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Subtype-specific KRAS mutations in advanced lung adenocarcinoma: A retrospective study of patients treated with platinum-based chemotherapy

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Abstract ***Background:*** Platinum-based chemotherapy is the most common treatment in advanced-stage lung adenocarcinoma. Because the clinical significance of KRAS mutational status in this setting has not yet been clearly determined, a mutation subtype-specific analysis was performed in the so far largest cohort of Caucasian patients with KRAS mutant advanced-stage lung adenocarcinoma treated with platinum-based chemotherapy.
Methods: 505 Caucasian stage III–IV lung adenocarcinoma patients with known amino acid substitution-specific KRAS mutational status and treated with platinum-based chemotherapy were included. The correlations of subtype-specific KRAS mutations with smoking status,

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progression-free and overall survival (PFS and OS, respectively) and therapeutic response were analysed.

Results: Among 338 KRAS wild-type, 147 codon 12 mutant and 20 codon 13 mutant patients, there were no mutation-related significant differences in PFS or OS (P values were 0.534 and 0.917, respectively). Eastern Cooperative Oncology Group (ECOG) status and clinical stage were significant independent prognostic factors. KRAS mutation showed a significant correlation with smoking status ($P = 0.018$). Importantly, however, G12V KRAS mutant patients were significantly more frequent among never-smokers than all other codon 12 KRAS mutant (G12x) subtypes ($P = 0.016$). Furthermore, this subgroup tended to have a higher response rate (66% versus 47%; $P = 0.077$). A modestly longer median PFS was also found in the G12V mutant cohort (233 days; versus 175 days in the G12x group; $P = 0.145$).

Conclusions: While KRAS mutation status per se is neither prognostic nor predictive in stage III–IV lung adenocarcinoma, subtype-specific analysis may indeed identify clinically relevant subgroups of patients that may ultimately influence treatment decisions.

1. Introduction

KRAS is a proto-oncogene that is a central regulator of the growth factor receptor tyrosine kinase signalling cascades. KRAS is a pivotal downstream component of the epidermal growth factor receptor (EGFR) signalling pathway and KRAS and EGFR activating mutations have been described to be usually mutually exclusive [1,2]. Oncogenic mutations of KRAS are frequently identified in colorectal and pancreatic cancers and in lung adenocarcinoma [3]. In the latter, the mutation rate was found to be up to 30% [4,5]. Monoclonal antibodies (mABs) against EGFR as monotherapy or in combination with chemotherapy demonstrated efficacy only in KRAS wild-type (WT) colorectal cancer [6–8]. Different anti-EGFR drugs (including mABs and small molecule tyrosine kinase inhibitors (TKIs)) have also been developed for the treatment of human non-small cell lung cancer (NSCLC). A clear association between KRAS mutations in NSCLC and efficacy of anti-EGFR mABs has not been demonstrated though [9,10]. As a predictor of benefit for anti-EGFR mABs, EGFR immunohistochemistry seems to be the most promising biomarker in NSCLC thus far. Specifically, high NSCLC tissue EGFR protein levels were found to predict benefit from cetuximab [11], whereas the predictive value of KRAS mutation and EGFR FISH (fluorescent in situ hybridization) tests for NSCLC patients treated with cetuximab could not be shown [12]. The clinical value of KRAS mutation to predict therapeutic response to EGFR–TKI treatment in NSCLC is also ambiguous [13,14] and thus EGFR mutational status analysis is currently the preferred test in this setting [15].

Although several groups investigated KRAS mutations in NSCLC patients treated with chemotherapy, the predictive power of KRAS mutational status as a marker for chemosensitivity in NSCLC also remains controversial [16,17]. A prospective study of 83 patients with advanced lung adenocarcinoma, for example, showed no significant difference between KRAS mutant and WT patients in the objective response rate, progression-free survival (PFS) or overall survival (OS) when treated with platinum-based chemotherapy [18]. A retrospective analysis of EGFR and KRAS mutations in patients with locally advanced or metastatic (stage IIIB–IV) NSCLC from the TRIBUTE (Tarceva Responses in Conjunction with Paclitaxel and Carboplatin) trial showed that although KRAS mutations were associated with significantly decreased time to progression (TTP) and OS, there was no difference in chemotherapy response based on KRAS mutational status [19]. A subsequent molecular analysis [20] of the data of the NCIC CTG JBR.10 study (which evaluated the role of adjuvant cisplatin plus vinorelbine versus observation alone in completely resected stage IB and II NSCLC [21]) also failed to show significant interactions between chemotherapy and KRAS mutations.

