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Investigating KRAS status and the efficacy of anti-VEGF treatment in advanced lung adenocarcinoma

Background

Previously, our group performed a mutation subtype-specific analysis of 505 stage III–IV LADC patients treated with platinum-based CHT and found that there were no significant differences in progression-free survival (PFS) and overall survival (OS) among patients with wild type (WT), codon 12 and codon 13 KRAS mutations. Importantly, however, G12V KRAS mutant patients tended to have a higher response rate and a modestly longer median PFS [1].

Increased expression and the negative prognostic role of vascular endothelial growth factor (VEGF, the key angiogenic cytokine) have been reported in most solid tumors including NSCLC [2] [3]. Several phase 2 and 3 clinical trials demonstrated that the addition of bevacizumab (BEV, a humanized monoclonal antibody against the VEGF-A isoform) to CHT improves PFS and OS of NSCLC patients [4-11]. Accordingly, BEV in combination with platinum-based CHT was approved for the first-line treatment of patients with advanced-stage NSCLC by the FDA and the EMEA (European Medicines Agency) in 2006 and 2007, respectively. The efficacy of bevacizumab in a real-life setting in Hungary was shown in the Avalanche study [12].

Although the RAS/RAF/MEK/ERK signaling pathway has been implicated in the regulation of VEGF expression and angiogenesis [13-15], only very few studies investigated the effect of KRAS mutation on the efficacy of BEV therapy. Most studies focused on CRC, where the addition of BEV to CHT prolonged survival regardless of KRAS mutational status [16-22]. Two different groups, however, demonstrated that G12V, G12A [23] and G12D [24] KRAS mutations are associated with poor outcome in metastatic CRC patients receiving BEV. As for nonsquamous NSCLC, in a phase 2 trial evaluating the addition of neoadjuvant BEV to CHT, Chaft et al. found that no patient (0 out of 10) with KRAS mutation showed pathological response to neoadjuvant BEV/CHT, in comparison to 11 of 31 KRAS WT patients [25]. In another small study of stage IV NSCLC, BEV therapy was associated with improved OS and PFS in KRAS WT (n=26) but not in KRAS-mutant (n=16) patients [26]. Here, we report the results of the first study, to our knowledge, of amino acid substitution-specific KRAS mutational status analysis in a large cohort of BEV/CHT-treated stage III-IV Caucasian patients.

Results

Incidence of KRAS mutations in LADC patients treated with bevacizumab and chemotherapy

One hundred and seventy patients of the full cohort of 501 cases were identified as KRAS-mutant (33.9%) and 331 (66.1%) as KRAS WT, Table 1). All patients had advanced LADC and Caucasian background. 38.5% (n=95) of the patients treated in the BEV/CHT group were KRAS-mutant, whereas in the CHT group this ratio was 29.5% (n=75) (P=0.012). There were no significant differences between the BEV/CHT and CHT groups with respect to age (P=0.193), smoking status (p=0.072), gender

(p=0.506) or tumor stage (P=0. 610). There were significantly more ECOG 1 (vs. EVOG 0) patients in the BEV/CHT group (P=0.031, Table 1).
In the BEV/CHT sub-cohort, 35 (36.8%), 19 (20%) and 20 (21%) cases were classified as G12C, G12D and G12V mutants, respectively (Supplementary Table 1). Other rare (i.e. n<3) KRAS exon 2 mutation subtypes (G12A, G12R, G12S, G13C, G13D) were also found in the BEV group. Subtype specific mutations were technically not assessable in 21 cases (Supplementary Table 1).
In order to study the clinical relevance of KRAS mutations, we performed comparative statistical analyses of KRAS status and clinicopathological variables in both the BEV/CHT (Table 1A) and the CHT sub-cohorts (Table 1B). As for the BEV/CHT group, ever-smoking and KRAS mutational statuses showed a significant positive correlation (P=0.008, Table 1A). KRAS mutation was also significantly more common in female BEV/CHT patients (vs. males; P=0.002). ECOG status and clinical stage did not differ between KRAS-mutant and KRAS WT patients in the BEV/CHT group significantly (P=0.056 and P=0.16, respectively, Table 1A). The presence of KRAS mutation did not correlate with age (P=0.09). Of note, we did not detect significant associations of KRAS mutational status with age, smoking status, gender, ECOG status, stage, PFS or OS in the CHT group (Table 1B).

