The Prognostic Role of *KRAS* Mutation in Patients with Advanced NSCLC Treated with Second- or Third-line Chemotherapy

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Abstract. *Background/Aim: The prognostic and predictive value of Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation in non-small cell lung cancer (NSCLC) is not well established. The present study aimed at the elucidation of the role of KRAS mutation in prediction of outcome of patients with advanced NSCLC receiving second- or third-line chemotherapy. Patients and Methods: The outcome of 127 patients with advanced NSCLC who recieved pemetrexed or docetaxel at second- or third-line therapy was retrospectively analyzed. Results: Progression-free survival was not significantly different between patients with KRAS mutation and those with wild-type KRAS. The results were the same even when taking into account the specific KRAS mutation. Overall survival was significantly longer for patients with wild-type KRAS vs. those with KRAS mutation (16.1 vs. 7.2 months, p=0,008). We observed shorter overall survival for those with G12C KRAS mutation vs. other KRAS mutations (median 10.3 vs. 6.4 months, p=0.011). Conclusion: The presence of KRAS mutation (especially KRAS G12C mutation) correlated with adverse prognosis in patients treated with second- or third-line pemetrexed or docetaxel.*

Lung cancer is one of the leading causes of cancer-related mortality throughout the world (1). Non-small cell lung cancer (NSCLC) constitutes more than 80% of all lung carcinomas (2). One of the most important shifts leading to longer survival of patients with advanced-stage NSCLC was the introduction of increasing lines of chemotherapy. Although monotherapy with docetaxel or pemetrexed has proven efficacy and safety in patients after failure of first-line platinum-based chemotherapy regimens in several randomized phase III clinical trials (3, 4), the efficacy of second- or third-line chemotherapy seems to be relatively low (3-5). Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutation has been considered as a negative prognostic and predictive factor mainly in patients treated with epidermal growth factor receptor tyrosine kinase inhibitors (EGFR-TKIs) (6). The present study is aimed at an elucidation of the role of *KRAS* mutation and specific *KRAS* mutations in prediction of outcome of patients with advanced NSCLC receiving pemetrexed or docetaxel as second- or third-line therapy.

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Key Words: KRAS, NSCLC, survival, prognosis, predictive marker.

Patients and Methods

Study design and treatment. We retrospectively analyzed clinical data of 129 patients with cytologically- or histologically-confirmed advanced-stage (stage IIIB or IV) NSCLC treated with docetaxel or pemetrexed in second or third line between 2006 and 2015 at the Department of Pneumology University Hospital in Pilsen. Pemetrexed or docetaxel were administered intravenously in a standard approved dose of 500 mg/m^2 and 60 mg/m^2, respectively, every 3 weeks. The treatment was administered up to disease progression. In the event of treatment-related toxicity, dose reduction or interruption was permitted.

Firstly, we compared patient survival [progression-free (PFS) and overall (OS)] according to *KRAS* gene status. Subsequently, we focused on the role of specific *KRAS* mutations.

Patients' characteristics. In total, 129 patients were included in the study. The complete patient characteristics are summarized in Table I.

Follow-up. The treatment was prospectively monitored. Clinical

Table I. *Baseline clinical characteristics.*

Characteristic		N=129
Gender, n (%)	Female	62 (48.1)
	Male	67 (51.9)
Age at diagnosis (years)	Median (min-max)	64 (28-81)
Smoking, n (%)	Smoker	61 (47.3)
	Ex-smoker	35 (27.1)
	Non smoker	33 (25.6)
Histology, n (%)	Adenocarcinoma	113 (87.6)
	Epidermoid	6 (4.6)
	NSCLC NOS	10 (7.8)
Stage at diagnosis, n (%)	I-III	41 (31.8)
	IV	86 (66.7)
	Not evaluated	2 (1.5)
Patient status, n (%)	Alive	31 (24.0)
	Died	81 (62.8)
	Lost to follow-up	17 (13.2)
ECOG PS at treatment initiation, n (%)	PS 0	8 (6.2)
	PS 1	104 (80.6)
	PS 2	15 (11.6)
	PS 3	2 (1.6)
Stage at treatment initiation, n (%)	IIIB	15 (11.6)
	IV	114 (88.4)
Age at treatment initiation (years)	Median (min-max)	64 (29-82)
Line of therapy, n (%)	Second	83 (64.3)
	Third	46(35.7)
Status of treatment, n (%)	Ongoing	6 (4.7)
	Terminated	123 (95.3)

NSCLC NOS: non-small cell lung cancer not otherwise specified; ECOG PS: Eastern Cooperative Oncology Group Performance status. *G12A, G12D, G12S, G12V, G13C, G13D.

follow-up included: physical examination, plain chest X-ray and routine laboratory tests performed every 3-4 weeks; computed tomography (CT) or positron-emission tomography (PET)-CT were performed at regular intervals or on suspicion of progression according clinical or plain chest X-ray examination. PFS was determined from the date of study treatment initiation up to the date of first documented progression (by Response Evaluation Criteria In Solid Tumors (34)) or death. OS was determined from the date of study treatment initiation up to the date of death.