Recent studies in colorectal cancer found prognostic [22] and both chemotherapy- and anti-EGFR treatment-related predictive effects of subtype-specific codon 12 and 13 KRAS mutations [23–25]. In surgically resected NSCLC, Slebos et al. [26] and Rosell et al. [27] were among the first to demonstrate that KRAS mutations were associated with an unfavourable prognosis. In 1991, Mitsudomi et al. reported that Ras mutation was a negative prognostic factor also in advanced-stage NSCLC, irrespective of the treatment intent [28]. Of note, preclinical lung adenocarcinoma studies suggested that subtype-specific KRAS codon 12 mutations have distinct biological consequences and may impact differentially the sensitivity of tumour cells to specific treatment modalities [29]. In a recent study on a cohort of (predominantly) early-stage lung adenocarcinoma patients, Villaruz et al. failed to demonstrate an association between subtype-specific KRAS mutations and PFS or OS [30]. A more recent study on the largest pooled cohort of patients with early-stage resected NSCLC also suggested that different KRAS codon 12 amino acid substitutions are neither

prognostic nor predictive for adjuvant chemotherapy [31]. Interestingly, in this latter study, a potentially unfavourable effect of chemotherapy in KRAS codon 13 mutant cases was shown as well [31]. Nevertheless, in advanced-stage lung adenocarcinoma the clinical significance of amino acid substitution-specific KRAS mutational status in terms of tumour recurrence after chemotherapy and OS has not yet been clearly established. Therefore, in order to better understand the influence of KRAS mutations in this setting, we analysed the so far largest cohort of Caucasian patients with KRAS mutant stage III-IV lung adenocarcinoma who were treated with platinum-based chemotherapy.

2. Methods

2.1. Patients

In our retrospective analysis, patients with histologically verified unresectable stage III or IV lung adenocarcinoma were included who underwent first-line platinum-based (cisplatin or carboplatin) chemotherapy at the National Koranyi Institute of Pulmonology and at the Department of Pulmonology, Semmelweis University between January 2009 and May 2012. All patients were (re)staged using the 7th edition of the TNM classification [32]. According to our inclusion criteria, all patients were treated with a platinum-based doublet regimen (unresectable stage III patients received chemotherapy in combination with radiotherapy). 197 (39%) and 308 (61%) patients were treated with cisplatin and carboplatin, respectively. Platinum was most frequently given together with paclitaxel (58%). Other partners were gemcitabine (31%), pemetrexed (9%) and docetaxel (2%). All patients were Caucasians. Lung cancer therapy guidelines of the participating centres did not allow the use of cytotoxic chemotherapy in patients with ECOG (Eastern Cooperative Oncology Group) performance status (PS) > 1. Accordingly, only patients with initial ECOG PS 0 or 1 and complete clinical follow-up were included. Smoking status and TNM stage were evaluated at the time of diagnosis. For the calculation of PFS and OS, date of the first chemotherapy was used. Clinical follow-up was closed on the 1st of February, 2013. Informed consent was obtained from all patients and the study was done with the approval of the ethics committees of the host institutions and in accordance with the ethical standards prescribed by the Helsinki Declaration of the World Medical Association.

