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Prognostic value of the *KRAS* G12V mutation in 841 surgically resected Caucasian lung adenocarcinoma cases

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Background: Identifying patients who will experience lung cancer recurrence after surgery remains a challenge. We aimed to evaluate whether mutant forms of epidermal growth factor receptor (*EGFR*) and Kirsten rat sarcoma viral oncogene homolog (*KRAS*) (*mEGFR* and *mKRAS*) are useful biomarkers in resected non-small cell lung cancer (NSCLC).

Methods: We retrospectively reviewed data from 841 patients who underwent surgery and molecular testing for NSCLC between 2007 and 2012.

Results: *mEGFR* was observed in 103 patients (12.2%), and *mKRAS* in 265 (31.5%). The median overall survival (OS) and time to recurrence (TTR) were significantly lower for *mKRAS* (OS: 43 months; TTR: 19 months) compared with *mEGFR* (OS: 67 months; TTR: 24 months) and wild-type patients (OS: 55 months; disease-free survival (DFS): 24 months). Patients with *KRAS* G12V exhibited worse OS and TTR compared with the entire cohort (OS: *KRAS* G12V: 26 months *vs* Cohort: 60 months; DFS: *KRAS* G12V: 15 months *vs* Cohort: 24 months). These results were confirmed using multivariate analyses (non-G12V status, hazard ratio (HR): 0.43 (confidence interval: 0.28–0.65), $P<0.0001$ for OS; HR: 0.67 (0.48–0.92), $P=0.01$ for TTR). Risk of recurrence was significantly lower for non-*KRAS* G12V (HR: 0.01, (0.001–0.08), $P<0.0001$).

Conclusions: *mKRAS* and *mEGFR* may predict survival and recurrence in early stages of NSCLC. Patients with *KRAS* G12V exhibited worse OS and higher recurrence incidences.

Lung cancer is the leading cause of cancer-related mortality worldwide. Non-small cell lung cancer (NSCLC) accounts for ~80% of all lung cancer cases (Jemal *et al*, 2011). The 5-year-overall survival (OS) rate across all stages is <15% (Bossard *et al*, 2007). Even stage IA and IB NSCLC patients, for whom surgery is the cornerstone of treatment, exhibit a relatively poor prognosis, with 27% to 42% of these patients, respectively, dying within 5 years (Izar *et al*, 2014), primarily due to recurrence. The identification of patients who may experience recurrence after surgery remains a challenge. The TNM staging indicates the level

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of disease progression and the malignant potential of primary lung cancer. However, even patients with disease at the same stage exhibit wide variations in their incidence of recurrence after curative resection. Consequently, the current TNM staging based on clinical and pathological findings may have reached the limits of its usefulness (Uramoto and Tanaka, 2014).

Recent years have witnessed an increased understanding of the molecular alterations in tumours. Notably, NSCLC classification shifted from histological subtypes towards molecular oncogenic alterations. The main genomic alterations observed in NSCLC adenocarcinomas are epidermal growth factor receptor (EGFR) mutations, which occur in 10–20% of Caucasian patients, *V-Ki-ras2* Kirsten rat sarcoma viral oncogene homolog (*KRAS*) oncogene, which is observed in 20–35% of patients, and anaplastic large cell lymphoma (ALK) gene fusion, which is observed in 5% of patients (Zhang *et al*, 2014).

The published data on the prognostic value of these mutations in resected NSCLC are contradictory. A recent meta-analysis concluded an absence of impact of *EGFR* mutation on OS and disease-free survival (DFS) in resected NSCLC (Zhang *et al*, 2014). However, the study included a heterogeneous set of patients (primarily Asian) whose diagnoses ranged from stages I to IIIB, limiting the interpretation of these results. Another meta-analysis indicated a reduced OS in cases of KRAS mutation (Meng *et al*, 2013). However, different methods of *KRAS* mutation detection and treatment regimens were considered. Recent publications have demonstrated that different *KRAS* mutations may be classified into KRAS-dependent and KRAS-independent groups (Singh *et al*, 2009) and that the type of amino-acid substitution leads to differential binding affinity for downstream effector molecules. These results suggest that specific amino-acid substitutions are associated with different outcomes. However, the clinical data on resected lung NSCLC are poor and contradictory (Izar *et al*, 2014; Nadal *et al*, 2014).

We evaluate the prognostic value of *EGFR* mutations (*mEGFR*) and *KRAS* mutations (*mKRAS*) with regard to specific amino-acid substitutions in 841 surgically treated French patients.

PATIENTS AND METHODS

The ethics committee of the French Society of Thoracic and Cardiovascular Surgeons approved this study (approval number: *2015-5-13-10-21-57-ReSt)*.

We retrospectively reviewed the data from 1971 patients who underwent molecular testing for *KRAS* and *EGFR* between January 2007 and December 2012 at the Molecular Biology Department of Strasbourg University Hospital (Strasbourg, France). Our study included 841 patients who underwent surgical resection with curative intent.

Molecular analysis. Samples were obtained from primary lung tumours. DNA was extracted from formalin-fixed paraffin-embedded tumour samples, and *EGFR*/*KRAS* analysis was performed at exons 18–21 for *EGFR* and codon 12 out of 13 for *KRAS*, as previously described.(Beau-Faller *et al*, 2009; Beau-Faller *et al*, 2013) Wild-type (WT) patients were defined as patients who harboured no m*EGFR* or m*KRAS*. More recently, patient samples were tested for BRAF, PI3KCA, HER2 and ALK mutations.

Covariates and data collection. Baseline patient characteristics were collected, including age, sex, smoking history, neo-adjuvant and adjuvant treatment. The Charlson comorbidity index (CCI) was calculated for each patient. We grouped patients into the following established categories according to their total score: (Charlson *et al*, 1987) 0 (no comorbidity); 1–2 (average); 3–4 (moderate); and ≥ 5 (severe). Smoking status was characterised as never a smoker, <100 cigarettes in their lifetime, a former smoker, quit >1 year before diagnosis and a current smoker with an ongoing smoking habit or who quit <1 year before diagnosis.

