who underwent surgical resection at Memorial Sloan-Kettering Cancer Center (MSKCC) for stage I–III lung adenocarcinoma collected from January 2002 to February 2006 were obtained with patients' consent under an institutional review board (IRB) approved protocol (#92-055). This protocol exists for fluid and tissue collection for research purposes only. Patients were excluded if they had nonadenocarcinoma histology, stage IV disease, or if they had received treatment with either gefitinib or erlotinib pre- or post-surgery. Five patients were excluded due to receiving TKI therapy. Some patients received neoadjuvant or adjuvant cytotoxic chemotherapy at the discretion of their treating physician (Table 1).

All tumors were snap frozen in the operating room in liquid nitrogen and stored at −80°C. Clinical information was extracted from IRB approved institutional databases. Age listed is the age at diagnosis. Stage listed is pathologic stage after surgical resection according to American Joint Committee on Cancer guidelines. Smoking status is defined as never smokers (<100 lifetime cigarettes), former smokers (quit ≥1 year before diagnosis), or current (quit <1 year before diagnosis). Internal databases and the Social Security Death Index (http://ssdi.rootsweb.com/cgi-bin/ssdi.cgi) were used to determine overall survival. Overall survival was from date of surgery until death by any cause. Patients without a date of death were censored at time of last follow-up.

Mutation Detection

DNA was extracted using a kit (DNeasy, Qiagen) or standard phenol extraction. *EGFR* mutations were assessed by direct dideoxynucleotide sequencing of exons 18 to 21,[13] polymerase chain reaction-based methods that detect exon 19 deletions and exon 21 L858R amino acid substitutions,[14] or mass spectrometry-based genotyping (Sequenom). *KRAS* mutations were assessed by direct sequencing of exon 2 or mass spectrometry-based genotyping (Sequenom). Data regarding the mutation status of some of these tumors was previously reported.[13,15,16]

Statistical Analysis

Patients were divided into three groups based on mutation type: *EGFR* mutant, *KRAS* mutant, or wild type for *EGFR/KRAS*. The associations were tested between the clinical characteristics and the mutation groups by using Fisher's exact test for the categorical variables. A *p* value of <0.05 was considered significant.

The Kaplan-Meier method was used to obtain the survival rates. The log-rank test was used to obtain the *p*-values for univariate survival analysis before controlling

for stage; whereas after stage controlling, the stratified log-rank test was applied to obtain the overall *p*-values. The hazard ratios were obtained by using a Cox proportional hazard model. All *p*-values were two-sided and a *p* value <0.05 was considered as statistically significant. The statistical analyses were performed using SAS 9.1 (SAS institute Inc. Cary, NC) and R 2.5 (http://www.r-project.org).

RESULTS

Two hundred ninety-six resected stage I–III lung adenocarcinomas were genotyped for *EGFR* kinase domain and *KRAS* mutations. Clinical characteristics of patients whose tumors were examined are listed in Table 1. *EGFR* and *KRAS* mutations were detected in tumors from 40 (14%) and 50 (17%) patients, respectively. No tumor had both mutations. Two patients with *EGFR* mutant tumors (Table 1) were excluded from subsequent survival analyses because they had rare atypical mutations which are less well studied in terms of their oncogenic properties and sensitivity to EGFR TKIs.

No differences were observed in the gender distribution between patients with *EGFR* mutant, *KRAS* mutant, or wild-type tumors ($p = 0.581$). The rates of induction and/or adjuvant therapy with cytotoxic chemotherapy were also similar among the three groups of patients ($p = 0.405$, see Table 1). More patients with *EGFR* mutation had a history of never smoking (48% versus 8% with *KRAS* mutations and 5% with wild type; $p < 0.001$). Patients with *KRAS* mutant tumors presented with later stage disease than those with *EGFR* mutant tumors ($p = 0.031$) and when compared with both *EGFR* mutant and wild-type combined ($p = 0.064$). Eighty-eight percent of patients with *EGFR* mutant adenocarcinomas presented with stage I disease, whereas 73% of patients with wild-type tumors and only 60% of patients with *KRAS* mutant tumors presented with stage I disease.