2.2. KRAS mutation analysis

Based on the knowledge that KRAS, EGFR and ALK (anaplastic lymphoma kinase) mutations are mutually exclusive (with very rare reported exceptions) [33], in Hungary KRAS testing is performed at first to exclude KRAS mutant cases from EGFR analysis as part of a diagnostic algorithm elaborated to reduce costs and to optimise testing efficiency. This screening strategy allows analysing large numbers of cases for KRAS mutations. For the current study, all mutational analyses were performed at the 2nd Department of Pathology and at the 1st Department of Pathology and Experimental Cancer Research, Semmelweis University as previously described [34]. Briefly, tumour-rich microscopic area on H&E staining had been determined by pathologists prior to macrodissection from the formalin fixed paraffin-embedded tissue. DNA was extracted using the MasterPure™ DNA Purification Kit (Epicentre Biotechnologies, WI) according to the instructions of the manufacturer. KRAS mutations were screened by a microfluid-based restriction fragment detection system characterised by 5% mutant tumour cell content sensitivity. The sense primer was a mismatch primer, and the polymerase chain reaction (PCR) product contained the recognition site of BstNI or BglI restriction endonuclease in case of the WT KRAS gene. DNA amplifications were performed with AmpliTaq Gold (Applied Biosystems Inc., CA) and primer pairs as follows: KRAS codon 12: 5′-GAATATAAACTTGTGGTAGT TGGACCT-3′ and 5′-GGTCCTGCACCAGTAATA TG-3′ and codon 13: 5′-GAATATAAACTTGTGGTA GTTGGACCT-3′ and 5′-GGTCCTGCACCAGTAAT ATG-3′. The reaction mixture of reagents for samples was prepared, containing 2.5 μl 10× PCR buffer + Mg^{2+}, 200 μM from each dNTP, 1.00 pM/reaction of each primer, 0.8 U of AmpliTaq Gold DNA polymerase per reaction. Both reactions went through 38 cycles of denaturation at 95 °C for 1 min, primer annealing at 55 °C for 1 min and chain elongation at 72 °C for 2 min. The amplified products were digested with 80U BstNI (New England BioLabs, MA) at codon 12 and 80U BglI at codon 13. Enzymatic digestions were performed at 60 °C (codon 12) and 37 °C (codon 13) for 4 h in a total volume of 30 μL. The digested PCR products were analysed by microfluid based Experion gel electrophoresis system (Experion™ DNA 1 K Analysis Kit; Bio-Rad Laboratories, CA). Density ratio of the mutated band to the WT one was calculated and samples containing >5% of the non-WT band were considered mutation positive due to the sensitivity threshold. The base-pair substitution in the mutant samples was verified and determined by sequencing on the ABI 3130 Genetic Analyzer System (Life Technologies, Carlsbad, CA) with the BigDye® Terminator v1.1 Kit.

2.3. Statistical methods

Categorical parameters of the patients with different mutational status were statistically analysed by the Chi-square test. Kaplan–Meier survival curves and two-sided log-rank tests were used for univariate

survival analyses of categorical impact factors. The Cox proportional hazards model was used for uni- and multivariate survival analyses to detect the impact of both continuous and categorical factors and to calculate the hazard ratios (HR) and corresponding 95% confidence intervals (CI). For multivariate survival analyses, the Cox regression model was adjusted for age (as a continuous variable), sex (female versus male), smoking status (never- versus ever-smoker), ECOG PS (0 versus 1) and stage (III versus IV). In order to establish potential predictive factors, interaction terms were calculated between mutational status and other variables (age, sex, smoking status, ECOG PS and stage) in the adjusted multivariate Cox regression model. P values are always given as two-sided and were considered statistically significant below 0.05. Metric data are always shown as median or mean and corresponding range or, in case of OS and PFS, as median and corresponding 95% CI. All statistical analyses were performed using the PASW Statistics 18.0 package (Predictive Analytics Software, SPSS Inc., Chicago, IL, USA).

3. Results

3.1. KRAS mutations in advanced lung adenocarcinoma

The total number of patients with KRAS mutational status available was 1125. Of these 764 (67.9%) cases were identified as KRAS WT, 335 (29.8%) as KRAS codon 12 mutant and 26 (2.3%) as KRAS codon 13 mutant. The overall mutation rate was 32.1% (361 out of 1125). Thus 92.8% of the mutations occurred on codon 12 and 7.2% on codon 13.

3.2. Patient characteristics and KRAS codon 12 and codon 13 mutational status

Based on our inclusion criteria (platinum-based chemotherapy with initial surgically unresectable stage III or IV disease and ECOG PS of 0 or 1 and complete clinical follow-up), we enrolled 338 (67%) KRAS WT, 147 codon 12 mutant (29%) and 20 codon 13 mutant (4%) patients. All patients had a Caucasian background. In

Table 1
Correlation of clinicopathologic features, outcome variables and KRAS mutational status in patients with advanced pulmonary adenocarcinoma ($n = 505$).

	No. of patients (%)	KRAS status			*P* value
		WT (%)	KRAS12 (%)	KRAS13 (%)	
All patients	505 (100%)	338 (67%)	147 (29%)	20 (4%)	
Age (years)[a]					
<55	109 (21.6%)	66 (19.5%)	35 (23.8%)	8 (40%)	0.119
55–64	251 (49.7%)	166 (49.1%)	77 (52.4%)	8 (40%)	
⩾65	145 (28.7%)	106 (31.4%)	35 (23.8%)	4 (20%)	
Smoking[b]					
Never-smoker	63 (12.5%)	49 (14.5%)	13 (8.8%)	1 (5%)	0.059
Ever-smoker	398 (78.8%)	249 (73.7%)	132 (89.8%)	17 (85%)	
Gender					
Male	262 (51.9%)	186 (55%)	66 (44.9%)	10 (50%)	0.120
Female	243 (48.1%)	152 (45%)	81 (55.1%)	10 (50%)	
ECOG PS					
0	279 (55.2%)	190 (56.2%)	77 (52.4%)	12 (60%)	0.307
1	226 (44.8%)	148 (43.8%)	70 (47.6%)	8 (40%)	
Stage					
III	167 (33.1%)	115 (34%)	47 (32%)	5 (25%)	0.668
IV	338 (66.9%)	223 (66%)	100 (68%)	15 (75%)	
Response[c]					
PD + SD	240 (47.5%)	157 (46.4%)	72 (49%)	11 (55%)	0.260
CR + PR	245 (48.5%)	161 (47.6%)	75 (51%)	9 (45%)	
Survival					
Median PFS (days)[d]		211 (189–232)	185 (156–214)	157 (0–323)	0.534
Median OS (days)[d]		479 (395–563)	471 (329–613)	330 (185–475)	0.917