Pre-operative staging was performed using computed tomography (CT) scans of the chest, brain and upper abdomen, coupled with whole-body 18-fluorodeoxyglucose positron emission tomography and fibre optic bronchoscopy. Treatment decisions were made by a multidisciplinary board in the presence of a certified thoracic surgeon and a certified onco-pneumologist and radiation oncologist. Neo-adjuvant treatment consisted of chemotherapy either alone or in combination with radiation therapy (RT). All chemotherapy regimens were platinum-based. Some patients were referred to our facility by physicians from different centres. Therefore, no uniform protocol of neo-adjuvant therapy was used. Tumour stage was categorised according to the American Joint Committee on Cancer Staging Manual version 7. Dates and types of surgeries were recorded. Appropriate anatomical resections and systematic radical mediastinal lymphadenectomy were performed in accordance with the recommendations of the French Society of Thoracic and Cardiovascular surgeons (Thomas *et al*, 2008). Histopathological baseline characteristics, namely, angioinvasion, R0/R1/R2 and the number of N2 stations involved, were included. Skip metastases were defined as N2 involvement without N1. Microscopic N2 was defined as nodal metastases ranging from 0.2 to 2 mm. Adjuvant chemotherapy consisted of platinum-based treatments, and RT was performed after CT-based three-dimensional treatment planning with a linear accelerator. The target volume included the area of loco-regional lymph nodes plus a margin of 2 cm. The dose per fraction was 2 Gray (Gy), given once daily, 5 days per week, up to a total dose of 60 Gy for R0 patients without extracapsular spread (ECS) or 66 Gy in cases of incomplete resection and/or ECS.

Patients were assessed for local and distant recurrence (DR), TTR and OS. The date of recurrence was defined as the first radiographic evidence of cancer relapse on imaging and/or pathological tumour evidence on biopsy. TKI was administered as a first-line treatment for recurrence in *EGFR*-mutated patients. The TTR was defined as time from surgery until the first diagnosis of recurrence on imaging or biopsy specimens. OS was defined as the time elapsed between surgery and either death or the last follow-up.

Statistical analyses. IBM SPSS (Armonk, NY, USA) v.20 was used for statistical analyses. Comparisons between groups were performed using χ^2, medians, or Fisher's or Student's *t*-tests where appropriate. Correlations between qualitative variables were assessed using Cramer's V. The prognostic influence of variables on OS and DFS was assessed using the log-rank test and Cox proportional hazards models, and the influence of each variable on recurrence was assessed using a step-wise binary logistic regression. All tests were two-sided, and variables were considered significant for P values <0.05. All variables with P values <0.2 were tested in multivariate analyses.

RESULTS

Population characteristics. Our population was primarily male (61.9%). The mean age at the time of diagnosis was 63.39 years (± 11.52). The median follow-up time was 39 months (min, 8; max, 80). Molecular analyses revealed 265 *mKRAS* patients (31.5%), 103 *mEGFR* patients (12.2%) and 473 WT patients (56.3%). *mEGFR* status correlated with female sex (Cramer's V: 0.26, $P<0.0001$) and non-smoking status (Cramer's V: 0.64, $P<0.0001$), and *mKRAS* status correlated with smoking status (Cramer's V: 0.1, $P=0.02$). Significantly more skip N and microscopic N types were observed in *mEGFR* patients ($P<0.0001$ for both). *mKRAS* patients exhibited significantly more pN+ ($P<0.0001$), involvement of two N2 stations

($P=0.004$), angioinvasion ($P<0.0001$) and the use of adjuvant treatment. There were more pT4 in WT patients ($P=0.002$). There were no differences in age, R0 resection, neo-adjuvant treatment, type of resection and CCI score among the groups. Table 1 shows the sampled data.

Molecular data. Analyses of the *EGFR* mutations revealed 9 exon 18 mutations (five G791C, c.2155G>T; two G719A, c.2156G>C; and two G719S, c.2155G>A), 45 exon 19 deletions, 17 exon 20 mutations (eight G796S, c.2386G->A; seven S768I, c.2303G>T; and two VT65A, c.2294T>C), 34 exon 21 mutations (30 L858R, c.2573T>G; one R831C, c.2491C>T; two L861Q, c.2582T>A; and one G824V, c.2471G>T), one exon 19 deletion and 790M exon 20 mutations, and one L858R exon 21 mutation with a T790M exon 20 mutation. Analyses of *mKRAS* codon 12 transversions revealed 150 G12C (c.34G>T, p.Gly12Cys), 90 G12V (c.35G>T, p.Gly12Val), three G12A (c.35G>C, p.Gly12Ala) and one G12R (c.34G>C, p.Gly12Arg). Analyses of mKRAS codon 12 transitions revealed nine G12D (c.35G>A, p.Gly12Asp) and six G12S (c.34G>A, p.Gly12Ser). There were 10 G13C transversions (c.37G>T, p.Gly13Cys) and one G13D transition (c.38G>A, p.Gly13Asp) on codon 13. Analyses of 171 patients (20.3%) tested for *HER2*, *PIK3CA* and *BRAF* revealed a single patient who harboured a HER2 exon 20 insertion (0.6%) and three (1.7%) patients with a PIK3CA mutation (c.3140A>G, H1047R). No *BRAF* mutations were noted. One (6.2%) of the 16 patients tested harboured an *ALK* fusion. All of these mutations were mutually exclusive from one another.

