KRAS Mutations in Advanced Nonsquamous Non–Small-Cell Lung Cancer Patients Treated with First-Line Platinum-Based Chemotherapy Have No Predictive Value

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Background: Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutation is thought to be related with dismal outcome for non–small-cell lung cancer (NSCLC) patients. The role of *KRAS* mutation as a predictor of response to chemotherapy for patients with metastatic NSCLC is poorly understood.

Methods: From a retrospective database of two university hospitals, all patients with advanced, nonsquamous NSCLC treated with first-line platinum-containing chemotherapy were selected. Mutation analysis for *KRAS* was performed and the relation with response to chemotherapy was assessed. Secondary endpoints were its relation with response to progression-free survival (PFS) and overall survival (OS).

Results: A total of 161 patients, 94 men and 67 women, were included in this study. Median age was 60 years. The majority of patients (79%) had stage IV disease, of which 60 patients (37%) had a *KRAS* mutation. Patients with a *KRAS* mutation had a similar response to treatment as patients with *KRAS* wild-type (wt) ($p = 0.77$). Median PFS in *KRAS*-mutated patients was 4.0 months versus 4.5 months in *KRAS* wt patients (hazard ratio = 1.3; [95% confidence interval, 0.9–1.8]; $p = 0.16$). Median OS in patients with *KRAS* mutation was 7.0 months versus 9.3 months in patients with *KRAS* wt (hazard ratio = 1.2; [95% confidence interval, 0.9–1.7]; $p = 0.25$). Type of *KRAS* mutation had no influence on response or outcome.

Conclusion: On the basis of our multicenter data presented here, we conclude that *KRAS* mutation is not predictive for worse response to chemotherapy, PFS, and OS in advanced NSCLC patients treated with platinum-based chemotherapy in first-line setting.

Key Words: Non–small-cell lung cancer, Kirsten rat sarcoma viral oncogene homolog mutation, Predictive biomarker, Prognostic biomarker.

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Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutation is the most common driver mutation in non–small-cell lung cancer (NSCLC) and is present in approximately 30% of all NSCLC patients, mainly in patients with adenocarcinoma (AC) histology. Other mutations in rat sarcoma viral oncogene homolog (*RAS*) gene family include mutations in Harvey rat sarcoma viral oncogene homolog (*HRAS*) and neuroblastoma rat sarcoma viral oncogene homolg (*NRAS*) but these are observed in less than 10% of the *RAS* mutations in NSCLC. RAS proteins circulate signals from growth receptors on the cell surface to intracellular effector pathways responsible for cell growth and proliferation. Mutations most often occur in codons 12, 13, or 61 of the *RAS* gene (located on chromosome 12) and result in an irreversible continuous activation of RAS protein.[1–3] Because of the critical role of RAS protein in cell proliferation, NSCLC patients with a *KRAS* mutation are believed to have a worse prognosis as compared with patients with a *KRAS* wild type (wt). The relation between *KRAS* mutational status and survival was first reported in 1990.[4] Since then, a variety of studies have investigated the influence of *KRAS* mutational status on survival, however, even today, conflicting results are reported.

It is believed that patients with a *KRAS* mutation do not respond to chemotherapy treatment, although at this moment, chemotherapy is the only treatment option for these patients.[5] In contrast, successful targeted agents are developed for patients with an *EGFR* mutation and EML4-ALK translocation.[6,7] Few studies have investigated the effect of *KRAS* mutation on response to first-line chemotherapy in patients with advanced NSCLC.[8–10] These studies conclude that patients with a *KRAS* mutation do not have worse response to chemotherapy treatment. Though, because of the small sample size and differences in type of chemotherapy used in these studies, this finding remains open to debate. The aim of our retrospective study is to evaluate the association of *KRAS* mutational status with response to chemotherapy, progression-free survival (PFS), and overall survival (OS) in patients with advanced NSCLC treated with platinum-based chemotherapy as first-line treatment.