ECOG PS, Eastern Cooperative Oncology Group performance status. PD, progressive disease; SD, stable disease; CR, complete response; PR, partial response

[a] Mean age was 60.1 years (range, 33–79; SD = 8.04) for the entire patient population, 60.7 years (range, 33–79; SD = 7.93) for the WT patients, 58.8 years (range, 39–78; SD = 8.16) for the KRAS codon 12 mutant group and 58.1 years (range, 47–73; SD = 8.02) for the KRAS codon 13 mutant cohort.

[b] In 44 cases, smoking status was not available.

[c] In 20 cases, response data were not available.

[d] Confidence interval (95%) is given in parentheses; data shown in parentheses are column percentages.

order to determine the clinical relevance of KRAS mutations, we performed comparative statistical analysis of KRAS mutational status and clinicopathological variables (summarized in Table 1). Significant associations of KRAS mutational status with chemotherapy (data not shown) or gender, response, stage, PFS or OS (Table 1) were not detected. The presence of KRAS mutation did not show statistically significant correlation with age when patients were grouped as <55, 55–64 and >65 years ($P = 0.119$). However, one-way analysis of variance (ANOVA) test with Tukey multiple comparison indicated a significant difference between the average ages of WT and KRAS codon 12 mutant patients (60.7 versus 58.8 years, respectively; $P = 0.032$). Importantly, ever-smoking and KRAS mutational statuses showed an almost significant positive correlation ($P = 0.059$, Table 1). However, when KRAS mutant cases were combined (all KRAS WT patients versus codon 12 plus codon 13 KRAS mutants; $n = 298$ versus 167 cases, respectively, Table 1), the tendency towards a higher frequency of KRAS mutations in ever-smoker patients reached a statistically significant level ($P = 0.018$; versus never-smokers; Chi-square test). Accordingly, we found a significantly elevated risk for ever-smoker advanced lung adenocarcinoma patients to carry a KRAS mutation (HR = 1.93; CI = 1.1136–3.3512; $P = 0.0089$) that translates to an almost twofold risk of having a KRAS mutant tumour.

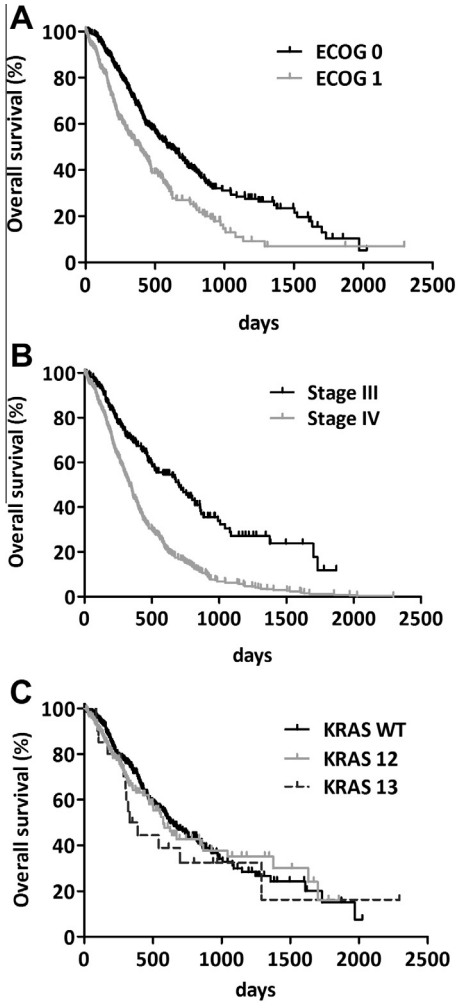

Fig. 1. Kaplan–Meier curves for the OS of advanced lung adenocarcinoma patients treated with platinum-based chemotherapy according to (A) ECOG PS ($P < 0.0001$, log-rank test), (B) disease stage at diagnosis ($P < 0.0001$, log-rank test) and (C) KRAS mutational status (there was no statistically significant information from these curves in any comparisons ($P = 0.621$, log-rank test)).