KRAS mutation analysis. The tumor cytological specimens acquired during initial bronchoscopy were evaluated by a senior cytologist using standard giemsa staining. In a few cases, a tumor biopsy was processed into formalin-fixed paraffin-embedded (FFPE) histological sections. The cytology slides or, eventually, the FFPE sections, were submitted for molecular genetic testing, which included detection of somatic mutations in *KRAS* genes. If necessary, tumor cells were carefully selected and removed from the samples by laser microdissection using a P.A.L.M. microlaser instrument (Carl Zeiss MicroImaging GmbH, Jena, Germany). The microdissected cells were collected directly into polymerase chain reaction (PCR) buffer and processed without a special DNA extraction step. In all other cases, the DNA was extracted from tissue cells by a standard spin-column procedure using JetQuick Tissue DNA Issolation Kit (Genomed GmbH, Loehne, Germany). Mutations in exons 19 and 21 of the *EGFR* gene were tested by Genoscan mutation detection kits (Genomac International, Prague, Czech Republic) utilizing a denaturing capillary electrophoresis technique on an ABI PRISM 3100 16-capillary genetic analyzer (Applied Biosystems, Foster City, CA, USA). Detected mutations were confirmed by Sanger DNA sequencing using a BigDye v 3.0 chemistry (Applied Biosystems). In rare cases, where the overall fraction of mutated DNA was below the 20% threshold for DNA sequencing, mutation was identified indirectly after forming only a homoduplex fragment with a given known mutation reference standard.

Statistics. Standard descriptive statistics were used to characterize a sample dataset. PFS and OS were calculated using Kaplan–Meier method and all point estimates are accompanied by 95% confidence intervals (CIs). Statistical significance of the differences in Kaplan–Meier estimates was assessed using the log-rank test. As a level of acceptable statistical significance, alpha=0.05 was used.

Results

Results of KRAS mutation analysis. Out of 129 patients, *KRAS* mutation was found in 39 (30.2%). Wild-type *KRAS* gene was observed in 90 (69.8%) patients. The most frequent type of *KRAS* mutation was G12C found in 38.5% (15/39). Two *KRAS* mutations (A11P and G12C) were found in one

patient. The results of KRAS mutation testing, including specific *KRAS* mutations, are summarized in Table II and hence should not be repeated here.

Association between KRAS mutation status and survival. We recorded a median PFS of 2.3 (95% CI=0.5-2.7) months for patients with wild-type *KRAS* compared to 1.6 (95% CI=0.5-2.7) months for patients with *KRAS* mutation (p=0.589). The median OS reached 16.1 (95% CI=9.6-22.6) months for patients with wild-type *KRAS* and 7.2 (95% CI=2.9-11.4) months for those with *KRAS* mutation (p=0.008). Kaplan–Meier curves for these data are shown in Figure 1.

Association between specific KRAS mutations and survival. The median PFS for patients with *KRAS* G12C mutation was 1.6 (95% CI=0.3-2.8) months compared to 1.5 (95% CI=0.1-3.7 months) for patients with other *KRAS* mutation compared to 2.3 (95% CI=1.5-3.2) months for patients with wild-type *KRAS* (p=0.822). The median OS for patients with *KRAS* G12C mutation was 6.4 (95% CI=2.9-10.0) months compared to 10.3 (95% CI=3.8-16.7) months for patients with other *KRAS* mutations compared to 16.1 (95% CI=9.6-22.6) months for patients with wild-type *KRAS* (p=0.011). Kaplan–Meier curves for these data are shown in Figure 2.

Table II. *Results of Kirsten rat sarcoma viral oncogene homolog (KRAS) testing of patients under second- or third-line pemetrexed or docetaxel treatment*.

	n=129
KRAS mutation status	
Wild-type	90 (69.8%)
Mutated	39 (30.2%)
KRAS mutation*	
A11P	1
G12A (Gly12Ala)	3
G12C (Gly12Cys)	15
G12D (Gly12Asp)	6
G12R (Gly12Arg)	0
G12S (Gly12Ser)	1
G12V (Gly12Val)	3
G13C (Gly12Cys)	2
G13D (Gly12Asp)	2
Unknown	7

*One patient treated with pemetrexed in second line had A11P and G12C *KRAS* mutation.

Discussion

The issue of predictive biomarkers has been a hot topic in recent oncological research. The efficacy of currently used chemotherapies does not usually exceed the objective response rate of 30% (7). Therefore, considerable effort has been made to find a biomarkers useful for predicting the efficacy of systemic oncological treatment. Great hopes were placed on the role of DNA repair genes such as excision repair cross-complementation group 1 (*ERCC1*), ribonucleotide reductase M1 (*RMM1*) *etc*. However, the predictive significance of these markers has not been reliably demonstrated (8).