PATIENTS AND METHODS

Study Patients

We retrospectively selected all consecutive nonsquamous (p63-negative) NSCLC patients by searching pathology reports

in two university hospitals from 2004 to January 2011. Patients were eligible for the study on the basis of the following criteria: proven incurable stage IIIb or IV (tumor, node, metastasis [TNM] 6th edition) NSCLC, treatment with first-line platinum-containing chemotherapy, availability of tumor material for *KRAS* mutation analysis, at least one target lesion according to Response Evaluation Criteria in Solid Tumours 1.0, and evaluable computed tomography before and after treatment. Palliative radiotherapy during chemotherapy treatment was allowed. The following data were retrieved from the medical records: age, sex, smoking history, World Health Organisation performance status (PS), histology (including p63 staining results), stage of disease (Union for International Cancer Control [UICC] 6th edition), chemotherapy treatment, number of courses, response to treatment according to the Response Evaluation Criteria in Solid Tumours 1.0, date of start of treatment, date of progression, and date of death or date of last contact.

Ethics Statement

This study was approved by the ethics committee of the VU University Medical Center Amsterdam; according to our local rules, informed consent was not needed for this retrospective study.

Immunohistochemistry

To distinguish between AC and squamous cell carcinoma and thyroid transcription factor 1 (TTF1) and p63, immunohistochemistry staining was performed on all patients, according to a previously described protocol.[11] For TTF1 clones 8G7G3/1 were used and for p63 clones 4A4 were used. A single observer scored the stained slides for intensity (0 = negative, 1 = weak, 2 = moderate, and 3 = strongly positive) and percentage of positive tumor cells with 10% increments. For each immunohistochemical stain, a total score was obtained by multiplying intensity and percentage of positive tumor cells. A score more than 240 was considered positive for p63. This threshold was chosen because it clearly delineates squamous cell carcinoma from AC differentiation.[12] For TTF1 a positive threshold of more than 30 was used, as even minor staining is associated with AC differentiation.

Mutation Analyses

Tumor tissue was manually macrodissected from serial sections guided by a hematoxylin-eosin–stained tissue section, on which the tumor area was marked by a pathologist. DNA was extracted and subjected to high-resolution melting and sequencing analysis for KRAS exon 2 and 3, according to routine protocol.[13,14]

Statistical Analysis

The primary objective was to evaluate the difference in probability of response between patients with *KRAS* wt and *KRAS* mutation. Secondary objectives were PFS, defined as time from start of treatment till objective disease progression or death, and OS defined as the time from start of treatment till death. The relationship between *KRAS* mutational status and patient characteristics, type of chemotherapy treatment, or response was calculated using Pearson's χ^2 test. Kaplan–Meier curve was used to estimate the distribution of survival according to *KRAS* mutational status. Log-rank test was used to calculate difference in survival among the subgroups. To estimate the hazard ratio (HR), Cox regression analysis was used. In addition, analysis was performed for different types of *KRAS* mutation. For the latter analysis, types of *KRAS* mutations that had a frequency of less than 5 were clustered in one group.

RESULTS

From the retrospective databases, 161 patients were eligible for the study. The patient characteristics are listed in Table 1. The median age was 60 years (range, 34–83 years), 17 patients (10.3%) had a PS of more than 1. The majority of patients had stage IV disease (79%). A total of 115 patients (71.4%) had an AC, 46 patients (29.6%) were diagnosed with NSCLC not otherwise specified favoring AC.

Treatment

According to the inclusion criteria, all patients received a platinum combination. Eighty-nine patients were treated with cisplatin, 69 patients were treated with carboplatin, and three patients received both regimens. Platinum was most frequently combined with gemcitabine (51.6%). Other partners were pemetrexed (31.1%), docetaxel (16.8%), and vinorelbine (0.6%). The median amount of chemotherapy courses was four. In total, there was one patient (0.6%) with a complete remission, 31 patients (19.3%) had a partial response (PR), 77 patients (47.8%) had a stable disease (SD), and 52 patients (30.7%) had progressive disease (PD).