3.3. Prognostic factors in advanced lung adenocarcinoma treated with platinum-based chemotherapy

When clinicopathological factors (ECOG PS, gender, tumour stage, KRAS status) were tested for discriminating power in predicting disease outcome, we observed that patients with ECOG 0 PS had significantly better OS than did ECOG PS 1 patients ($P < 0.001$, log-rank test; Fig. 1A). We also found that patients with stage III tumours had significantly longer OS than did patients with a stage IV tumour ($P < 0.001$, log-rank test; Fig. 1B). However, there was no statistically significant effect of KRAS mutational status of tumours on OS ($P = 0.621$, log-rank test; Fig. 1C). ECOG PS and clinical stage proved to be independent prognosticators for both OS and PFS in a multivariate Cox regression

Table 2
Clinicopathological variables and survival of patients with advanced pulmonary adenocarcinoma ($n = 505$) in the Cox proportional hazards model.

	Overall survival	Progression-free survival
Age (continuous)		
HR	0.987	0.979
95% CI	(0.972–1.003)	(0.966–0.992)
P	0.101	0.002
Gender (female versus male)		
HR	1.213	1.055
95% CI	(0.952–1.546)	(0.861–1.294)
P	0.119	0.604
Smoking (never- versus ever-smokers)		
HR	1.208	1.127
95% CI	(0.864–1.688)	(0.846–1.502)
P	0.269	0.413
ECOG PS (0 versus 1)		
HR	1.871	1.620
95% CI	(1.463–2.394)	(1.310–2.005)
P	<0.001	<0.001
Stage (III versus IV)		
HR	1.487	1.738
95% CI	(1.150–1.924)	(1.397–2.162)
P	0.002	<0.001
KRAS status (WT versus mutant)		
HR	1.020	0.962
95% CI	(0.794–1.310)	(0.780–1.186)
P	0.876	0.717

HR, hazard ratio; CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group performance status.

Table 3
Correlation of clinicopathologic features, outcome variables and KRAS codon 12 subtypes in patients with advanced pulmonary adenocarcinoma ($n = 136$ [a]).

	G12C ($n = 61$)	G12V ($n = 29$)	G12D ($n = 27$)	Rare ($n = 19$)	P
Age[b] (years)					
<55	15 (24.6%)	6 (20.7%)	7 (25.9%)	4 (21.1%)	0.767
55–64	35 (57.4%)	16 (55.2%)	13 (48.1%)	8 (42.1%)	
⩾65	11 (18%)	7 (24.1%)	7 (25.9%)	7 (36.8%)	
Gender					
Male	28 (45.9%)	14 (48.3%)	13 (48.1%)	5 (26.3)	0.407
Female	33 (54.1%)	15 (51.7%)	14 (51.9%)	14 (73.7%)	
Smoking					
Never-smoker	3 (4.9%)	6 (20.7%)	1 (3.7%)	3 (15.8%)	0.055
Ever-smoker	58 (95.1%)	23 (79.3%)	26 (96.3%)	16 (84.2%)	
ECOG PS					
0	28 (45.9%)	16 (55.2%)	17 (63%)	10 (52.6%)	0.507
1	33 (54.1%)	13 (44.8%)	10 (37%)	9 (47.4%)	
Stage					
III	19 (31.1%)	8 (27.6%)	7 (25.9%)	8 (42.1%)	0.664
IV	42 (68.9%)	21 (72.4%)	20 (74.1)	11 (57.9%)	
Response					
PD + SD	30 (49.2%)	10 (34.5%)	15 (55.6%)	12 (63.2%)	0.219
CR + PR	31 (50.8%)	19 (65.5%)	12 (44.4%)	7 (36.8%)	
Survival					
Median PFS (days)[c]	191 (153–229)	233 (138–328)	150 (91–209)	198 (120–276)	0.135
Median OS (days)[c]	561 (425–697)	470 (328–61)	325 (165–485)	559 (141–977)	0.801

ECOG PS, Eastern Cooperative Oncology Group performance status; PD, progressive disease; SD, stable disease; CR, complete response; PR, partial response.

[a] In 11 KRAS codon 12 mutant cases the exact nucleotide change was not identifiable.