With a median time to follow-up of 35 months, the median overall survival for patients with *EGFR* mutant, *KRAS* mutant, or wild-type tumors for both genes has not yet been reached. On univariate analysis, patients with *EGFR* mutant tumors had a longer overall survival than patients with *KRAS* mutant tumors ($p = 0.009$, Figure 1) and versus *KRAS*

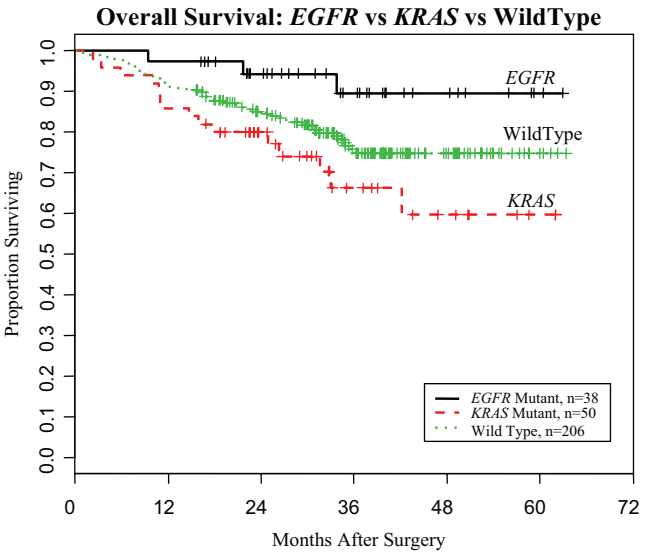

FIGURE 2. Kaplan-Meier survival curves for 294 patients. The median follow-up time is 35 months. The median survival was not reached for any group. Unadjusted $p = 0.031$, stage adjusted $p = 0.18$.

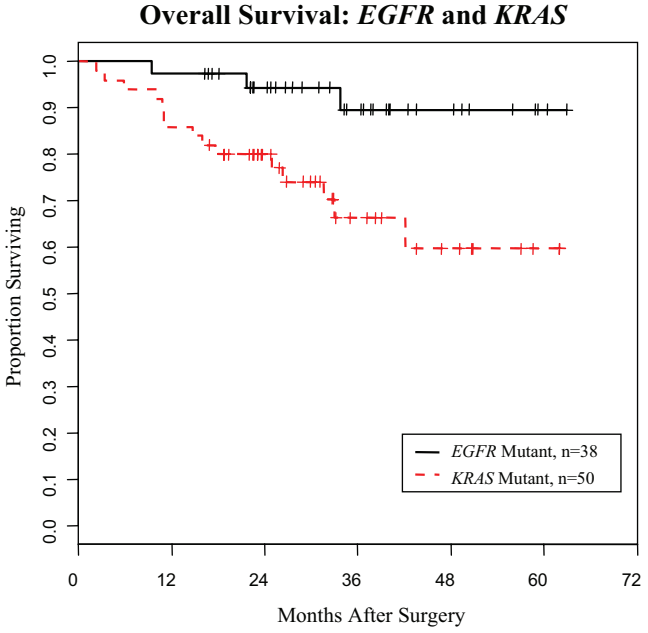

FIGURE 1. Kaplan-Meier survival curves for 88 patients with an *EGFR* or *KRAS* mutant tumor. Unadjusted $p = 0.009$, stage adjusted $p = 0.108$.

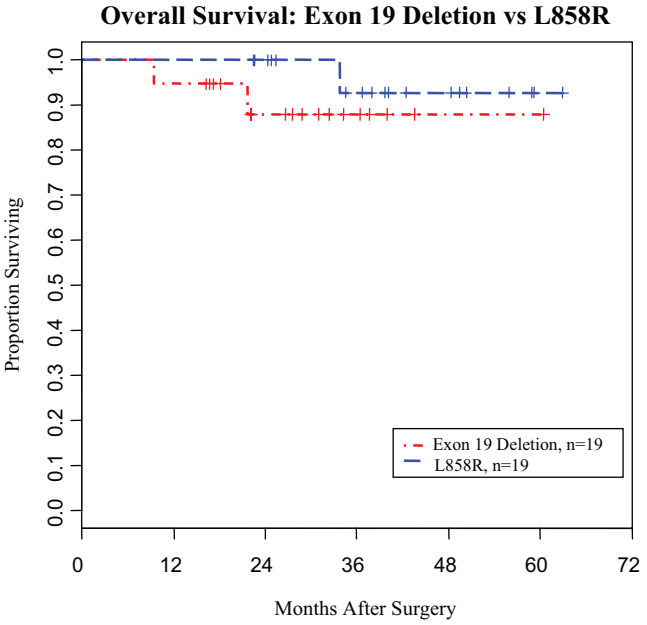

FIGURE 3. Kaplan-Meier survival curves for 38 patients with either L858R mutation or exon 19 deletion in *EGFR*. Unadjusted $p = 0.499$.