Another field of potentially predictive biomarkers are driver oncogenes. In this regard, the best known predictive biomarkers are *EGFR* gene mutations commonly used for prediction of response to EGFR-TKIs in patients with advanced NSCLC (9). The second most frequently investigated driver gene in NSCLC is probably *KRAS*. Many publications deal with its predictive significance in relation to EGFR-TKIs with equivocal results (10, 11). It is possible that this was due to differences in the impact of specific *KRAS* mutations as shown by several recently published studies (12, 22, 25).

Predictive value of *KRAS* mutation in patients treated with chemotherapy was investigated mainly for first-line treatment (13). However, the results of previous studies are contradictory as mentioned in a review by Martin *et al*. (14). Some authors point to the heterogeneity of patients from Asian and Caucasian populations, the various stages of the disease, different chemotherapy schedules and the small number of patients in these studies (14-16). Most of these studies did not record *KRAS* mutation as a significant predictive factor for first-line chemotherapy (10, 14, 17-20). The role of specific *KRAS* mutations was also investigated. Although Metro *et al*. described the greatest influence of mutations of codon 13, anothers published the effect of mutations of codon 12 on PFS (21-23). Nevertheless, even after testing the effect of *KRAS* mutations from liquid biopsy (to exclude tumor heterogeneity), unequivocal verification of predictive value of *KRAS* mutations for first-line chemotherapy failed (21).

There is much less evidence of the predictive utility of *KRAS* mutation for second-line treatment. We did not observe any significant difference in PFS for patients with *KRAS* mutation and those with wild-type *KRAS*. Similar results were obtained when we considered the possible effect of specific *KRAS* mutations. On the contrary, Sun *et al*. described a trend to shorter PFS for patients with *KRAS* mutation (24). The trend was more evident for those treated with gemcitabine regimens than with taxane regimens. However, patients in that study were treated with various lines of chemotherapy (first-, second- and third-line). Jänne *et al*. described a trend for longer PFS for patients with *KRAS* G12C or G12V mutations in a phase II study with a combination of docetaxel and novel Mitogen-activated protein kinase kinase inhibitor, selumetinib (25). Although the effect of selumetinib must be taken into account, these results are in contrast not only with our work but also with

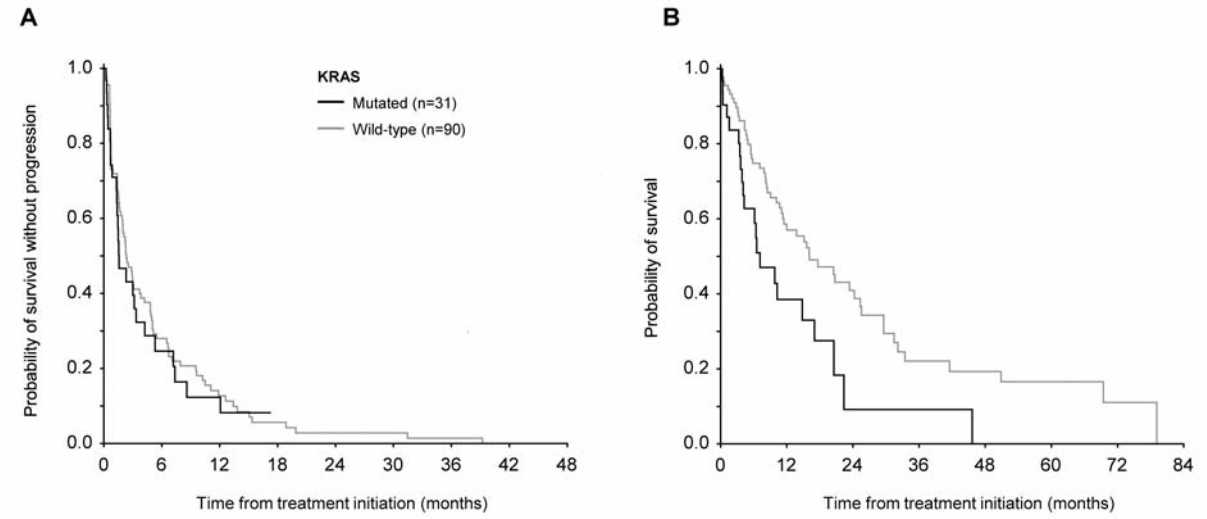

Figure 1. *Progression-free (A) and overall (B) survival from treatment initiation according to Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation status of patients treated with pemetrexed/docetaxel in second or third line.*