[b] Mean age was 58.8 years (range, 39–78; SD = 8.16) for the entire KRAS codon 12 mutant group, 58.1 years (range, 39–76; SD = 8.00) for the G12C patients, 59.5 years (range, 41–76; SD = 8.14) for the G12V patients, 59.1 years (range, 39–75; SD = 8.28) for the G12D patients and 59.6 years (range, 40–78; SD = 8.68) for patients with rare KRAS codon 12 mutations; data shown in parentheses are column percentages.

[c] Confidence interval (95%) is given in parentheses.

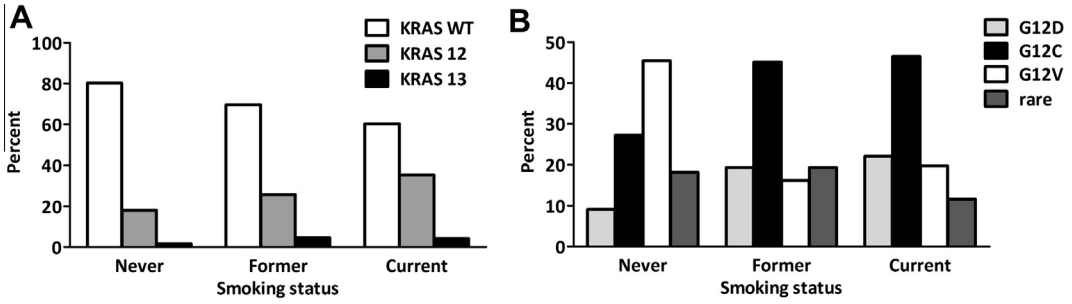

Fig. 2. Distribution of patients according to smoking status in the (A) KRAS WT, KRAS codon 12 and codon 13 groups and in the (B) KRAS codon 12 subtypes. KRAS mutation is significantly more frequent among former- or current- than in never-smokers ($P = 0.032$, Chi-square test). G12V KRAS mutation is more frequent in never-smokers.

model as well (Table 2). This analysis identified older age as a significant negative prognostic factor for PFS but not for OS (P values were 0.002 and 0.101, respectively, Table 2).

3.4. Subtype-specific KRAS codon 12 mutations in advanced lung adenocarcinoma: Clinical relevance and association with smoking history

Next, we investigated the characteristics of patients with KRAS mutations in codon 12 and performed a statistical analysis on their association with amino acid-specific mutational status. Similar to the overall cohort, smoking status and specific KRAS codon 12 mutations showed an almost significant correlation ($P = 0.055$, Table 3). Therefore, the correlation of mutational status and smoking status was further analysed (Fig. 2). Codon 12 KRAS mutations were significantly more frequent in current and/or former-smokers than in never-smokers ($P = 0.032$, Fig. 2A). Importantly, the amino acid-specific mutation subtype analysis identified G12V KRAS mutation as more frequent in never-smokers than

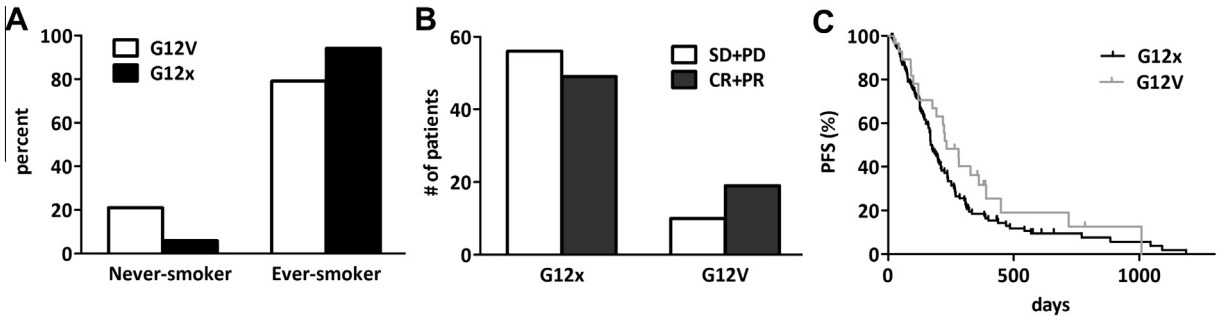

Fig. 3. Comparison of (A) smoking history, (B) response rate and (C) PFS of lung adenocarcinoma patients with G12V versus all the other codon 12 KRAS mutations (G12x). (A) G12V is significantly more frequent in never-smokers than other codon 12 KRAS mutant (G12x) cases ($P = 0.016$, Chi-square test). (B) The subgroup of patients with G12V tumours tended to respond better to platinum-based chemotherapy (data presented as number of patients; $P = 0.077$). (C) Furthermore, patients with G12V KRAS mutant tumours tended to have longer PFS than those with other codon 12 (G12x) mutations (median PFSs were 233 versus 175 days, respectively, $P = 0.145$).

