Prognostic Impact of *KRAS* Mutation Subtypes in 677 Patients with Metastatic Lung Adenocarcinomas

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Background: We previously demonstrated that patients with metastatic *KRAS* mutant lung cancers have a shorter survival compared with patients with *KRAS* wild-type cancers. Recent reports have suggested different clinical outcomes and distinct activated signaling pathways depending on *KRAS* mutation subtype. To better understand the impact of *KRAS* mutation subtype, we analyzed data from 677 patients with *KRAS* mutant metastatic lung cancer.

Methods: We reviewed all patients with metastatic or recurrent lung cancers found to have *KRAS* mutations over a 6-year time period. We evaluated the associations among *KRAS* mutation type, clinical factors, and overall survival in univariate and multivariate analyses. Any significant findings were validated in an external multi-institution patient dataset.

Results: Among 677 patients with *KRAS* mutant lung cancers (53 at codon 13, 624 at codon 12), there was no difference in overall survival for patients when comparing *KRAS* transition versus transversion mutations ($p = 0.99$), smoking status ($p = 0.33$), or when comparing specific amino acid substitutions ($p = 0.20$). In our dataset, patients with *KRAS* codon 13 mutant tumors ($n = 53$) had shorter overall survival compared with patients with codon 12 mutant tumors ($n = 624$) (1.1 versus 1.3 years, respectively; $p = 0.009$), and the findings were confirmed in a multivariate Cox model controlling for age, sex, and smoking status (hazard ratio: 1.52, 95% confidence interval: 1.11–2.08; $p = 0.008$). In an independent validation set of tumors from 682 patients with stage IV KRAS mutant lung cancers, there was no difference in survival between patients with KRAS codon 13 versus codon 12 mutations (1.0 versus 1.1 years, respectively; $p = 0.41$).

Conclusions: Among individuals with *KRAS* mutant metastatic lung cancers treated with conventional therapy, there are no apparent differences in outcome based on *KRAS* mutation subtype.

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RAS mutations are identified in 25%–30% of lung adenocarcinomas; the vast majority is *KRAS* mutations occurring at codon 12 or 13. In small cohorts, specific point mutations such as G12V and G12R and *KRAS* codon 12 have been associated with trends toward poorer outcomes.[1–3] It is difficult to make definitive conclusions about *KRAS* mutations as a prognostic marker as published studies use differing molecular diagnostic techniques, comparing populations and endpoints.[1–9] Moreover, the discovery of other oncogenes such as epidermal growth factor receptor (*EGFR*) and *ALK* with potent targeted therapy available[10,11] must be taken into account when evaluating the prognostic significance of any biomarker as these discoveries highlight the heterogeneity of the *KRAS* wild-type designation.

Studies report superior survival for never-smokers compared with current or ex-smokers,[12] which may be due to a differing distribution of oncogenic drivers with *KRAS* more frequent in smokers and *EGFR* and *ALK* alterations more common in never smokers.[13] There are no differences in survival between current/former smokers and never smokers when their tumors harbor the same driver oncogene.[13] Transversion mutations refer to the substitution of a purine nucleotide to a pyrimidine, or vice versa. Transitions refer to a purine to purine or pyrimidine to pyrimidine nucleotide change. Within *KRAS*, transversion mutations are more common in current or ex-smokers, and never-smokers have a higher frequency of transition mutations.[14,15] Data with regard to outcomes for *KRAS* transition versus transversion mutations are conflicting.[2,16]

In colorectal cancer, the specific *KRAS* point mutation present may be a prognostic and predictive marker with G12V conveying an increased risk of disease recurrence and death.[17–19] Patients with *KRAS* codon 12 mutant colorectal tumors had shorter overall survival compared with patients with *KRAS* codon 13 mutant tumors.[19] The biologic basis of these findings is not fully understood but may be related to differences in downstream signaling or protein expression.[20,21] In colorectal cancer, *KRAS* mutation status has been validated as a predictive marker of response to *EGFR*-targeted therapies

with the presence of a *KRAS* mutation predicting a lack of response to cetuximab or panitumumab,[22] and newer data raise the possibility that specific *KRAS* point mutations may induce differential responses to *EGFR*-directed therapies.[23]