mutant tumors and *EGFR/KRAS* wild-type tumors together ($p = 0.031$, Figure 2). The 3-year overall survival rates for patients with *EGFR* mutant, *EGFR/KRAS* wild type, and *KRAS* mutant tumors were 90% (95% CI 70–97%), 76% (95% CI 69–81%), and 66% (95% CI 48–79%), respectively. There was no difference in survival between those patients with *EGFR* L858R versus those with an exon 19 deletion (Figure 3). The overall survival between never and ever smokers was similar (Figure 4).

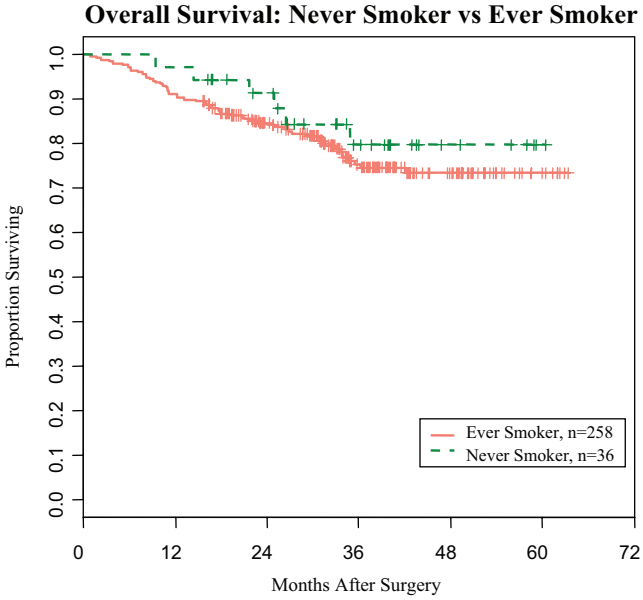

FIGURE 4. Kaplan-Meier survival curves for ever versus never smokers. Unadjusted $p = 0.426$, stage adjusted $p = 0.586$.

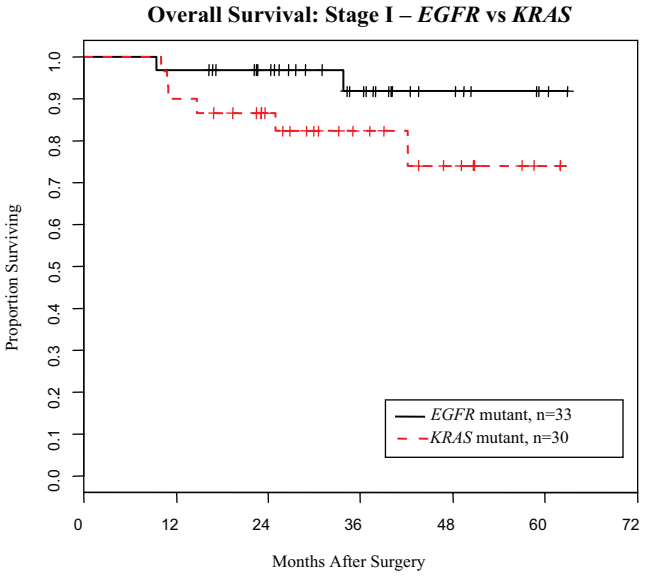

FIGURE 5. Kaplan-Meier survival curves for patients with stage I lung adenocarcinoma: *EGFR* mutant ($n = 33$), *KRAS* mutant ($n = 30$). Unadjusted $p = 0.11$, stage adjusted (IA, IB) $p = 0.147$.

After adjustment for pathologic stage, patients whose tumors harbored *EGFR* mutations displayed a trend toward longer survival when compared with patients whose tumors harbored *KRAS* mutations alone ($p = 0.108$) and those with wild-type tumors ($p = 0.18$, Table 2). For stage I disease only, patients with *EGFR* mutant tumors showed a trend toward longer overall survival compared with patients with *KRAS* mutant tumors, on both univariate analysis ($p = 0.11$) and after stage adjustment ($p = 0.147$) (Figure 5).