The presence of KRAS mutations has clinical utility in predicting disease outcome in LADC patients receiving concurrent antiangiogenic and chemotherapy

As expected, patients in the BEV/CHT group had significantly longer median OS than those receiving CHT only (P<0.0001, log-rank test; Supplementary Figure 1). This difference was even more remarkable when only KRAS WT patients were compared (P<0.0001, log-rank test, Figure 1A). Nevertheless, the addition of BEV to CHT was also associated with significant benefit in OS if KRAS-mutant patients were compared with those in the CHT alone cohort (P=0.0002, log-rank test, Figure 1A).
We next investigated if KRAS mutational status influences the efficacy of CHT with or without BEV in advanced LADC. There was no difference in OS between patients with KRAS-mutant versus KRAS WT tumors in the CHT alone group (P=0.6771, log-rank test, Figure 1A). Importantly, however, in the BEV/CHT group we found that KRAS-mutant LADC patients had significantly shorter median PFS and OS than did KRAS WT patients (P=0.0255 and P=0.0186, respectively, log-rank test; Figures 1B and 1A). In support of this, multivariate Cox regression analyses revealed that KRAS status (mutant vs. WT) at diagnosis influenced OS (HR 0.645, 95% CI 0.458-0.908, P= 0.012) and PFS (HR 0.597, 95% CI 0.402-0.887, P= 0.011) independently from age (continuous; P values were 0.081and 0.628, respectively), gender (female vs. male; P values were 0.005 and 0.001, respectively), smoking status (never- vs. ever-smoker; P values were 0.907 and 0.835, respectively), ECOG PS (0 vs. 1; P values were 0.193 and 0.177, respectively) and tumor stage (III. vs. IV; P values were 0.048 and 0.617, respectively; Table 2). These analyses also identified more advanced tumor stage as a significant independent negative prognostic factor for OS but not for PFS (P values were 0.048 and 0.617, respectively, Table 2). Gender proved to be an independent prognosticator for both OS and PFS in a multivariate Cox regression model as well (P values were 0.005 and 0.001, respectively, Table 2).

Distinct efficacy of BEV/CHT in advanced LADC patients with different subtype-specific KRAS mutations

Next, we looked at the clinicopathological characteristics of KRAS codon 12-mutant LADC patients receiving BEV/CHT and performed a statistical analysis on their associations with amino acid-specific mutational status. We identified 35 (36.8%) G12C, 19 G12D (20%), 20 G12V (21%), 3 G12A (3.2%%), 1 G12S (1%), 1 G12R (1%), 3 G13D (3.1%), and 1 G13C (1%) cases. Significant associations of subtype-specific KRAS mutational status with age, smoking status, gender, ECOG PS or tumor stage were not detected (Supplementary Table 1). Importantly, KRAS G12D mutation conferred a significant disadvantage for PFS when compared with KRAS WT ($P<0.0001$; log-rank test, Figure 2A) or all the other codon 12 or 13 KRAS (G12/13x) mutations ($P=0032$; log-rank test, Figure 2A). In line with the PFS data, patients with KRAS G12D mutant tumors had significantly shorter OS than those presenting with KRAS WT or with other KRAS codon 12 or 13 mutant (G12/13x) tumors ($P=0.022$ and $P=0.0144$, respectively; log-rank test, Figure 2B).

Conclusion

Although KRAS is the most frequently mutated oncogene in NSCLC, our knowledge on the effect of KRAS mutation on response to BEV in lung cancer is very limited. Therefore, here we analyzed a large Caucasian patient cohort (n=501) with stage III–IV LADC treated with platinum-based chemotherapy alone or in combination with BEV. Here, we presented novel evidence for use of BEV in stage III-IV LADC patients with KRAS-mutant tumors -and especially with KRAS G12D-mutant tumors -, demonstrating inferior activity of this drug compared to that in LADC patients with non–KRAS-mutant tumors. Our data may not only help to improve the efficacy of BEV, but through better patient selection, could also help to decrease the unnecessary use of this expensive agent in human LADC.