In lung cancers, the predictive utility of *KRAS* mutations as a marker of response to both targeted therapy and standard cytotoxic chemotherapy has been of great interest (Table 1). The presence of a *KRAS* mutation suggests a lack of response to *EGFR* tyrosine kinase inhibitors,[24,25] but has not been helpful in selecting patients for treatment with *EGFR* monoclonal antibodies.[26,27] In the BATTLE trial, patients with *KRAS* G12C and G12V mutant lung cancers were found to have a shorter progression-free survival than other *KRAS* genotypes with certain targeted therapies such as erlotinib, vandetanib, bexarotene, and sorafenib.[28] Recent data suggest that the specific *KRAS* mutation present may predict response to adjuvant chemotherapy

TABLE 1. *KRAS* as a Predictive Marker

Author	Trial Design	Treatment	% KRAS Mutant	Findings
Rodenhuis et al.[30]	Single arm phase II of patients with metastatic disease	Carboplatin, etoposide, mesna, ifosfamide	26% (n = 16)	*No difference*. Similar OS and PFS among KRAS and KRAS wild-type
Schiller et al.[5]	E4592, completely resected pts randomized postoperative RT alone or with chemo	RT ± chemo	24% (n = 44)	*No difference*. Similar OS and PFS among KRAS and KRAS wild-type
Eberhard et al.[44]	TRIBUTE, untreated patients with metastatic disease randomized to chemo alone or with erlotinib	Carboplatin, paclitaxel ± erlotinib	21% (n = 55)	*No difference*. Similar OS, TTP, and ORR among KRAS and KRAS wild-type
Tsao et al.[4]	JBR.10, completely resected pts randomized to observation or chemo	Cisplatin, vinorelbine	26% (n = 117)	*No apparent benefit with adjuvant chemo in RAS mt (HR: 1.02)*, but chemo, RAS interaction test nonsignificant
Massarelli et al.[45]	Metastatic pts treated with EGFR TKI	Gefitinib or erlotinib	16% (n = 23)	*Nonsignificant association with poor response in RAS mt (p = 0.06)*
Shepherd et al.[29]	ANITA, JBR.10, IALT, CALGB-9633	Platinum-based chemotherapy	19% (n = 300)	*No difference in OS or DFS* when comparing RAS mt and wild-type, possible worse OS with chemo in KRAS codon 13 mt patients
Zhu et al.[46]	BR.21, metastatic pts randomized to erlotinib or placebo	Erlotinib	15% (n = 30)	*Lack of response in RAS mt*, HR 1.67 for RAS mt treated with erlotinib compared with HR 0.69 for RAS wild-type
Douillard[47]	INTEREST, metastatic pts randomized to gefitinib or docetaxel	Gefitinib or docetaxel	18% (n = 49)	*No difference between treatments in RAS mt*

KRAS as a Prognostic Marker

Author	Endpoint	Comparison	Number of Pts	Findings
Slebos et al.[1]	Overall survival, disease-free survival	KRAS wild-type early stage adenocarcinomas	28% (n = 19)	*Shorter survival* (p = 0.002), *DFS* (p = 0.038) in KRAS mt
Keohavong et al.[2]	Overall survival	KRAS wild-type early stage adenocarcinomas	32% (n = 41)	*No difference*, but with KRAS G12C/V having a trend (p = 0.07) toward poorer prognosis compared with wild-type or other KRAS mts
Villaruz et al.[3]	Overall survival, recurrence-free survival	KRAS wild-type predominantly early stage (79%) adenocarcinomas	32% (n = 318)	*No difference* by KRAS point mutation in overall survival (p = 0.612) or RFS (p = 0.089), trend toward better OS for KRAS codon 13 mts (p = 0.052)
Tsao et al.[4]	Overall survival	KRAS wild-type early stage NSCLC	26% (n = 117)	*No difference* in overall survival (p = 0.40)
Schiller et al.[5]	Overall survival, progression-free survival	KRAS wild-type early stage NSCLC	24% (n = 44)	*No difference* in overall survival (p = 0.38) or progression-free survival
Graziano et al.[6]	Overall survival	KRAS wild-type early stage NSCLC	16% (n = 35)	*No difference* in overall survival (p = 0.33)
Johnson et al.[7]	Overall survival	KRAS, EGFR wild-type advanced adenocacinomas	23% (n = 241)	KRAS mutant had *shorter survival* compared with KRAS/EGFR wild-type (p = 0.048)
Mascaux et al.[8]	Overall survival, meta-analysis	Not consistent over studies	28 total studies	KRAS mutants with *shorter survival* (HR: 1.35, 95% CI: 1.16–1.56)

