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Retrospective Data Analysis of Patients With Metastatic Lung Adenocarcinoma With or Without *KRAS*-Mutation or *TTF1*-Expression

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Abstract

Introduction: Patients with lung adenocarcinoma not expressing *TTF1* and those with a *KRAS* mutation have worse prognosis. However, available data are limited and sometimes contradictory. Therefore, this retrospective cohort analysis aimed to clarify whether there was a difference in overall survival and progression-free survival between these groups of patients.

Methods: In total, data derived from 181 patients with metastatic lung adenocarcinoma treated at the Martha-Maria Halle-Dölau Hospital from 2016 to 2019 were analyzed. Kaplan-Meier curves were generated, and associated values, such as median survival and its confidence intervals, were determined using the log-rank test.

Results: A benefit in overall survival (OS) (8.4 vs 5.8 months; HR, .8; 95% CI, .53-1.19; P = .267) was associated with positive *TTF1* expression, but this was not statistically significant. The same trend was shown with the progressive free survival (PFS) (6.5 vs 4.6 months; HR, .76; 95% CI, .51-1.20; P = .162). In patients with a *KRAS* mutation, there was no difference in OS compared to those with a wildtype *KRAS*. The median survival was almost identical at 7.5 months (*KRAS* mutation, 95% CI, 3.32-11.74) and 7.0 months (*KRAS* wildtype, 95% CI, 3.59-10.41). Additionally, in PFS, there was no difference between the 2 groups (5.8 vs 6.3 months).

Conclusions: Our analysis did not show a worse prognosis in patients with a *KRAS* mutation or in those with missing *TTF1* expression, which is most likely related to the new therapeutic options. As a result of the administration of immunotherapy in patients with a *KRAS* mutation and the change from a regimen containing pemetrexed to a regimen containing no pemetrexed, the corresponding patients no longer seem to have a worse prognosis.

Keywords

lung-cancer, adenocarcinoma, KRAS, TTF1, overall survival

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Introduction

Lung carcinoma remains the most common cause of cancer-related deaths with almost 1.8 million deaths worldwide every year.[1] At the time of initial diagnosis, half of the patients are already in a metastatic stage, with approximately 5% and 3% being the 5-year survival rate for women and men, respectively (Koch-Institut, Robert and Gesellschaft Der Epidemiologischen Krebsregister In Deutschland E.V. 2019). There are various histological subtypes, with adenocarcinoma (AC) being the most common subtype at approximately 40%.[2]Typical markers of AC include napsin A and thyroid transcription factor 1 (*TTF1*).[3] *TTF1* is particularly relevant as an immunohistochemical marker for the identification of the primary tumor metastasis or differentiation of carcinoma, as it indicates the development of lungs AC.[4] If another primary tumor is ruled out clinically, lung AC can also be *TTF1*

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negative as a result of a dedifferentiation of the tumor. Other studies have shown that approximately 80% of primary pulmonary AC are *TTF1* positive.[5-7] According to new findings, patients with *TTF1*-negative tumors have a worse prognosis, partly because they respond poorly to chemotherapy containing pemetrexed.[8]

Furthermore, driver alterations occur more frequently in AC than in other histological subtypes, which is why appropriate molecular testing should be performed before initiating systemic therapy for metastatic AC.[9] It is currently recommended to test for at least *EGFR* mutations in exons 18-21, *ALK* fusions, *ROS1* fusions, and *BRAF V600* mutations at the metastatic tumor stage.[9] Targeted therapy for the corresponding driver changes has decisively improved the prognosis of these patients.

The most common mutation in AC is detected in the *KRAS* gene. In general, *KRAS*-mutated carcinomas are associated with poor prognosis. Several meta-analyses have reported an increased risk of death in patients with a *KRAS* mutation.[10,11] It is unclear whether this association will continue to be applied in the future, as there are also studies showing that patients with the corresponding *KRAS* mutation respond better to immunotherapy, especially with *TP53* comutation.[12,13] Since January 2022, the first targeted therapy for *KRAS-G12 C* mutations in patients with progressive disease under at least 1 previous systemic therapy has been approved.[14] This could also decisively improve the prognosis of patients.