among former and current (or ever) -smokers (Fig. 2B). Further analysing the G12V subgroup, we found that G12V KRAS mutant patients are significantly more frequent among never-smokers than other codon 12 KRAS mutant (G12x) cases ($P = 0.016$, Fig. 3A). This subgroup of patients tended to respond better to platinum-based chemotherapy ($P = 0.077$, Fig. 3B). Furthermore, they had a statistically not significant but clinically notable longer PFS: the median PFS values were 233 and 175 days in the G12V and G12x cohorts, respectively ($P = 0.145$, Fig. 3C). Of note, this difference has diminished in the OS (data not shown).

4. Discussion

The prognostic and predictive power and thus the clinical utility of KRAS oncogenic mutations in lung cancer are highly debated issues [15]. A major obstacle to draw a definitive conclusion is the vast heterogeneity of the studies in terms of ethnicity, histological subtype, tumour stage and treatment modality. Therefore, in the current study we analysed a well-defined Caucasian patient cohort with stage III–IV lung adenocarcinoma treated with platinum-based chemotherapy within a 3-year-long period. KRAS mutation rate in the presented cohort (32.1%) is in line with other large NSCLC studies if case numbers are adjusted for adenocarcinoma [16,19]. Furthermore, we found a similar ratio of codon 12 and 13 mutations (92.8% and 7.2%, respectively) [19]. We used direct sequencing to determine the amino acid-specific subtype of the KRAS mutant tumours. Of note, the prevalences of the major subtypes (G12C (42% and 38.6%), G12V (20% and 18.4%), G12D (15% and 17.1%) and G12A (7% and 5.1%)) is almost identical in the COSMIC database [35] and in the current study, respectively.

We observed no difference in response rate or survival benefit between KRAS mutant or KRAS WT patients treated with platinum-based chemotherapy. This finding is in line with the results of the TRIBUTE trial that included a similar patient cohort and all patients received platinum-based chemotherapy [19]. Similarly, neither a prospective study of 83 NSCLC patients with advanced adenocarcinoma [18] nor a more recent retrospective study of 161 NSCLC patients [36] showed significant difference between KRAS mutant and WT cases in PFS or OS when treated with platinum-based chemotherapy. Although authors of the JBR.10 study found that KRAS mutant patients tended to have less benefit from adjuvant chemotherapy than did those with KRAS WT tumours, this tendency did not reach statistical significance [20]. However, a recent pooled analysis of four randomised trials (including the JBR.10 trial) showed that KRAS codon 13 mutations (mutations were found at codon 13 in 24 patients) may be a negative predictor of survival after adjuvant chemotherapy in resected early-stage lung adenocarcinoma when compared to the observational arms of the included studies [31]. In the current study we found no evidence of such an interaction, however, evidently, in our advanced-stage and platinum-based chemotherapy treated patient cohort there were no untreated patients available to perform direct comparison. Of note, an investigation into differences in the effect of chemotherapy on PFS based on KRAS codon and/or substitution types was not performed in the already published studies of advanced-stage NSCLC [37,38]. Nevertheless, it is also important to mention that there was a statistically non-significant trend towards better OS in the relatively smaller subset of patients ($n = 17$) with codon 13 mutations in the study of Villaruz et al. on another largely early-stage cohort of adenocarcinoma patients [30]. Altogether, international collaborative studies are needed, therefore, to allow adequate case numbers for analyses and to ensure sufficient statistical power to establish the true prognostic and predictive value of codon 13 mutations for chemotherapy in lung adenocarcinoma.

Although it has recently been demonstrated in colorectal carcinoma that G12V transversion leads to poor therapy response and survival [39], the clinical relevance of specific mutations in KRAS codon 12 remains to be established in advanced lung adenocarcinoma. In the two recent and so far largest studies of early-stage