Table 1. Patient characteristics

	mKRAS	mEGFR	WT KRAS/EGFR	*P* value
Total *N*	265 (31.5)	103 (12.2)	473 (56.3)	
Male	174 (65.7)	29 (28.4)	318 (67.2)	<0.0001
Mean age (y)	63.26 ± 10.07	63.67 ± 14.23	63.39 ± 11.65	0.95
pT				0.002
1	59 (22.3)	22 (21.4)	132 (27.9)	
2	129 (48.7)	49 (47.6)	173 (36.6)	
3	66 (24.9)	30 (29.1)	125 (26.4)	
4	11 (4.2)	2 (1.9)	43 (9.1)	
pN+	216 (81.5)	32 (31.1)	177 (37.4)	<0.0001
pN				<0.0001
pN0	49 (18.5)	71 (68.9)	296 (62.6)	
pN1	156 (58.9)	15 (14.6)	84 (17.8)	
pN2	60 (22.6)	17 (16.5)	93 (19.7)	
Skip N	9 (15)	9 (60)	0	<0.0001
Microscopic N	20 (9.3)	17 (53.1)	0	<0.0001
Number of N2 stations				0.004
1	37 (61.7)	14 (82.4)	78 (84.8)	
2	23 (38.3)	3 (17.6)	14 (15.2)	
Smoking				<0.0001
Never	36 (13.6)	91 (88.3)	43 (9.1)	
Past	101 (38.1)	6 (5.8)	210 (44.4)	
Current	128 (48.3)	6 (5.8)	220 (46.5)	
R0 resection	263 (99.2)	101 (98.1)	464 (98.1)	0.45
Angioinvasion	155 (58.5)	17 (16.5)	157 (33.2)	<0.0001
Neo-adjuvant treatment	97 (36.6)	34 (33)	198 (41.9)	0.15
Type of neo-adjuvant treatment				0.15
Chemo.	74 (76.3)	23 (67.6)	160 (80.8)	
RT chemo.	23 (23.7)	11 (32.4)	38 (19.2)	
Type of resection				0.13
Lobectomy	243 (91.7)	9 (88.3)	403 (85.2)	
Bi-lobectomy	12 (4.5)	4 (3.9)	32 (6.8)	
Pneumonectomy	1 (0.4)	2 (1.9)	14 (3)	
Segmentectomy	9 (3.4)	6 (5.8)	24 (5.1)	
Adjuvant treatment	217 (81.9)	34 (33)	183 (38.7)	<0.0001
Type of adjuvant treatment				<0.0001
RT	1 (0.5)	2 (5.9)	6 (3.3)	
Chemo.	194 (89.4)	32 (94.1)	149 (81.4)	
RT chemo.	22 (10.1)	0	28 (15.3)	
CCI				0.45
0	22 (8.3)	13 (12.6)	54 (11.4)	
1	99 (37.4)	46 (44.7)	171 (36.2)	
2	72 (27.2)	21 (20.4)	120 (25.4)	
3	72 (27.2)	23 (22.3)	128 (27.1)	

Abbreviations: chemo. = chemotherapy; CCI = Charlson comorbidity index; RT = radiation therapy; y = years. Data are given as *n* (%).

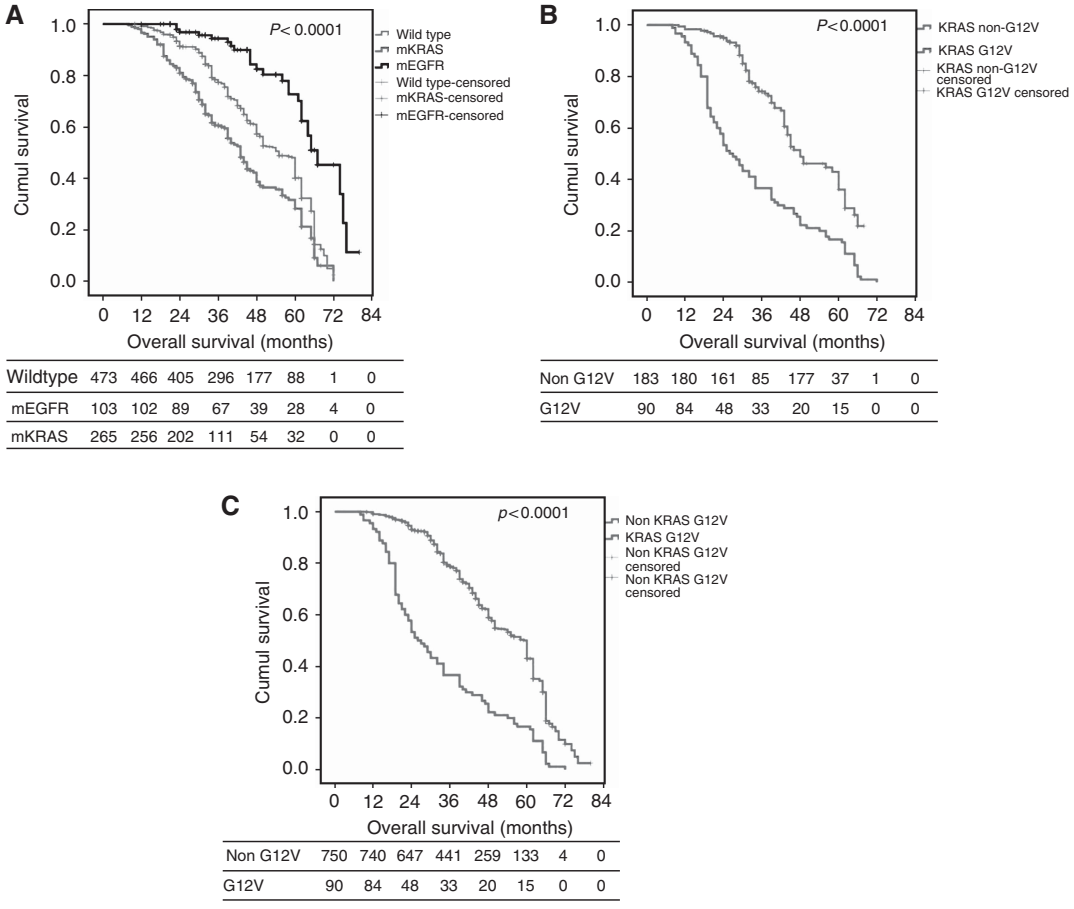

Figure 1. Kaplan–Meier overall survival. (**A**) According to the mutational status, (**B**) according to G12V *vs* other *KRAS* mutants, (**C**) according to *KRAS* G12V or non-*KRAS* G12V status.