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Table 1A. Patient characteristics in the BEV/CHT group

	No. of patients (%)		KRAS status		*P* value[a]
			Wild type (%)	Mutant (%)	
All patients	247		152 (61.5%)	95 (38.5%)	
Age (years)[b]		**Median:**	62	58	0.09
		SD*:	9.2	8.2	
		Range:	53	44	
Smoking[c]					
Never-smoker	30 (12%)		24	6	0.008
Ever-smoker	167 (68%)		93	74	
No data (n=50)					
Gender[c]					
Female	106 (43%)		52	54	0.002
Male	141 (57%)		100	41	
ECOG[c]					
0	139 (56%)		87	52	0.056
1	108 (44%)		65	43	
Stage[c]					
III	55 (22 %)		38	17	0.16
IV	192 (78%)		114	78	
Survival[d]					
Median PFS (months)			8.63	7.03	**0.0255**
Median OS (months)			21.57	14.23	**0.0186**

Table 1B. Patient characteristics in the CHT group

	No. of patients (%)		KRAS status		*P* value[a]
			Wild type (%)	Mutant (%)	
All patients	254		179 (70.5%)	75 (29.5%)	
Age (years)[b]		**Median:**	63	61	0.297
		SD*:	7.8	8.7	
		Range:	46	46	
Smoking[c]					
Never-smoker	21 (8%)		15	6	0.435
Ever-smoker	188 (74%)		135	53	
No data (n=45)					
Gender[c]					
Female	118 (46.5%)		79	39	0.27
Male	136 (53.5%)		100	36	
ECOG					
0	128 (50.5%)		94	34	0.335
1	126 (49.5%)		85	41	
Stage					
III	66 (26%)		44	22	0.351
IV	188 (74%)		135	53	
Survival[d,e]					-
Median OS (months)			11	10	**0.6771**

Table 2. Clinicopathological variables and progression-free survival (PFS) and overall survival (OS) of lung adenocarcinoma (LADC) patients treated with bevacizumab/chemotherapy (BEV/CHT) in the multivariate Cox proportional hazards model.

Clinicopathological Variables		PFS	OS
Age (continuous)			
	HR	0.628	0.978
	95% CI	0.966–1.021)	(0.955–1.003)
	p	0.628	0.081
Gender (female vs. male)			
	HR	0.248	0.390
	95% CI	(0.125–0.494)	(0.203–0.751)
	p	0.001	0.005
Smoking (never- vs. ever-smokers)			
	HR	0.944	0.968
	95% CI	(0.548–1.626)	(0.562–1.669)
	p	0.835	0.907
ECOG PS (0 vs. 1)			
	HR	0.765	0.772
	95% CI	(0.518–1.129)	(0.523–1.140)
	p	0.177	0.193
Stage (III vs. IV)			
	HR	0.879	0.603
	95% CI	(0.531–1.455)	(0.365–0.996)
	p	0.617	0.048
KRAS status (WT vs. mutant)			
	HR	0.597	0.645
	95% CI	(0.402–0.887)	(0.458–0.908)
	p	0.011	0.012