KRAS Mutation

KRAS mutations were present in 60 patients (37.3%), of which the majority of patients had stage IV disease (85.0%). There was no significant relationship among patient characteristics as listed in Table 1 and *KRAS* mutational status. Also, there were no differences in treatment among patients with or without a *KRAS* mutation ($p = 0.90$) or in amount of courses administered ($p = 0.31$). For *KRAS* wt patients, platinum doublet chemotherapy resulted in 1 complete remission (1.0%), 21 PRs (20.8%), 48 SDs (47.5%), and 31 PDs (30.7%). Responses in the *KRAS*-mutated group included 10 PRs (16.7%), 29 SDs (48.3%), and 21 PDs (35.0%; $p = 0.77$) (Fig. 1).

The different types of mutation are presented in Figure 2. The most frequent mutations observed were: p.G12C (41.7%), p.G12V (16.7%), p.G12D (11.7%), and p.G13C (10.0%). There were no differences between type of mutation and response to therapy ($p = 0.98$).

PFS and OS

The median follow-up was 48 months. Date of death was assessed on October 1, 2011. Median PFS in *KRAS*-mutated patients was 4.0 months (95% CI, 2.8–5.2 months) versus 4.7 months (95% CI, 3.2–6.1 months) in *KRAS* wt patients (HR = 1.3; [95% CI, 0.9–1.8]; $p = 0.12$) (Fig. 3*A*). Median OS in patients with *KRAS* mutation was 7.0 months (95% CI, 3.9–10.2) versus 9.3 months (95% CI, 6.6–11.9) in patients with *KRAS* wt (HR = 1.2; [95% CI, 0.9–1.7]; $p = 0.25$) (Fig. 3*B*). Classic prognostic factors, such as stage

TABLE 1. Patient Characteristics

	KRAS Wild-Type		*KRAS* Mutation		
	n	**%**	***n***	**%**	**Sign ($p < 0.05$)**
Median, age (range, yr)	61	(34–80)	58	(35–83)	0.61
Sex					0.33
M	56	55.4	38	63.3	—
F	45	44.6	22	36.7	—
Smoking history					0.09
Never	12	13.5	2	3.5	—
Former	41	46.1	25	43.9	—
Current	36	40.4	30	52.6	—
WHO PS					0.61
0	43	45.7	23	40.4	—
1	40	42.6	28	49.1	—
2	9	9.6	6	10.5	—
3	2	2.1	0	0.0	—
Histology					0.68
Adenocarcinoma	71	70.3	44	73.3	—
NSCLC-NOS	30	29.7	16	26.7	—
Stage					0.18
IIIb	24	23.8	9	15.0	—
IV	77	76.2	51	85.0	—
Platinum regimen					0.91
Carboplatin	2	2.0	1	1.7	—
Cisplatin	42	41.6	27	45.0	—
Both	57	56.4	32	53.3	—
Chemotherapy regimen					0.90
Gemcitabine	52	51.5	31	51.7	—
Pemetrexed	31	30.7	19	31.7	—
Docetaxel	17	16.8%	10	16.7	—
Vinorelbine	1	1.0	0	0.0	—

WHO PS, World Health Organisation performance status; NSCLC-NOS, non–small-cell lung cancer not otherwise specified.

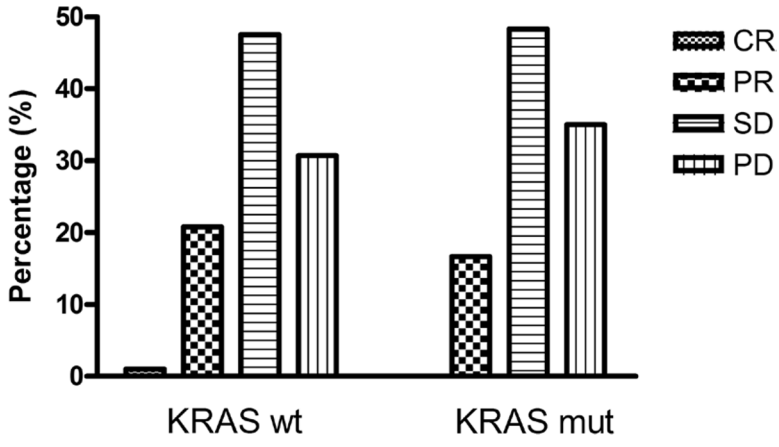

FIGURE 1. Histogram of response (in %) in patients with *KRAS* wt or *KRAS* mut. CR, complete remission; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; mut, mutation; PD, progressive disease; PR, partial response; SD, stable disease; wt, wild type.