RT, radiotherapy; EGFR, epidermal growth factor receptor; NSCLC, non–small-cell lung cancer; HR, hazard ratio; CI, confidence interval; OS, overall survival; PFS, progression-free survival.

for patients with resected non–small-cell lung cancer. Patients harboring *KRAS* codon 13 mutations appear to have poorer outcomes with adjuvant cisplatin-based chemotherapy.[29] In the metastatic setting, *KRAS* mutations do not appear to independently predict response or resistance to chemotherapy treatments.[30–33]

We hypothesized that with a large number of patients with *KRAS* mutant advanced lung cancers, we would have sufficient power to identify any clinically meaningful differences in outcome related to specific *KRAS* mutation subtypes. To evaluate this hypothesis, we reviewed specific *KRAS* point mutation status, clinical characteristics and survival of patients with metastatic *KRAS* mutant lung cancers identified at our institution and then evaluated key findings in an independent group of patients from other institutions.

METHODS

Patients

Consecutive patients with metastatic or recurrent lung cancers found to have a *KRAS*-mutation by routine molecular testing performed between January 2005 and January 2011 were included in this analysis. An electronic medical record search was used to identify individuals seen at Memorial Sloan-Kettering with a primary tumor diagnosis of lung cancer by ICD-0 code with available diagnostic molecular pathology reports that indicated the presence of a *KRAS* mutation. The list was then manually reviewed to exclude patients who did not have metastatic or recurrent disease or a tumor diagnosis of a primary lung cancer. Data collection was approved by the MSKCC Institutional Review Board/Privacy Board. We collected clinical characteristics and treatment course for all patients. Overall survival was defined as the time from date of advanced disease (stage IV or recurrent) until date of death or last follow-up. *KRAS* mutation analysis was performed before this retrospective review on available tissue by standard Sanger sequencing or by a mass spectrometry based mutation profiling assay.[34,35]

Information on mutation status and outcomes for patients with stage IV *KRAS*-mutant lung cancers identified by routine molecular sequencing during the same time period were collected from Dana Farber Cancer Institute, Massachusetts General Hospital Cancer Center, and Vanderbilt Ingram Cancer Center. This dataset was compiled with the intent to verify any significant findings in our institutional dataset.

Statistical Methods

We compared characteristics of patients with *KRAS* codon 12 and codon 13 mutant tumors using *t* test (for continuous variables) and χ^2 test (for categorical variables). Overall survival following diagnosis of stage IV lung cancer was estimated using Kaplan–Meier methodology. Patients were followed until death; patients alive at the end of the study were censored at the time of the last available follow-up. Univariate group comparisons were performed using log-rank tests. A multivariate Cox proportional hazards model was used to assess the independent effect of *KRAS* mutation type, controlling for potential confounding factors associated with overall survival in univariate analysis. All associations found significant were validated using the external validation set.

RESULTS

Clinical Characteristics

We evaluated tumor specimens from 3357 unique patients with lung cancers for *KRAS* mutations. During that period, 677 patients were identified to have metastatic lung cancer that harbored a *KRAS* mutation. Patient demographics are noted in Table 2. The majority (59%) had chemotherapy treatment details available. Of those with available data, there was no difference in the frequency of platinum-based chemotherapy, pemetrexed and/or bevacizumab among patients with *KRAS* codon 12 versus codon 13 mutations. The median line of treatment in those who received chemotherapy at MSKCC was 2 (range 1–9).