The aim of this retrospective cohort study was to clarify whether there is a difference in overall survival (OS) and progression-free survival (PFS) between patients with and without a *KRAS* mutation or *TTF1* expression.

Methods

A total of 181 patients with metastatic lung AC were treated at the Martha-Maria Halle-Dölau Hospital between 2016 and 2019 and complete mutation analysis was performed. In addition, age at initial diagnosis (ED), ECOG performance status, sex, stage at initial diagnosis (stages IVA and IVB or in patients with ED in 2016 only stage IV), smoking history, medical history, and possible occupational exposure to asbestos were recorded. The type of first- and second-line therapies (chemotherapy, immunotherapy, immunochemotherapy, tyrosine kinase inhibitors, radiation only, and none) was also noted. The end of the observation period was November 2020. Patients who are lost to follow up at 1 point in time and patients who were still alive or progression-free at the end of the observation period were listed as censored.

The methods used for mutation analysis were nucleic acid amplification by PCR of the sections coding for *KRAS* exon 2 codon 12/13, *EGFR* exon 18, 19, 20, 21, and *BRAF* exon 15 codon 600 with specific probes and subsequent sequencing with direct detection of potential point mutations with a sensitivity of 5% (*KRAS*, *BRAF*) or 20% (*EGFR*) mutated DNA.

Table 1. Patient demographics.

	Number of Patients
Age (median) (years)	66
Sex	
Male, n (%)	120 (66%)
Female, n (%)	61 (34%)
ECOG	
0	89 (49.2%)
1	75 (41.4%)
2	15 (8.3%)
3	1 (.6%)
4	1 (.6%)
Smoker status	
Never smoker	34 (18.8%)
Smoker	147 (81.2%)
UICC (7) IV	27 (14.9%)
UICC (8) IVA	74 (40.9%)
UICC (8) IVB	80 (44.2%)

To determine *ALK* and *ROS* status, chromogenic hybridizations (CISH) were used with the ZytoDot 2C SPEC ALK or ROS DNA probe combinations spanning the corresponding gene loci from Zytovision, showing a translocation by assigning the different colored signals in the sense of a so-called break-apart probe and subsequent counting of at least 50 tumor cells.

Statistical analyses were performed using SPSS 27 (IBM Corp., Armonk, NY, USA). Survival analyses first comprised a descriptive presentation of the cumulative survival functions according to Kaplan-Meier analysis, and differences among the curves were evaluated using the log-rank test. Hazard ratios were determined using Cox regression analysis. The chi-squared test was used to determine whether there could be an association between certain characteristics. The reporting of this study conforms to STROBE guidelines.[15]

Results

A total of 181 patients with metastatic lung AC were treated at the Martha-Maria Halle-Dölau Hospital between 2016 and 2019 and complete mutation analysis was performed.

The median age at the time of the initial diagnosis was 66 years (range 41-87 years). Approximately two-thirds of the patients were men (120%–66%) and one-third were women (61%–34%). The ECOG performance status was 0, 1, 2, 3, and 4 in 89 (49.2%), 75 (41.4%), 15 (8.3%), 1 (.6%), and 1 (.6%) patient, respectively. A total of 147 patients (81.2%) were current or former smokers and 34 patients (18.8%) had never smoked. The tumor stage at the time of the initial diagnosis was IVA in 74 patients, IVB in 80 patients by UICC8, and IV in 27 patients by UICC7. In 2016, according to UICC7, there was still no classification into stages IVA or IVB. Patient demographics are summarized in Table 1.