(HR = 1.6; [95% CI, 1.0–2.5]), histology (AC versus large cell; HR = 1.6; [95% CI, 1.1–2.3]), and PS (HR = 1.5; [95% CI, 1.2–2.0]) were found to be prognostic factors. The 1-year survival was 36.7 and 46.1% in *KRAS*-mutated and *KRAS*-wt patients, respectively. The PFS and OS were comparable in the different types of *KRAS* mutation ($p = 0.90$ and $p = 0.99$, respectively).

DISCUSSION

In this retrospective study of a consecutive cohort of patients, we observed no differences in response to first-line platinum-based chemotherapy treatment in advanced NSCLC patients with or without a *KRAS* mutation. Although median OS and the 1-year survival rate was worse in patients

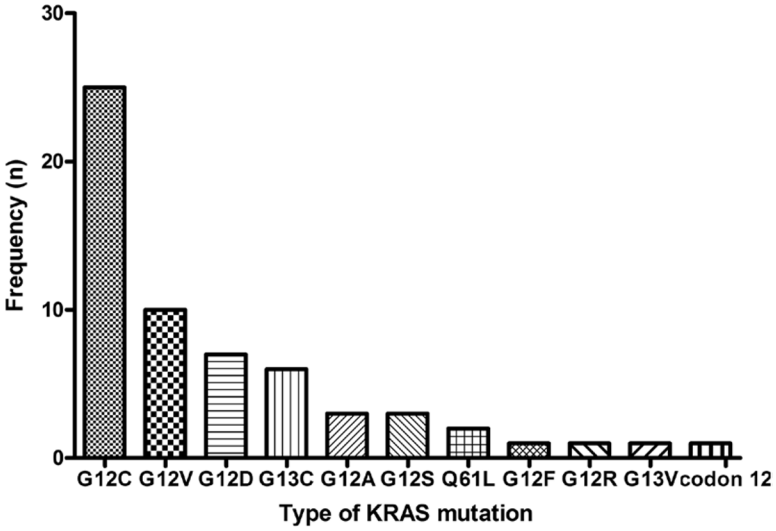

FIGURE 2. Histogram of type of *KRAS* mutation. *KRAS*, Kirsten rat sarcoma viral oncogene homolog.

harboring a *KRAS* mutation, this was not statistically significantly different.

The aggressive behavior of *KRAS* mutation in NSCLC patients was first suggested by Slebos et al.[4] in 1990, who reported worse survival of *KRAS*-mutated NSCLC patients. The hypothesis is provided by the function and downstream effectors of the RAS protein family, including the mitogen-activated protein kinase (MAPK) pathway. An activating *KRAS* mutation could, therefore, result in continuous stimulation of tumor proliferation, resulting in early progression and poor survival. In accordance with other studies, we found that response and PFS were not significantly worse in advanced NSCLC patients with a *KRAS* mutation. (Table 2). In a prospective trial, 62 patients with inoperable stage III or IV NSCLC were treated with mesna, ifosfamide, carboplatin, and etoposide. Sixteen patients (25.8%) had a *KRAS* mutation. In three of 16 patients (19%) with a *KRAS* mutation there was response to treatment, compared with a response rate of 26% in *KRAS*-wt patients. This difference in response was not significant ($p = 0.49$). The PFS and OS were not significantly different in patients with *KRAS* wt and *KRAS* mutation.[8] Another study evaluated mutational status and response to treatment using data of the TRIBUTE trial. This was a randomized phase III study in advanced NSCLC, comparing first-line chemotherapy with first-line chemotherapy with concurrent erlotinib. Of 274 patients, 264 had tumor material available for *KRAS*-mutation analysis. In 55 patients (21%) a *KRAS* mutation was present. No differences in response were seen between *KRAS*-mutated patients treated in the erlotinib-containing arm and the chemotherapy-only arm (8% versus 23%, respectively; $p = 0.16$). Also, no differences in PFS and OS was observed in patients either with, or without a *KRAS* mutation.[9] Recently, a study evaluated clinical outcome in advanced NSCLC patients receiving first-line chemotherapy according to *EGFR* and *KRAS* mutational status. In this study, 162 patients were treated with first-line chemotherapy. Thirty of 133 patients (22.6%) had a *KRAS* mutation. No difference in response to chemotherapy was found between patients with *KRAS* mutation or *KRAS* wt (25.0% versus 26.5%, respectively;