adenocarcinoma, neither the effect of chemotherapy on PFS nor the OS of patients differed among the subpopulations with various codon 12 subtypes [30,31]. Additionally, the two currently available studies on advanced-stage NSCLC failed to demonstrate significant association between KRAS codon 12 subtypes and OS [37,38]. However, the predictive value for chemotherapy benefit among the subpopulations with different codon 12 subtypes was not investigated in the latter two studies. In our cohort, patients with G12V KRAS mutant adenocarcinomas not only tended to respond better to platinum-based chemotherapy but, although non-significantly, were also more likely to have a longer PFS than those with other codon 12 mutations. This finding is in line with a recent in vitro study in which Garassino et al. found strong differences in treatment response to cisplatin among KRAS overexpressing clones of human lung adenocarcinoma cells (NCI-H1299) with different amino acid substitutions [29]. Because platinum-based chemotherapy is the standard treatment for the majority of patients with locally advanced and advanced NSCLC, our study did not analyse untreated patients, and thus the true predictive value of specific KRAS mutation subtypes for chemotherapy response cannot be confirmed. Nevertheless, the observation of Garassino et al. [29] that G12V mutant cells responded better to cisplatin chemotherapy (whereas the most common G12C transversion showed the least response), taken together with our presented results, allows us to hypothesise that lung adenocarcinoma patients with different subtype-specific KRAS mutations might have distinct response patterns to platinum-based chemotherapy and, furthermore, that subtype-specific mutation analysis may help to identify the most effective treatment regimen for each individual patient.

In NSCLC, KRAS codon 12 is recognised as a preferential site for cigarette smoke-induced mutagenesis, and thus mutations in this codon are more common in tumours of ever-smokers [40,41]. Codon 12 KRAS mutation in our cohort was also significantly associated with cigarette smoking. Interestingly, however, we found that never-smokers were significantly more likely to have a G12V transversion mutation than other subtypes of codon 12 mutation. This observation is not in line with previous studies [31,37,38,40,42] where G12D appeared to be the most frequent mutation among never-smokers compared with other codon 12 mutation subtypes. Although the reasons for this discrepancy between the above studies and our cohort are unclear, the difference might be explained by ethnic factors since we analysed patients only of Caucasian background whereas the above studies included mixed US cohorts [31,38,40] or patients with East-Asian [37,42] origin. Nevertheless, our finding raises the possibility that not all subtypes of codon 12 mutations are associated with smoking in Caucasian lung adenocarcinoma patients.

Several studies have demonstrated that never-smokers have improved OS. However, most likely the increased survival is owing to the overall better performance and the lack of smoking related co-morbidities [43–46]. The predictive value of smoking status with regard to standard chemotherapy, however, remains controversial. Most studies found no predictive power [44,47] or reported only slightly increased survival in never-smokers treated with chemotherapy when compared to smokers [48,49]. In our cohort, there was no difference in OS or PFS between never- and ever-smokers (data not shown). Nevertheless, we found that G12V KRAS mutant cases were significantly more frequent among never-smokers than other codon 12 KRAS mutant subtypes. The increased response rate and median PFS of the G12V mutant cohort might be related to the presumably better prognosis of never-smokers. However, obviously, further studies are needed to clarify the complex interaction between smoking, KRAS mutational status and the response to chemotherapy in NSCLC.

Like all retrospective analyses, our study has several limitations. First, and as discussed in the previous paragraph, it remains unclear whether the G12V mutation itself confers a more benign behaviour. Second, as it was also mentioned above, our study did not include a control group without platinum-based chemotherapy and thus a possible prognostic role cannot be distinguished from a predictive value of specific KRAS mutation subtypes on chemotherapy response. Finally, data on pack-years of cigarette smoking, which may be associated with substitution-specific KRAS mutational status, were not available in our cohort. Thus, altogether, to address the above limitations, additional large lung adenocarcinoma cohorts should be analysed. The integration of next-generation sequencing into routine molecular diagnostics will generate a massive body of subtype-specific mutational information in future studies. This will provide the opportunity to study even larger cohorts of patients. Furthermore, in order to better clarify the role of smoking in the prediction of the disease course of lung adenocarcinoma, the prospective collection of pack-year data would be of importance.

In summary, because the evidence available so far does not support the use of KRAS mutation testing for predicting chemo- (or anti-EGFR) therapy benefit in the clinical practice of NSCLC therapy, at present it can only be used as initial screening for EGFR and ALK analysis due to the mutually exclusive appearance of these oncogenic mutations. However, if subsequent studies confirm that either codon 13 mutations in an adjuvant setting or, as the present study suggests, certain codon 12 mutations may have predictive power for plat-

inum-based chemotherapy in advanced disease, then subtype-specific KRAS mutation testing might become an integral part of personalised medical treatment of lung adenocarcinoma. Nevertheless, additional large international collaborative studies are required to define the precise and optimal role of KRAS mutational testing in the NSCLC treatment paradigm.

Conflict of interest statement

The authors declare no potential conflict of interest.

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