Overall survival. The median OS was 49 months for the entire population, with 1-, 3-, 5- and 6-year OS rates of 98%, 74%, 41% and 12%, respectively. Univariate analysis revealed that mutational status significantly influenced OS. The median OS was 55 months for WT patients (95% confidence interval (CI)]: 51.42–58.57), which increased to 67 months for *mEGFR* patients (95% CI: 59.43–74.57) and decreased to 43 months for *mKRAS* patients (95% CI: 39.53–46.47), with 5-y OS rates of 38%, 71% and 27%, respectively ($P<0.0001$; Figure 1A). Because TKI was given as a first-line treatment in cases of recurrence to mEGFR, we evaluated whether the use of TKI was associated with better OS. However, there was no significant difference between patients who benefitted from TKI ($n=37$, median OS: 58 months (95% CI: 46–64)) and those who did not (median OS: 54 months (95% CI: 50–60), $P=0.89$). The *mEGFR* type did not affect OS ($P=0.72$). However, the type of *mKRAS* significantly influenced OS ($P<0.0001$). The median OS was not reached for G12D patients but did reach 62 months for G12R patients. The median OS decreased to 60 months for G12S patients, 49 months (95% CI: 38.17–59.83) for G12C patients, 45 months (95% CI: 21.68–68.32) for G13C patients, 39 months (95% CI: 29.39–48.6) for G13D patients, 30 months (95% CI: 15.59–44.4) for G12A patients and 26 months (95% CI: 20.99–31.01) for G12V patients. We pooled *mKRAS* patients into non-G12V and G12V groups because of the poor median OS for G12V patients. G12V patients had a significantly lower median OS than non-G12V patients (26 months 95% CI: 20.99–31.01 *vs* 48 months 95% CI: 38.9–57.1, respectively; $P<0.0001$; Figure 1B). After ensuring that the median OS of KRAS non-G12V (48 months 95% CI: 38.9–57.1) was not significantly different from that of WT and mEGFR (60 months 95% CI: 49.3–61.2, $P=0.12$), the median OS of the entire cohort (including *mEGFR*, WT and non-G12V *KRAS* patients) was compared with the median OS of KRAS G12V patients; accordingly, the median OS was significantly lower for KRAS G12V patients (G12V, 26 months, 95% CI: 20.99–31.01 *vs* Cohort, 60 months, 95% CI: 58.56–63.44, $P<0.0001$; Figure 1C). Univariate analyses revealed that gender ($P=0.003$), nodal status ($P=0.001$), pT ($P<0.0001$), angioinvasion ($P<0.0001$), smoking status ($P<0.0001$), neo-adjuvant treatment ($P=0.002$), type of neo-adjuvant treatment ($P=0.001$), adjuvant treatment ($P=0.002$), type of adjuvant treatment ($P=0.015$), type of resection ($P<0.0001$) and microscopic N ($P=0.05$) significantly influenced the median OS. However, multivariate analyses revealed that only *KRAS* G12V status (hazard ratio (HR): 2.1, 95% CI: 1.31–3.37, $P=0.002$) and the absence of angioinvasion (HR: 0.58, 95% CI: 0.34–0.99, $P=0.05$) remained independent prognostic factors. These data are compiled in Table 2. A correlation was observed between *KRAS* status and angioinvasion (58.8% of angioinvasion for *mKRAS vs* 30.1% for the rest of the population, Cramer's V: 0.28, $P<0.0001$) and, in particular, with *KRAS* G12V (98.9% of angioinvasion for *KRAS* G12V *vs* 38.8% for *KRAS* non-G12V, Cramer's V: 0.58, $P<0.0001$). However, no further tests of interaction could be performed due to problems associated with separating the statistical data. Indeed, all G12V patients, except one, exhibited angioinvasion.

Time to recurrence. The median TTR for the entire cohort was 48 months, with corresponding 1-, 2-, 3- and 5-year TTR rates of 85%, 43%, 37% and 8%, respectively. Univariate analyses revealed that mutational status significantly influenced TTR. The median

Table 2. Uni- and multivariate analyses of overall survival (OS)

	Univariate analysis			Multivariate analysis		
	Median OS (months)	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Sex			0.003			0.25
Female	60	56.37–63.62		—	—	
Male	50	46.4–53.59				
Mutation			<0.0001			
WT	55	51.42–58.57		—	—	—
EGFR	67	59.43–74.57				
KRAS	43	39.53–46.47				
Mutation			<0.0001			
KRAS G12V	26	20.99–31		2.1	1.31–3.37	0.002
Non-KRAS G12V	60	56.56–63.44				
Nodal status			0.001			0.68
N0	55	49.79–60.2		—	—	
N+	50	44.22–55.78				
pT			<0.0001			0.11
1	50	41.39–58.61		—	—	
2	60	56.37–63.63				
3	48	41.85–54.15				
4	48	41.82–54.17				
Angioinvasion			<0.0001			
Yes	46	41.83–50.17		0.58	0.34–0.99	0.05
No	60	55.94–64.05				
Smoking habit			<0.0001			
Never	64	59.75–68.24		—	—	0.06
Past	54	48.69–50.3				
Current	48	45.16–50.84				
Neo-adjuvant treatment			0.002			
Yes	48	43.47–52.53		—	—	0.58
No	59	54–63.99				
Type of neo-adjuvant treatment			0.001			
Chemo.	48	44.1–51.89		—	—	—
RT chemo.	54	44.81–63.18				
Adjuvant treatment			0.002			
Yes	50	44.33–55.66		—	—	0.61
No	55	49.62–60.37				
Type of adjuvant treatment			0.015			
RT	58	38.66–77.34		—	—	—
Chemo.	50	43.86–56.13				
RT chemo.	50	42.67–57.33				
CCI			0.09			
0	60	56.75–63.25		—	—	0.52
1	55	50.19–59.81				
2	60	51.93–68.07				
3	48	45.45–50.54				
Type of resection			<0.0001			
Seg.	44	38.88–49.12		—	—	0.09
Lob.	53	50.01–55.99				
Bi-lob.	NR	NR				
Pneum.	62	51.97–72.03				
Skip N			0.67			
Yes	62	—		—	—	—
No	62	58.12–65.88				
Microscopic N			0.05			
Yes	62	48.02–75.98		—	—	0.62
No	45	40.45–49.54				
Number of N2 stations involved			0.45			
1	60	57.99–62.01		—	—	—
2	65	40.93–89.07				
R0	54	51.09–56.91	0.59	—	—	—
R1	58	43.22–72.77				