KRAS Mutation Subtype

The most frequent nucleotide change in tumor specimens was a guanine to thymidine (G>T) seen in 366 patients. The prevalence of specific *KRAS* point mutations is summarized in Figure 1. Mutations were found at codon 12 in 624 patients (92%), and codon 13 in 53 patients (8%). Twenty-three percent of patients (157 of 677) had transition mutations (G12D, G12S, G13D, G13S). The prevalence of specific point mutations differed between former/current smokers and never-smokers (Fig. 1). Patients with transition mutations were more likely to be never-smokers, compared with patients with transversion mutations ($p < 0.001$). Clinical characteristics were similar between patients with *KRAS* codon 12 versus 13 mutations (Table 2). No concurrent *EGFR* mutations or *ALK* rearrangements were found in any patients.

KRAS Mutation and Survival

The median follow-up among 197 patients alive at the data cutoff of June 2012 was 17 months (range 1–207 months). The median overall survival for all patients with *KRAS* mutant advanced lung cancers in our cohort was 1.2 years (95% confidence interval [CI]: 1.2–1.4 years). Median overall survival for specific *KRAS* point mutations ranged from 0.7 years (G13C) to 1.5 years (G12F), although no significant

TABLE 2. Patient Characteristics in Original *KRAS* Dataset

Characteristic	Total, *n* = 677	*KRAS* Codon 12, *n* = 624	*KRAS* Codon 13, *n* = 53
Age at diagnosis (stage IV)			
Median	66	66	64
Range	31–89	31–89	44–87
Sex (%)			
Men	260 (38)	240 (38)	20 (38)
Women	417 (62)	384 (62)	33 (62)
Smoking history (%)			
Never-smoker	48 (7)	46 (7)	2 (4)
Former/current smoker	625 (92)	576 (92)	49 (93)
Median pack year	38	35	40
Range	1–245	1–245	7–120
Unknown	4	2	2

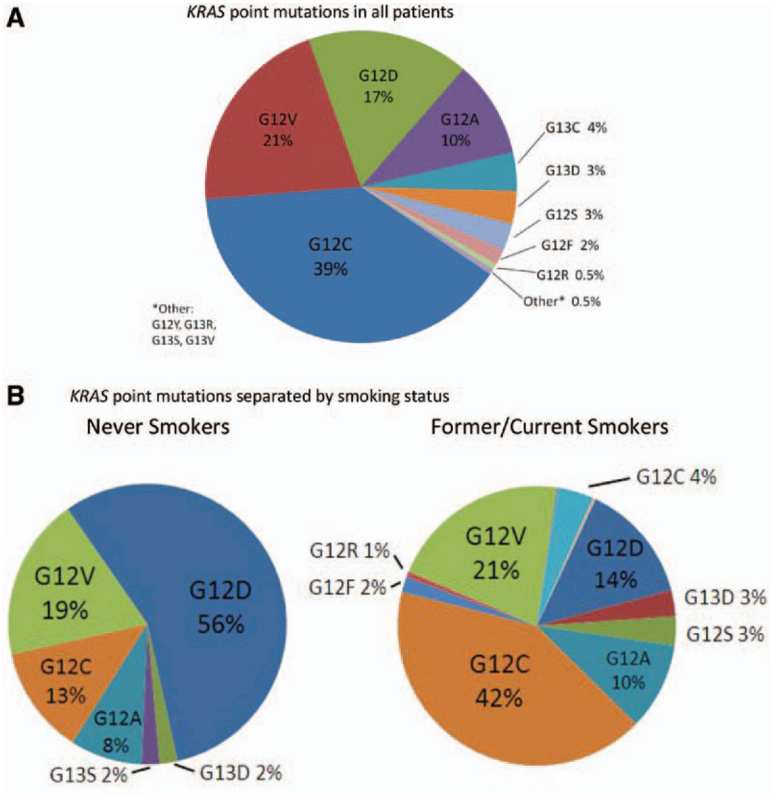

FIGURE 1. *KRAS* point mutations in the original *KRAS* dataset. *A, KRAS* point mutations in all patients. *B, KRAS* point mutations separated by smoking status.

difference in survival was seen when comparing different *KRAS* point mutations (Fig. 2). There was no difference in outcome for patients with *KRAS* transition versus transversion mutations, with a median survival of 1.2 years for both (p = 0.66) (Fig. 3*A*). No difference in overall survival was seen in patients with *KRAS* mutant lung cancers who are current or former-smokers (n = 625) compared with never-smokers (n = 48), median overall survival 1.2 years (95% CI: 1.1–1.4 years) and 1.6 years (95% CI: 0.9–2.6), respectively (p = 0.34; Fig. 3*B*). The median overall survival of patients with G12C/G12V mutant tumors was not different from patients with all other point mutations, 1.2 years for both (p = 0.74; Fig. 3*C*).