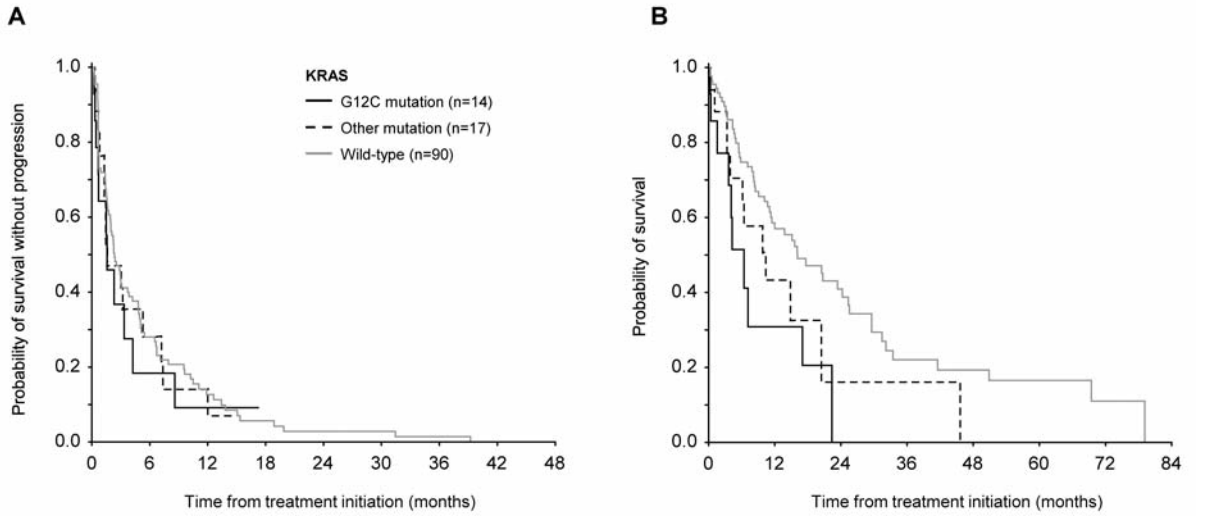

Figure 2. *Progression-free (A) and overall (B) survival from treatment initiation according to Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation of patients treated with pemetrexed/docetaxel in second or third line.*

other authors that reported worse outcomes in patients with *KRAS* G12C mutation (21, 23). In concordance with our study, there was no significant association between *KRAS* mutation and efficacy of docetaxel in second-line treatment in the TAILOR clinical trial (26). An association between folate metabolism and *KRAS* mutations, that could positively affect treatment with pemetrexed, has been documented (28, 29). A better overall response rate was published for patients with *KRAS* mutation treated with pemetrexed compared to erlotinib in the Hellenic Oncology Research Group clinical trial (27). However our results did not confirm such findings.

Due to the incoherent results in the field of the prognostic value of *KRAS* mutation, a large meta-analysis based on data from 12 randomized clinical trials was recently conducted by Ying *et al*. It showed worse OS for patients with *KRAS* mutation (19). However, it was focused on a relatively wide spectrum of patients with different stages, ethnicities, treatment protocols *etc*. Several studies of patients with advanced NSCLC treated with first-line chemotherapy showed poor prognosis of patients with *KRAS* mutation (22, 30, 31). On the other hand there are some studies with different results (14, 18).

Our data on patients receiving chemotherapy in the second and third line indicate the prognostic value of *KRAS* mutation. We recorded significant differences in OS between patients with *KRAS* mutation and those with wild-type *KRAS*. Sun *et al*. published similar results for patients treated with chemotherapy, but the study also included patients treated in first line (24). The study with selumetinib and docetaxel also mentioned the possible impact of *KRAS* mutation on prognosis in patients with higher lines of treatment (25). In contrary, the TAILOR trial did not document any prognostic effect of docetaxel (or erlotinib) in second-line treatment (26). However, neither of these studies calculated the potential effect of specific *KRAS* mutations on prognosis (23) and it is not clear how patients with various *KRAS* mutations were represented. We demonstrated a significantly worse OS for patients with *KRAS* G12C mutation compared to patients with other *KRAS* mutations. This could be related to the different metabolic pathways that are affected by different *KRAS* mutations (32, 33).

It is necessary to admit that there exist several limitations to our study. The most important limitation is its retrospective design. The next limitation is the relatively small number of patients in the study. Finally we cannot exclude the possibility that the results could have been partially influenced by subsequent treatment.

In conclusion, we found that the presence of *KRAS* mutation (especially *KRAS* G12C mutation) correlated with adverse prognosis in patients treated with second- or third-line pemetrexed or docetaxel.

Acknowledgements

This study was supported by the National Sustainability Program I (NPU I) Nr. LO1503 provided by the Ministry of Education Youth and Sports of the Czech Republic.

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Received December 16, 2015
Revised January 21, 2016
Accepted January 28, 2016