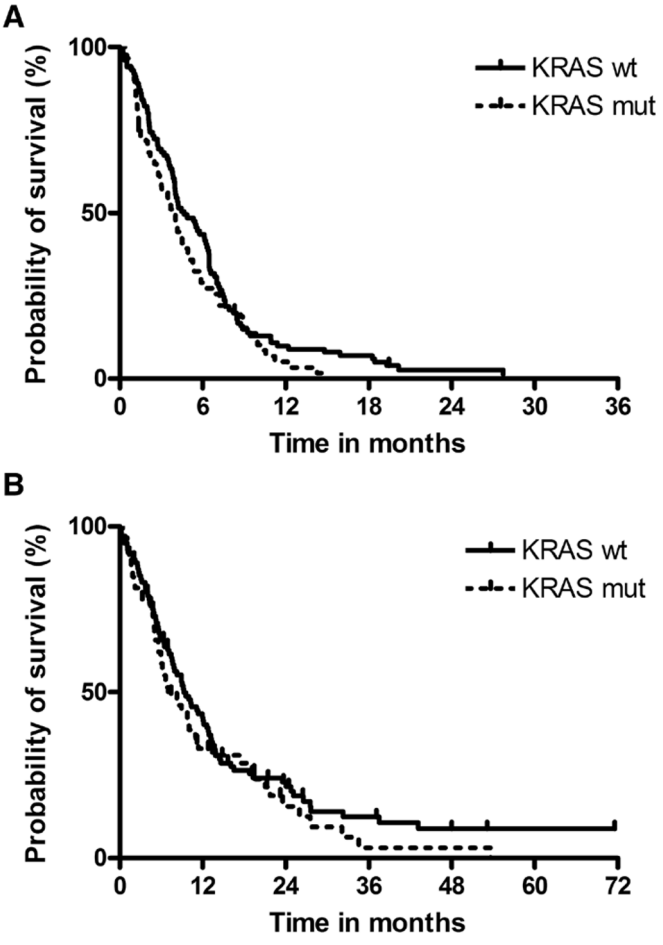

FIGURE 3. *A*, PFS in months. Continuous line *KRAS* wt (median PFS 4.7 months), dashed line *KRAS* mut (median PFS 4.0 months). HR = 1.3 (95% CI, 0.9–1.8). *B*, OS in months. Continuous line *KRAS* wt (median OS 9.3 months), dashed line *KRAS* mut (median OS 7.0 months). HR = 1.2 (95% CI, 0.9–1.7). CI, confidence interval; HR, hazard ratio; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; mut, mutation; os, overall survival; PFS, progression-free survival; wt, wild type.

TABLE 2 Predictive Value of *KRAS* Mutational Status

Study	Histology	Type of Chemotherapy	No. of Patients[a]	Response			Predictive
				KRAS Mut (%)	*KRAS* Mut (%)	*KRAS* Wt (%)	
Rodenhuis et al. 1997[8]	Adenocarcinoma	Mesna, ifosfamide, carboplatin, and etoposide	62	26	19	26	No
Eberhard et al. 2005[9]	All	Carbo/paclitaxel ± erlotinib	264	21	23	26	No
Kalikaki et al. 2010[10]	All	Several	133	23	25	27	No

[a]Number of patients eligible for *KRAS* mutation analysis.
Wt, wild type; Mut, mutation; *KRAS*, Kirsten rat sarcoma viral oncogene homolog.