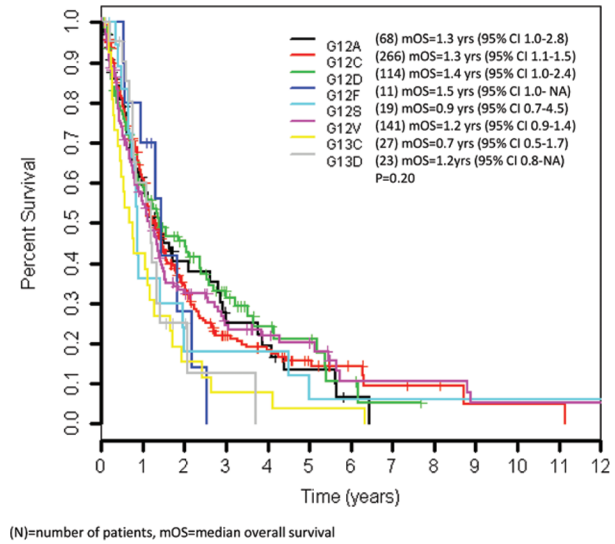

FIGURE 2. Overall survival by different *KRAS* point mutations in the original *KRAS* dataset. *n*, number of patients; mOS, median overall survival.

Patients with *KRAS* codon 13 mutant tumors (n = 53) had inferior survival compared with patients with codon 12 mutant tumors (n = 624), median 1.1 years (95% CI: 0.8–1.3) and 1.3 years (95% CI: 1.1–2.4), respectively (p = 0.008; Fig. 3*D*). When comparing patients with G12D versus G13D mutant tumors, there was no difference in overall survival, 1.4 and 1.2 years, respectively (p = 0.16). Among patients with *KRAS* transition mutations, there is no difference in overall survival when comparing smokers versus never-smokers, 1.2 and 1.4 years, respectively (p = 0.95). Among patients with *KRAS* G12D mutations, there is no difference in outcome when comparing current/former-smokers to never-smokers (p = 0.66).

Multivariate Analysis

We evaluated sex, age, smoking history, and *KRAS* codon to determine impact on survival (Table 3). Sex, age, and *KRAS* codon were associated with survival in univariate analysis. Men had increased risk of death, compared with that of women (hazard ratio [HR]: 1.28, 95% CI: 1.07–1.55; p = 0.008). Older age was significantly associated with increased risk of death, with each five additional years at diagnosis increasing the risk of death by 5% (HR: 1.05 for each added years, 95% CI: 1.01–1.10; p = 0.026). Smoking history did not affect outcome among those with *KRAS* mutations (HR: 1.2, 95% CI: 0.83–1.72; p = 0.34). *KRAS* codon was associated with overall survival, with *KRAS* codon 13 mutant