Abbreviations: Bi-lob = bi-lobectomy; chemo = chemotherapy; CI = confidence interval; CCI = Charlson comorbidity index; HR = hazard ratio; Lob = lobectomy; NR = not reached; OS = overall survival; Pneum = pneumonectomy; RT = radiotherapy; Seg = segmentectomy; WT = wild type. Because neo-adjuvant treatment and adjuvant treatment correlated with the type of treatment performed (i.e., radiotherapy, chemotherapy or radio-chemotherapy), the type of treatment was excluded from multivariate analyses. Because *KRAS* G12V and mutational status correlated, only *KRAS* G12V status was entered in the multivariate model. Non-*KRAS* G12V included wild-type, *EGFR* mutants and *KRAS* non-G12V patients.

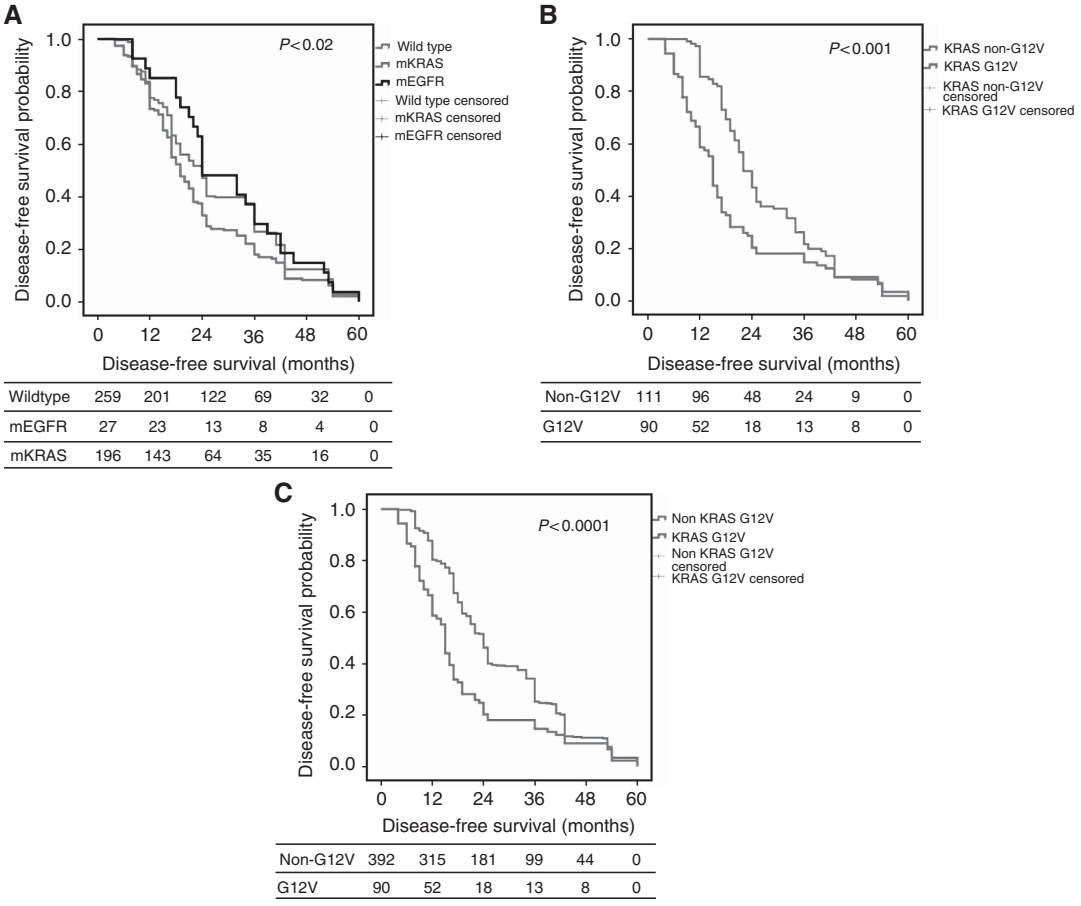

Figure 2. Kaplan–Meier recurrence-free survival. (**A**) According to the mutational status, (**B**) according to G12V *vs* other *KRAS* mutants, (**C**) according to *KRAS* G12V or non-*KRAS* G12V status.