TABLE 2. Overall Survival Analysis Results for 294 Patients Before and After Adjusting for Stage

				Unadjusted		Stage Adjusted	
Variable	**Category**	***N***	**3 yr OS Rate% (95% CI)**	**Hazard Ratio (95% CI)**	***p***	**Hazard Ratio (95% CI)**	***p***
Stage	IA	139	89 (82–93%)	Ref. level	<0.001	N/A	
	IB	75	75 (63–84%)	2.9 (1.4–6.0)			
	II	29	51 (28–70%)	6.8 (3.1–14.9)			
	III	51	52 (34–68%)	5.5 (2.8–10.9)			
Gender	F	176	80 (73–86%)	Ref. level	0.009	Ref. level	0.043
	M	118	69 (59–77%)	1.9 (1.2–3.1)		1.7 (1.0–2.7)	
Mutation type	Wild type	206	76 (69–81%)	Ref. level	0.031	Ref. level	0.18
	EGFR	38	90 (70–97%)	0.4 (0.1–1.1)		0.4 (0.1–1.4)	
	KRAS	50	66 (48–79%)	1.6 (0.9–2.9)		1.3 (0.7–2.4)	
	EGFR	38	90 (70–97%)	Ref. level	0.009	Ref. level	0.108
	KRAS	50	66 (48–79%)	4.5 (1.3–15.7)		2.8 (0.8–10.1)	
Smoking history	Never	36	80 (60–91%)	Ref. level	0.426	Ref. level	0.586
	Current/former	258	75 (69–81%)	1.4 (0.6–3.3)		1.3 (0.5–3.0)	

Never smokers defined as having smoked <100 lifetime cigarettes. All *p* values are obtained by either using log-rank test for the unadjusted setting or the stratified log-rank test for the adjusted setting.

OS, overall survival.

DISCUSSION

Among patients with completely resected NSCLC in the United States, 30% of stage I, 60% of stage II, and 75% of stage IIIA patients, respectively, die within 5 years of resection.[17] Adjuvant cisplatin-based chemotherapy has been shown to increase overall survival.[18] A recent meta-analysis from nearly 5000 patients randomized to either surgery followed by cisplatin-based adjuvant chemotherapy or surgery alone demonstrated that higher stage patients garner more benefit from adjuvant chemotherapy.[19] However, there are no data to support adjuvant chemotherapy in patients with stage IA disease, little support for treating stage IB patients, no data for stage II–III patients who are not candidates for cisplatin, and no data for patients more than the age of 75.[20] Clearly, better prognostic and predictive biomarkers are needed to help oncologists decide which patients to treat and which drugs to use.

Many clinical factors (e.g., age, gender, performance status, tumor size, pathologic stage, extent of resection, histologic subtype, etc.) have been shown to have prognostic value in resected NSCLC.[21,22] However, although these characteristics can be used to help decide whether patients should receive adjuvant therapy, none of them predict sensitivity or resistance to specific types of adjuvant treatment. Recently, several studies using mRNA profiling of resected lung tumors have sought further to identify molecular subtypes associated with patient outcome. Gene expression signatures ranging from 5 to 64 genes have shown predictive value.[23–25] However, these studies have identified nonoverlapping gene sets and are not available for widespread use. How many of the individual genes within these profiles that contribute to tumor biology is not known. Moreover, whether any chemotherapy can effectively alter the prognosis defined by a significant molecular signature remains to be established.

In this study, we examined the prognostic significance of two genetic alterations in lung adenocarcinoma—*EGFR* and *KRAS* mutations—that already have known biologic and therapeutic relevance. Both mutations are oncogenic and importantly, *EGFR* mutations are associated with benefit from EGFR TKIs, whereas *KRAS* mutations predict for resistance to these drugs. In patients with metastatic disease, *EGFR* mutations may also have prognostic significance, but the impact of either mutation on overall survival has not yet been well studied in patients with resected disease. Others have examined only the prognostic significance of tumors mutant versus wild type for *EGFR*[8,10] or *KRAS,*[12,26,27] but not in direct comparison with one another.