$p = 0.87$). In patients treated with platinum-based chemotherapy (96 patients, of which 18 patients were with a *KRAS* mutation) there were also no differences in response (29.2% versus 30.2%, respectively; $p = 0.95$). The PFS and OS was comparable in patients with or without a *KRAS* mutation.[10]

A variety of studies have investigated the prognostic role of *KRAS* mutational status. Unfortunately, these studies were equivocal because of differences in patient selection, stage of disease, histology, and type of treatment. In the early 1990s, *KRAS* mutation was reported to be a poor prognostic factor in early-stage NSCLC patients who underwent surgery.[4,15,16] However, in subgroup analysis of prospective studies in NSCLC, *KRAS* mutational status was not found to be prognostic.[17,18] In a meta-analysis on the prognostic role of *KRAS* mutational status in NSCLC, reviewing 53 studies and 5216 patients, *KRAS* mutational status was found to be a poor prognostic factor. Patients with a *KRAS* mutation had a worse survival with an HR of 1.40 (95% CI, 1.18–1.65).[19] Unfortunately, a multivariate analysis with prognostic factors such as PS and stage of disease was not performed.

A post hoc power analysis for response rate and 1-year survival rate (SAS 9.2; $\beta = 0.9$; $\alpha = 0.05$; weight is 3 for *KRAS*-mutated patients and 5 for *KRAS* wt) demonstrated that with our sample size, a reduction of 20% in 1-year survival could be detected with a power of 0.80. Prospective studies should be conducted to answer the question whether *KRAS* mutational status has prognostic or predictive value. However, based on the studies discussed above and our findings, *KRAS* mutational status does not seem to have a clinically relevant impact on PFS and OS. Therefore, the value of *KRAS* mutational status in NSCLC as predictor of poor outcome has to be reviewed.

A very interesting hypothesis is that different types of *KRAS* mutation are associated with different responses to chemotherapy regimens. Garassino et al.[20] described this in a study on *KRAS*-mutated NSCLC cell lines. Differences were found in three types of *KRAS* mutation: p.G12C, p.G12V, and p.G12D. In comparison with the WT clones, p.G12C mutation was associated with a reduced response to cisplatin, but increased sensitivity to taxol and pemetrexed, whereas p.G12V mutation showed a strong sensitivity to cisplatin, less sensitivity to pemetrexed. Cell lines harboring a p.G12D mutation showed resistance to taxol, but sensitivity to sorafenib. Another recently published study reported poor survival in patients with *KRAS* p.G12C and p.G12V, treated with molecular targeted therapy compared with other types of *KRAS* mutation.[21] This study used data of the Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination (BATTLE) trial, in which a total of 255 patients were randomized to erlotinib, vandetanib, bexarotene, and erlotinib or sorafenib.[22] A total of 43 patients had a *KRAS* mutation; of these, 24 patients had a p.G12C or p.G12V mutation. *KRAS* mutational status was not associated with OS or PFS ($p = 0.09$). When *KRAS* mutations were clustered in p.G12C + p.G12V and others, there was a significant difference in median PFS when compared with *KRAS* wt (1.8, 3.4, and 2.0 months, respectively; $p = 0.046$). In our patient population, we could not confirm these data, but the sample size was probably too small to find relevant differences between the types of *KRAS* mutation. We encourage further study on type of *KRAS* mutation and its relation to response to chemotherapy and prognosis in a larger cohort of patients.

CONCLUSION

On the basis of our multicenter data presented here, we conclude that *KRAS* mutational status is not likely to be predictive for worse response or PFS in advanced nonsquamous NSCLC patients treated with platinum-based chemotherapy in first-line setting.

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