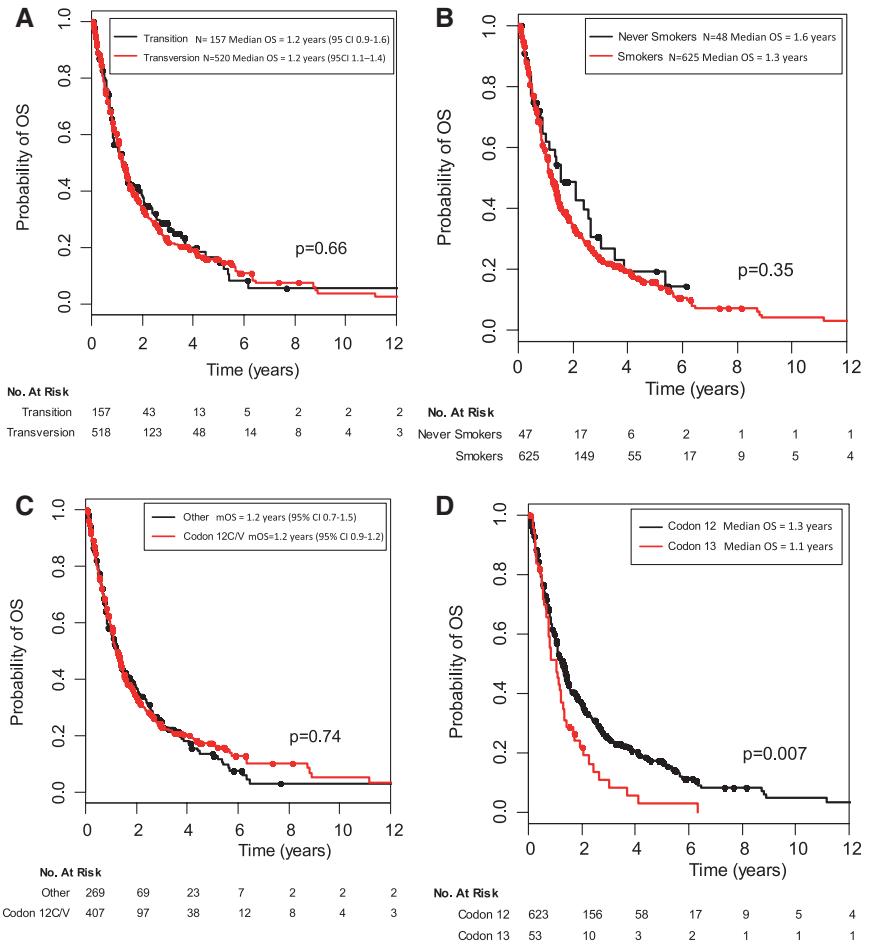

FIGURE 3. Overall survival from diagnosis of stage IV cancer in the original *KRAS* dataset. *A,* Overall survival of transitions versus transversions. *B,* Overall survival of smokers versus never-smokers. *C,* Overall survival of G12C/V versus all others. *D,* Overall survival of codon 12 versus codon 13.

tumors having an increased risk of death, compared with patients with *KRAS* codon 12 (HR: 1.50, 95% CI: 1.11–2.04; $p = 0.009$). In a multivariate analysis controlling for age, sex, and smoking history, *KRAS* codon 13 was associated with shorter overall survival (HR: 1.52, 95% CI: 1.11–2.08; $p = 0.008$; Table 3).

TABLE 3. Univariate and Multivariate Analysis of Original *KRAS* Dataset

Univariate Analysis			
Variable	**Survival (Yrs)**	**HR**	***p* Value**
KRAS (G13 vs. G12)	1.1 vs. 1.3	1.50 (1.11–2.04)	0.009
Sex (M vs. W)	1.0 vs. 1.4	1.28 (1.07–1.54)	0.008
Age		1.05*	0.026
Smoking (Y vs. N)	1.2 vs. 1.6	1.20 (0.83–1.72)	0.34
Multivariate Analysis			
Variable		**HR**	***p* Value**
KRAS (G13 vs. G12)		1.52 (95% CI: 1.11–2.08)	0.008
Sex (M vs. W)		1.29 (95% CI: 1.07–1.55)	0.007
Age		1.01 (95% CI: 1.001–1.02)	0.025
Smoking (Y vs. N)		1.18 (95% CI: 0.82–1.69)	0.39

HR, hazard ratio; M, men; W, women; Y, yes; N, no; CI, confidence interval.

External Validation Set

To verify the survival difference in patients with *KRAS* codon 13 versus codon 12 mutant lung cancer, we used an external validation set consisting of patients with *KRAS* mutant lung cancers treated at other institutions (Table 4). In total, 682 patients were analyzed: 354 patients from Dana Farber Cancer Institute, 242 patients from Massachusetts General Hospital, and 86 patients from Vanderbilt Ingram Cancer Center. In this collected set, there was no difference in overall survival from time of advanced disease in patients with KRAS codon 13 versus codon 12 mutant metastatic lung cancers, 1 year (95% CI: 0.7–1.5 years) versus 1.1 year (95% CI: 0.9–1.2 years), respectively (Fig. 4). Median follow-up was similar in our dataset and the validation dataset. Median overall survival was longer in our dataset compared with the validation set, 15 versus 13 months, respectively ($p = 0.05$).