Our analysis of nearly 300 cases of genotyped lung adenocarcinomas shows that *EGFR* and *KRAS* mutations delineate biologically distinct subsets of lung adenocarcinoma. In the absence of targeted therapy, patients whose tumors contain an *EGFR* mutation had a 90% (95% CI 70–97%) 3-year overall survival compared with a 66% (95% CI 48–79%) 3-year overall survival for patients with *KRAS* mutant tumors. The survival difference approaches significance on multivariate analysis even though 40% of the patients with *KRAS* mutant tumors presented with stage II or greater disease, while 88% of the patients with *EGFR* mutant tumors presented with stage I disease. The 3-year overall survival for stage I patients only was 92% (95% CI 70–98%) for the *EGFR* mutant group ($n = 33$) and 82% (95% CI 62–92%) for the *KRAS* mutant group ($n = 30$).

We observed no difference in survival in patients whose resected tumors harbored the two most common *EGFR* mutations (i.e., the L858R mutant or an exon 19 deletion mutant). By contrast, in the metastatic setting, we previously reported that patients whose tumors harbor exon 19 deletion mutations seem to have a longer overall survival compared with those with the L858R mutation when treated with gefitinib or erlotinib.[16] If validated in other studies, these data suggest that existing EGFR TKIs differentially affect the natural history of patients with *EGFR* mutant tumors.

We also found no difference in survival between never versus ever smokers (Figure 4). Several others have reported that among patients with NSCLC, never smokers have a better overall survival than ever smokers.[28,29] The exclusion of patients with nonadenocarcinoma histology in this study could underlie the discrepant results, as most never smokers who develop lung cancer have tumors with adenocarcinoma histology. The high percentage of females seen at our institution and the limited follow-up of largely patients with stage I disease may also help explain this finding.

This study has several limitations. The data represents a retrospective review of a cohort of patients seen at a single academic institution over a period of 4 years. Our physicians routinely see more women with lung cancer than the national average and this is reflected in our study population. Also, we included only patients with adenocarcinoma histology and those with stage I–III disease who underwent a complete resection. Other histologic types of NSCLC were excluded because the frequency of *EGFR* mutations in nonadenocarcinoma NSCLC is so low (~1%) that such subtypes are not routinely tested for *EGFR* mutations at MSKCC.[30] In the future, we plan to enlarge our sample size and potentially include other histologic subtypes to better delineate the differences that exist between *EGFR* and *KRAS* mutant lung tumors in NSCLC.

Another limitation of the study is sample size. We studied survival rates of only 294 patients. However, our aim was to identify any differences between patients with *EGFR* and *KRAS* mutant tumors, and these sample sizes ($n = 40$ and 50, respectively) are comparable with other North American series.[8,31] Approximately another 600 patients would need to be genotyped and analyzed to validate the survival difference seen here on univariate analysis between those with *EGFR* and *KRAS* mutant tumors.

Because *EGFR* and *KRAS* mutations seem to have both prognostic and predictive significance, we now routinely perform mutation testing on all adenocarcinomas resected at MSKCC. We believe the survival of the subset with *EGFR* mutant tumors could in theory be further extended with EGFR TKIs. Thus, we plan to conduct a single-arm, phase two clinical trial of erlotinib as adjuvant chemotherapy for patients whose tumor harbors an *EGFR* mutation and who have completed standard adjuvant chemotherapy.

Conversely, there is presently no targeted therapy effective for patients with *KRAS* mutant tumors. A recent prospective analysis demonstrated that for this subset of patients, even adjuvant cisplatin-based chemotherapy does not confer a survival advantage.[18] For these individuals, we plan to conduct a clinical trial involving mutant KRAS-specific vaccines after noncisplatin-based chemotherapy.[32]

This study represents the first direct comparison of the survival of resected patients with either *EGFR* or *KRAS* mutant lung adenocarcinomas compared with those that are wild type for both genes, in the absence of targeted therapy. The data indicate further that *EGFR* and *KRAS* mutations define clinically distinct molecular subsets of lung adenocarcinoma. Importantly, because *EGFR* and *KRAS* mutations can predict sensitivity and resistance, respectively, to EGFR TKIs, they are important tumor characteristics that can be readily incorporated into clinical trials to help guide the administration of specific types of adjuvant therapy.

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