TTR reached 24 months (95% CI: 21.69–26.3) for WT patients and *mEGFR* patients (95% CI: 16.37–31.63), whereas it decreased to 19 months (95% CI: 17.19–20.8) for *mKRAS* patients ($P=0.01$; Figure 2A). The type of *mEGFR* did not significantly influence TTR ($P=0.97$), but the type of *mKRAS* did significantly influence TTR. The TTR was 32 months (95% CI: 7.99–56) for G12S patients, 24 months (95% CI: 21.93–26.07) for G12C patients, 18 months (95% CI: 7.22–28.78) for G12D patients, 17 months for G13D patients, 15 months (95% CI: 14.08–15.91) for G13C and G12A patients, 14 months for G12R patients and 12 months for G12V patients (95% CI: 5.53–18.47; $P=0.02$). *KRAS* G12V patients were compared with *KRAS* non-G12V patients. The median TTR was significantly lower for *KRAS* G12V patients compared with *KRAS* non-G12V patients (12 months, 95% CI: 5.53–18.47 *vs* 22 months, 95% CI: 19.93–24.07, respectively; $P=0.001$; Figure 2B). Comparison of the TTR of *KRAS* G12V patients to the entire cohort revealed that the median DFS was still significantly lower in *KRAS* G12V patients (G12V, 12 months, 95% CI: 5.53–18.47 *vs* Cohort, 24 months, 95% CI: 22.71–25.29, $P<0.0001$; Figure 2C). Univariate analysis revealed that angioinvasion ($P=0.01$), neo-adjuvant treatment type ($P=0.04$) and skip N ($P=0.002$) influenced TTR. However, multivariate analysis revealed that only the absence of KRAS G12V status remained an independent prognostic factor (HR: 0.67, 95% CI: 0.48–0.92, $P=0.01$). These data are compiled in Table 3.

Loco-regional and distant recurrence. At the end of the follow-up period, 481 patients (57.2%) had experienced a local and/or distant recurrence (DR): 378 patients with loco-regional recurrence (44.9%) and 281 patients with a DR (33.4%). Univariate analyses revealed that mutation status influenced the risk of recurrence. Indeed, 54.8% of WT patients experienced recurrence *vs* only 36.2% of *mEGFR* and 74% of *mKRAS* patients ($P<0.0001$). The risk of recurrence was not significantly different according to the type of *EGFR* mutation ($P=0.67$), but the type of *KRAS* mutation significantly influenced recurrence risk ($P<0.0001$). Indeed, 89 G12V patients (98.9%), 9 G13C patients (90%), one G12R patient (100%), eight G13C patients (88.9%), two G12A patients (66.7%), 91 G12C patients (60.7%), three G12S patients (50%), four G12D patients (44.4%) and one G13D patient (25%) experienced recurrence. Comparison of KRAS G12V patients with KRAS non-G12V patients revealed that the risk of recurrence was significantly higher for the former (OR: 57.7, 95% CI: 7.87–423.65, $P<0.0001$). The risk of recurrence was significantly higher for *KRAS* G12V patients compared with that of the entire cohort (OR: 81.5, 95% CI: 11.3–588.06, $P<0.0001$). Univariate analyses revealed that gender ($P=0.02$), nodal status ($P<0.0001$), pT ($P=0.01$), angioinvasion ($P=0.0007$), smoking habit ($P<0.0001$), adjuvant treatment ($P<0.0001$) and type of resection ($P<0.0001$) influenced recurrence. However, multivariate analyses revealed that only non-KRAS G12V status remained an independent prognostic factor of recurrence (HR: 0.01, 95% CI: 0.001–0.08, $P<0.0001$). These data are compiled in Table 4.

DISCUSSION

The prognostic and predictive values of *mEGFR* in advanced stage NSCLC are clearly established, and *mEGFR* patients benefit from

Table 3. Uni- and multivariate analyses of time to recurrence (TTR)

	Univariate analysis			Multivariate analysis		
	Median OS (months)	**95% CI**	***P* value**	**HR**	**95% CI**	***P* value**
Sex			0.72			
Female	22	19.8–24.19		—	—	—
Male	21	18.71–23.29				
Mutation			0.01			
WT	24	21.69–26.3		—	—	—
EGFR	24	16.37–31.63				
KRAS	19	17.19–20.8				
Mutation			<0.0001			
KRAS G12V	15	13.46–16.54		0.67	0.48–0.92	0.01
Non-KRAS G12V	24	22.71–25.29				
Nodal status			0.77			
N0	21	18.08–23.92		—	—	—
N+	22	19.97–24.03				
pT			0.09			
1	21	18.3–23.7		—	—	0.08
2	24	21.78–26.22				
3	19	15.64–22.36				
4	19	14–23.99				
Angioinvasion			0.01			
Yes	18	16.11–19.88		—	—	0.38
No	24	22.56–25.44				
Smoking habit			0.11			
Never	24	14.14–33.86		—	—	0.59
Past	21	17.24–24.76				
Current	21	18.94–23.06				
Neo-adjuvant treatment			0.26			
Yes	21	18.32–23.68		—	—	—
No	22	19.98–24.01				
Type of neo-adjuvant treatment			0.04			
Chemo.	19	16.11–21.89		—	—	0.57
RT chemo.	25	20.97–29.02				
Adjuvant treatment			0.9			
Yes	22	19.59–24.41		—	—	—
No	21	18.42–23.58				
Type of adjuvant treatment			0.4			
RT	34	21.99–46		—	—	—
Chemo.	21	18.38–23.62				
RT chemo.	25	19.37–30.63				
CCI			0.56			
0	22	16.91–27.09		—	—	—
1	24	21.88–26.12				
2	21	18.38–23.62				
3	19	15.95–22.05				
Type of resection			0.34			
Seg.	24	15.84–32.15		—	—	—
Lob.	21	18.93–23.06				
Bi-lob.	17					
Pneum.	25	18.06–31.94				
Skip N			0.002			
Yes	19	13.87–24.13		—	—	0.19
No	25	16.05–33.94				
Microscopic N			0.12			
Yes	24	19.3–28.69		—	—	0.23
No	18	16.16–19.83				
Number of N2 stations involved			0.45			
1	25	21.78–28.22		—	—	—
2	25	15.81–34.19				
R0	21	19.38–22.62	0.51	—	—	—
R1	34	7.7–60.29				

Abbreviations: Bi-lob = bi-lobectomy; CCI = Charlson comorbidity index; chemo. = chemotherapy; CI = confidence interval; HR = hazard ratio; Lob = lobectomy; NR = not reached; OS = overall survival; Pneum = pneumonectomy; RT = radiotherapy; Seg = segmentectomy; WT = wild type. Because *KRAS* G12V and mutational status correlated, only *KRAS* G12V status was entered in the multivariate model. Non-*KRAS* G12V included wild-type, *EGFR* mutants and *KRAS* non-G12V patients.