TABLE 4. Overall Survival by KRAS Subgroup in Original *KRAS* Dataset

Subset	Median Survival (Yrs)	*p* Value
Specific point mutations	0.5–1.5	
Transition vs. transversion	1.2 vs. 1.2	0.66
Smoker vs. never-smoker	1.2 vs. 1.6	0.34
G12C/V vs. other	1.2 vs. 1.2	0.74
KRAS (G13 vs. G12)	1.1 vs. 1.3	0.009
G12D vs. G13D	1.4 vs. 1.2	0.16
Transition: smoker vs. never-smoker	1.2 vs. 1.4	0.95
KRAS G12D: smoker vs. never-smoker		0.66

DISCUSSION

In this series of patients, the largest reported series of patients with *KRAS* mutant lung cancers, we validated clinical prognostic factors known to be relevant in advanced lung cancers, but did not observe any difference in outcomes based on individual *KRAS* genotype (specific point mutation, transition versus transversion, G12 versus 13). Although others have noted that the specific *KRAS* point mutations present is associated with outcomes in smaller series,[2] such differences were not observed in our group of patients. The importance of independent validation of observed prognostic differences is underscored by our identification of a difference in outcomes for *KRAS* G13 versus G12, which was not confirmed in a cohort of similar patients seen at three other cancer centers.

Similar to our findings, Shepherd et al.[29] found that *KRAS* mutation was not prognostic in patients with early stage lung cancer. Other factors besides mutation subtype might be influencing survival. The presence of concurrent mutations may also influence the clinical phenotype seen with different *KRAS* mutation subtypes. *LKB1 (STK11)* and *p53* mutations are seen concurrently with *KRAS* mutations and portend a poorer prognosis in patients, although the prevalence of concurrent mutations has only been assessed in small series.[36–40] Preclinical data indicate that loss of *LKB1* leads to a more aggressive tumor phenotype, with short tumor latency and greater rate of metastasis.[37] The presence of concurrent mutations may also have treatment implications as preclinical data suggest concurrent *LKB1* inactivation alters downstream signaling and sensitivity to mammalian target of rapamycin and MEK inhibition.[41–43] More clearly characterizing the frequency of concurrent mutations and understanding their correlation with clinical behavior will be helpful in further defining the prognosis of patients with *KRAS* mutant lung cancers.

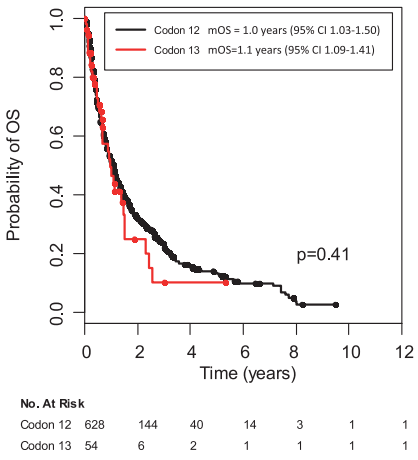

FIGURE 4. Overall survival of codon 12 versus codon 13 in external validation dataset.

There are limitations to our analysis. All patients were identified based upon molecular testing at a single institution during a time (beginning in 2005) in which molecular analysis was not broadly performed, and therefore these patients may not be representative of a general population. They received diverse treatment and the vast majority did not receive any *KRAS*-directed targeted therapy. Patients with different KRAS mutation subtypes may respond differently to chemotherapy, and our analysis was not powered to identify any predictive effects of KRAS mutation subtype. Performance status, a powerful prognostic factor, was not included in our analysis. In addition, even with a larger dataset, we are limited in our ability to make conclusions regarding KRAS mutation subsets that are rare. Due to these limitations, we attempted to validate our findings in an external dataset, in which we did not find a difference in survival when comparing patients with *KRAS* G12 versus G13 mutant tumors. Further validation would be needed to draw definitive conclusions regarding the prognostic value of *KRAS* mutation subtype.

KRAS mutation subtype does not appear to be associated with overall survival from the diagnosis of lung cancer. Investigation into other areas such as variable gene expression and identifying concurrent mutations may identify potential molecular prognostic markers in patients with *KRAS* mutant lung cancers.

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