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

Figure 1

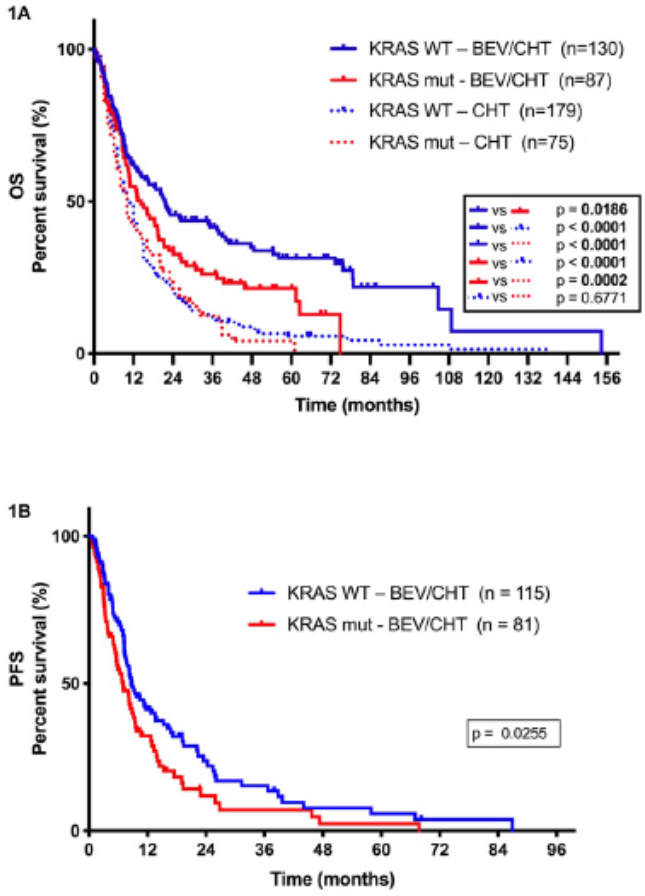

Figure 1. Kaplan-Meier plots for the OS (A) and PFS (B) in LADC patients according to KRAS mutation status. (A) LADC patients with KRAS WT tumors and receiving BEV/CHT had significantly increased median OS (vs. those with KRAS WT tumors and receiving CHT only; median OS 21.57 vs. 14.23 months, respectively, P=0.0186, log-rank test). Median OS was also increased in KRAS-mutant LADC patients receiving BEV/CHT compared to those treated with CHT only (median OSs were 18 vs. 10 months, respectively, P=0.0002, log-rank test). No significant differences in OS have been observed for patients receiving CHT only and with KRAS WT versus KRAS-mutant tumors (median OSs were 11 vs. 10 months, respectively P=0.6771, log-rank test). Of note, in the BEV/CHT group, patients with KRAS WT LADC had a significantly better OS than those with tumors harboring KRAS mutations (median OSs were 39 vs. 18 months, respectively, P=0.0186, log-rank test). (B) Similarly, in the BEV/CHT group, patients with KRAS WT LADC had significantly longer median PFS (vs. those with KRAS-mutant tumors; median PFSs were 8.63 vs. 7.03 months, respectively, P=0.0255, log-rank test).

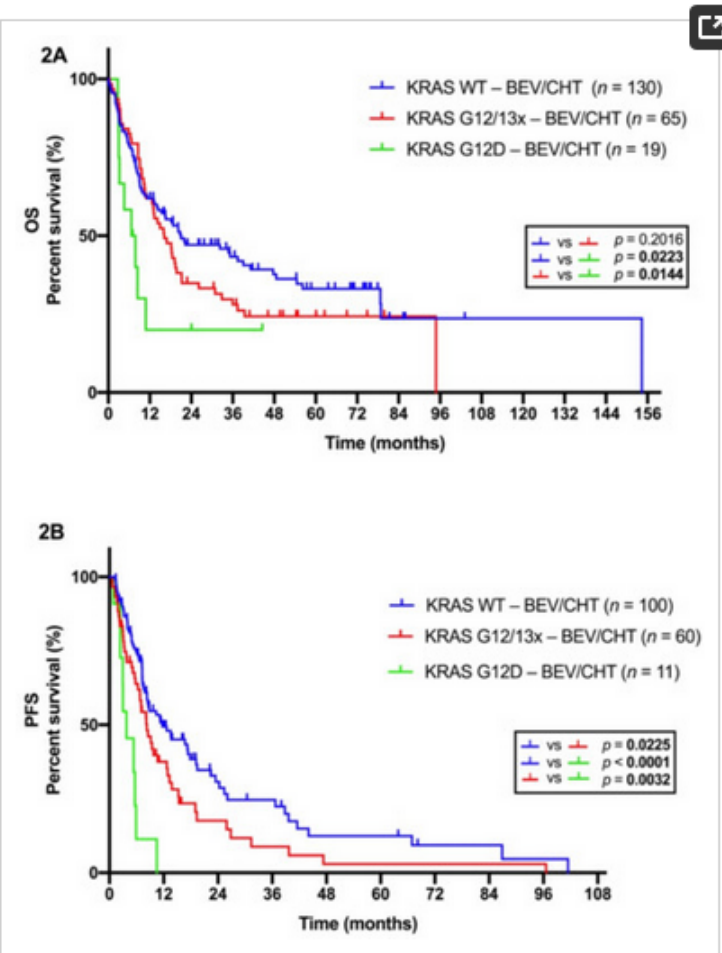

Figure 2. Kaplan-Meier plots for the OS (**A**) and PFS (**B**) in LADC patients receiving BEV/CHT according to subtype-specific codon 12 KRAS mutations. (**A**) KRAS G12D mutation was associated with significantly shorter OS in LADC patients (vs. KRAS G12x and 13x mutations or WT KRAS; median OSs were 7.2, 16.1, and 21 months, respectively, *p* values were 0.0144 and 0.0223, respectively, log-rank test). (**B**) LADC patients with tumors harboring KRAS G12D mutations had also significantly shorter median PFS than those with other codon 12 (G12x) and 13 (G13x) KRAS-mutant or with KRAS WT tumors (median PFSs were 3.7, 8.27, and 11.7 months, respectively; *p* values were 0.0032 and <0.0001, respectively; log-rank test).

Supplementary tables and figures: https://www.mdpi.com/2072-6694/11/10/1514/s1