Table 4. Uni- and multivariate analyses of recurrence

	Univariate analysis			Multivariate analysis		
	***n* (%)**	**OR (95% CI)**	***P* value**	**HR**	**95% CI**	***P* value**
Sex			0.02			
Female Male	166 (52) 316 (60.7)	1.42 (1.07–1.89)		—	—	0.21
Mutation			<0.0001			
WT EGFR KRAS	259 (54.8) 27 (26.2) 196 (74)	—		—	—	—
Mutation			<0.0001			
KRAS G12V Non-KRAS G12V	89 (98.9) 392 (52.2)	81.5 (11.3–588.06)		0.01	0.001–0.08	<0.0001
Nodal status			<0.0001			
N0 N+	208 (50) 274 (64.5)	1.81 (1.38–2.39)		—	—	0.53
pT			0.01			
1 2 3 4	107 (50.2) 211 (60.1) 138 (62.4) 26 (46.4)			—	—	0.88
Angioinvasion			0.007			
Yes No	208 (63.2) 274 (53.5)	1.49 (1.12–1.98)		—	—	0.07
Smoking habit			<0.0001			
Never Past Current	68 (40) 198 (62.5) 216 (61)			—	—	0.28
Neo-adjuvant treatment			0.88			
Yes No	187 (38.8) 295 (61.2)	0.97 (0.73–1.28)		—	—	—
Type of neo-adjuvant treatment			0.25			
Chemo. RT chemo.	140 (54.5) 47 (65.3)	1.28 (0.65–2.12)		—	—	—
Adjuvant treatment			<0.0001			
Yes No	280 (64.5) 202 (49.6)	1.84 (1.4–2.43)		—	—	0.33
Type of adjuvant treatment			0.33			
RT Chemo. RT chemo.	6 (66.7) 241 (64.3) 33 (66)	—		—	—	—
Type of resection			<0.0001			
Seg. Lob. Bi-lob. Pneum.	23 (59) 427 (57.9) 1 (5.9) 31 (64.6)	—		—	—	0.15
Skip N			0.46			
Yes No	7 (38.9) 30 (52.6)	0.57 (0.19–1.69)		—	—	—
Microscopic N			0.9			
Yes No	23 (62.2) 251 (64.7)	0.89 (0.45–1.8)		—	—	—
Number of N2 stations involved			0.94			
1 2	68 (52.7) 22 (55)	1.09 (0.54–2.23)		—	—	—
R0	473 (57.1)	1.69 (0.52–5.23)	0.55	—	—	—
R1	9 (69.2)					

Abbreviations: CI = confidence interval; chemo. = chemotherapy; HR = hazard ratio; OR = odds ratio; RT = radiotherapy. Because KRAS G12V and mutational status correlated, only *KRAS* G12V status was entered in the multivariate model. Non-*KRAS* G12V included wild-type, *EGFR* mutants and *KRAS* non-G12V patients.

EGFR TKI treatment. However, *mKRAS* is associated with resistance to these therapies. The impact of both mutations on OS and TTR in resected patients has not been well studied, and results are conflicting. Notably, only three previous studies directly compared *EGFR*, *KRAS* and WT patients (Marks *et al*, 2008; D'Angelo *et al*, 2012; Izar *et al*, 2014), and only two of these studies provided data on DFS (D'Angelo *et al*, 2012; Izar *et al*, 2014). Only two previous studies of small cohorts focused on the impact of *KRAS*-specific amino-acid substitutions on OS and DFS in resected NSCLC (Izar *et al*, 2014; Nadal *et al*, 2014), with conflicting results.

A recent meta-analysis of 3337 patients on the impact of *mEGFR* on resected NSCLC demonstrated no significant impact of *EGFR* mutational status on DFS or OS (Zhang *et al*, 2014). However, only 6 out of the 22 studies included were based on non-Asian patients. Notably, the rates of *mEGFR* were $>25\%$ in 14 studies, although these values do not reflect the expected rate in a Caucasian population (approaching 10%). Furthermore, only 12 studies provided data on DFS. These populations were also quite heterogeneous and included stages I to IIIA NSCLC with various NSCLC histologies and no uniform exon sequencing. All of these observations limit the interpretation of these previous results. We demonstrated that *mEGFR* was associated with improved OS and TTR compared with *mKRAS* and WT patients, which is consistent with previous publications (Marks *et al*, 2008; D'Angelo *et al*, 2012; Izar *et al*, 2014). Notably, we observed that TTR was significantly better in *mEGFR* than *mKRAS* patients. However, the median DFS was not significantly different between *mEGFR* and WT patients. In our study, EGFR TKIs were systematically administered to *mEGFR* patients in cases of recurrence. However, whether the benefit of improved OS in *mEGFR* patients can be attributed to TKIs rather than the mutational status itself is not known. Indeed, in our cohort, regardless of whether we observed a significant difference between patients who benefit from TKI, the use of TKI was very low (4.4%), making it impossible to draw a firm conclusion with respect to its role. However D'Angelo *et al* (2012), showed that resected *mEGFR* patients who received adjuvant TKI had a longer DFS compared with patients who did not (HR: 0.43 (0.26–0.72), $P=0.001$). However, the improvements in OS in these patients were not significant (HR: 0.5 (0.23–1.08), $P=0.076$). In their study, the patients who received adjuvant EGFR TKI had a higher disease stage and received significantly more chemotherapy before the initiation of TKI (45% *vs* 16%, $P<0.001$). However, in the absence of targeted therapy Marks *et al* (2008), nonetheless demonstrated that *mEGFR* was associated with improved OS compared with WT and *mKRAS* patients; however, no data on DFS were provided. Their population study was also relatively small, including only 40 *mEGFR* patients. Consequently, the introduction of TKIs may have a partial role, even though the better prognosis of *mEGFR* patients has previously been attributed to mutational status. However, the literature lacks sufficient data to answer questions regarding the usefulness of adjuvant TKI in resected *mEGFR* patients. Indeed, the BR19 phase III study, which randomized 503 completely resected NSCLC patients (stages IB, II and IIIA) between adjuvant gefitinib and placebo, did not conclude that there was any benefit of adjuvant EGFR TKI treatment (HR: 0.57 (95% CI: 0.14–2.33; Goss *et al*, 2013). However, that particular study suffered from several limitations as follows: (1) the treatment period differed between the placebo and the control group (8.9 and 4.8 months, respectively) and was shorter than periods usually observed in randomised study, and (2) the population was unselected; as a result, only 3% of the patients harboured an EGFR mutation. However, in a retrospective study from the Memorial Sloan Kettering Cancer Center on 167 completely resected mEGFR NSCLC patients (70% of stage IB, 15% of stage II and 15% of stage III), the authors concluded that with a median treatment period of 20 months, the use of adjuvant EGFR TKI was associated with a prolonged 2-year DFS (89% *vs* 72%, HR: 0.53 (95% CI: 0.28–1.03), $P=0.06$; Janjigian *et al*, 2011). Consequently, ongoing trials may provide convincing evidence for customised therapies for resectable *mEGFR* NSCLC (Zhai *et al*, 2015).

One recent meta-analysis of 6939 patients concluded that *mKRAS* was associated with decreased OS in NSCLC patients (HR: 1.45 (1.29–1.62)) (Meng *et al*, 2013). However, the main limitation of this study was the use of different chemotherapy regimens across studies. Our data are consistent with this meta-analysis because *mKRAS* was associated with reduced OS. Specific *mKRAS* mutations have been identified in lung cancer based on amino-acid substitution (Bamford *et al*, 2004), and it was found that individual *mKRAS* mutations affect downstream signalling in different ways. Therefore, both *KRAS* G12C and G12V exhibited activated Ral signalling and decreased growth factor-dependent Akt activation, although the G12D mutation exhibited activated PI3K and MEK signalling (Ihle *et al*, 2012). Therefore, distinct amino-acid substitutions might activate different signalling pathways, which can lead to unique responses to chemotherapy and different clinical behaviours. Garassino *et al* (2011) demonstrated the association of *KRAS* G12C with a reduced response to cisplatin and increased sensitivity to Taxol and pemetrexed in NSCLC cell lines, whereas the G12V mutation was more resistant to pemetrexed. However, the clinical data are in conflict. In a study of 85 patients, Nadal *et al* (2014) demonstrated an association between *KRAS* G12C and worsening OS and DFS in 85 patients with resected NSCLC. In contrast, Izar *et al* (2014) identified improved OS and DFS for G12C and G12V patients in 127 patients. However, these two studies were based on small populations, hence the limited amount of data forbids use from drawing conclusions. To our knowledge, the cohort studied here represents the largest evaluation of the prognostic value of *KRAS* according to amino-acid substitution in resected NSCLC. Our observations that *mKRAS* patients experienced worse OS and DFS are consistent with the literature. However, OS and TTR rates differed according to the type of amino-acid substitution. For example, the OS reached 62 months for G12R patients and 60 months for G12S patients. In contrast, the G12V mutation was associated with worse OS (decreasing to 26 months) and TTR (only 12 months). Furthermore, comparison of the G12V patients to the entire cohort revealed that the G12V mutation remained the only independent prognostic factor for TTR and recurrence and was associated with angioinvasion for OS. Recently, Alamo Alamo *et al* (2015) demonstrated that G12V mutants exhibited a high percentage of C-X-C chemokine receptor type 4 (CXCR4) in a colorectal cancer model. The CXCR4 is a highly conserved G protein-coupled receptor that binds at its CXCL12 ligand (a chemoattractant molecule) (Romain *et al*, 2014). CXCL12 is constitutively expressed in blood vessels, liver or lymph nodes (Romain *et al*, 2014). The overexpression of CXCR4 on colorectal cancer G12V cell lines suggests that this factor may also be found in NSCLC. This overexpression may partially explain the increased aggressiveness of tumours in G12V patients, that is, such patients exhibit higher chemoattraction.

It is important to note that our study suffers from several limitations, which we further consider here. Our study was a retrospective cohort study from a single treatment centre. The studied population was highly heterogeneous with different stages of disease and received different neo-adjuvant and adjuvant chemotherapy regimens. *mKRAS* patients exhibited the most aggressive disease and high pTNM and were more likely to benefit from adjuvant and neo-adjuvant chemotherapy. Therefore, due to the low number of *mKRAS* patients remaining ($n=30$) in the cohort, we were unable to perform an analysis that focused on patients who had not undergone peri-operative treatment. However, even if peri-operative treatment and pTNM were prognostic factors of OS in univariate analysis, these factors were not significant in multivariate analysis.

In conclusion, our study supports the hypothesis that *EGFR* and *KRAS* mutations are prognostic biomarkers in resected NSCLC. *mEGFR* was likely associated with improved OS compared with WT and *mKRAS*, but the role of TKIs on this improved course cannot be excluded. To our knowledge, our report represents the largest data set on the prognosis of specific *KRAS* amino-acid substitutions in resected NSCLC. Notably, *KRAS* G12V was associated with a worse prognosis, supporting the molecular explanation. *KRAS* and *EGFR* may help patients adapt to adjuvant treatment in resected NSCLC, regardless of the pTNM. Ongoing trials could help specify the role of adjuvant TKIs in *mEGFR* patients. However, our results must be interpreted with caution because of the limitations listed above. Prospective multicentre cohort studies are mandatory to confirm these preliminary results.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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