No mutations were found in approximately 60% of the tested patients. Among the driver changes, *KRAS* made up the largest proportion by far, with almost a quarter (24.9%) of all those tested, followed by *EGFR* (10.5%). *BRAF*, *ALK*, and *ROS* were rarely detected, accounting for 2.2%, 1.7%, and .6% of all the patients with mutation analysis, respectively (Table 2). The corresponding subtype was determined for 45 patients who tested positive for *KRAS* (Table 3).

The most common mutations were *G12 V* and *G12 C* in 15 and 14 patients, respectively, accounting for 33% and 31% of all *KRAS* mutations, respectively. The second most common mutation was *G12D* detected in 8 patients (18%). These 3 types together accounted for over 80% of all mutations in the *KRAS* gene in this sample. The remaining 18% were accounted for by *G12S*, *G12 A*, *G12 F* and 2 mutations in codon 13.

There was no association between sex and *KRAS* mutation (26% of men and 23% of women had KRAS mutations).

Table 2. Mutation analysis of all 181 patients.

Mutation	Number absolute	Percentage, %
None	109	60.2
KRAS	45	24.9
EGFR	19	10.5
BRAF	4	2.2
ALK	3	1.7
ROS1	1	0.6

The first-line therapies with respect to the *KRAS* status are listed in Table 4. About 20% of the patients in each group did not receive tumor-specific therapy. Mostly because of death before the start of the therapy, the high ECOG or as a patient decision. About 50% of the patients with *KRAS* mutation received immunotherapy or immunochemotherapy. When excluding patients with a treatable driver alteration, it is about the same percentage in patients with *KRAS* wildtype.

Among the 181 patients, 81.2% were current or former smokers and 18.8% had never smoked. Over 91% of patients with a *KRAS* mutation were current or former smokers, while only 78% of those with wildtype *KRAS* had ever smoked. This difference becomes even clearer when examining these values from a different perspective. The rate of *KRAS*-mutated tumors at 28% among current or former smokers was about twice as high as that of non-smokers (12%). The odds ratio was 2.90 (95% CI, .96-8.75). Using the chi-square test, a *P*-value of .05 was determined for the question of a possible association between smoking status and the presence of a *KRAS* mutation (Fisher: 0.076).

A comparison between patients with and without occupational exposure (OE) in terms of asbestos contact shows a very similar picture. Among the *KRAS* mutants, the proportion of patients with OE was 13.3%, approximately 2.5 times as high as that among the *KRAS* wildtypes (5.1%). At 46.2%, occupationally exposed individuals had *KRAS* mutations almost twice as often as patients without OE. The odds ratio therefore is 2.84 (95% CI, .90-8.93, P = .065; Fisher 0.092). If 1 compares patients with both OE and a history of smoking with nonsmokers without asbestos contact, the difference

Table 3. Analysis of the *KRAS* subtypes.

KRAS mutation	Number absolute	Percentage of all Patients, %	Percentage of the *KRAS* mutations, %
G12 V	15	8.3	33.3
G12 C	14	7.7	31.1
G12D	8	4.4	17.8
G12 A	3	1.7	6.7
G12S	2	1.1	4.4
G12 F	1	0.6	2.2
G13 C	1	0.6	2.2
G13X	1	0.6	2.2
Negative	136	75.1	

Table 4. First-line therapies with respect to *KRAS* status.

first-Line therapy	*KRAS* wildtype	*KRAS* mutated	*KRAS G12 C*	*KRAS G12 V*
Immunotherapy	7 (5.1%)	4 (8.9%)	3 (21.4%)	0
Chemotherapy	30 (22.1%)	12 (26.7%)	2 (14.3%)	7 (46.7%)
Immunochemotherapy	47 (34.6%)	19 (42.2%)	6 (42.9%)	5 (33.3%)
Tyrosine kinase inhibitor	18 (13.2%)	0	0	0
Only radiation	6 (4.4%)	0	0	0
None	28 (20.6%)	10 (22.2%)	3 (21.4%)	3 (20.0%)

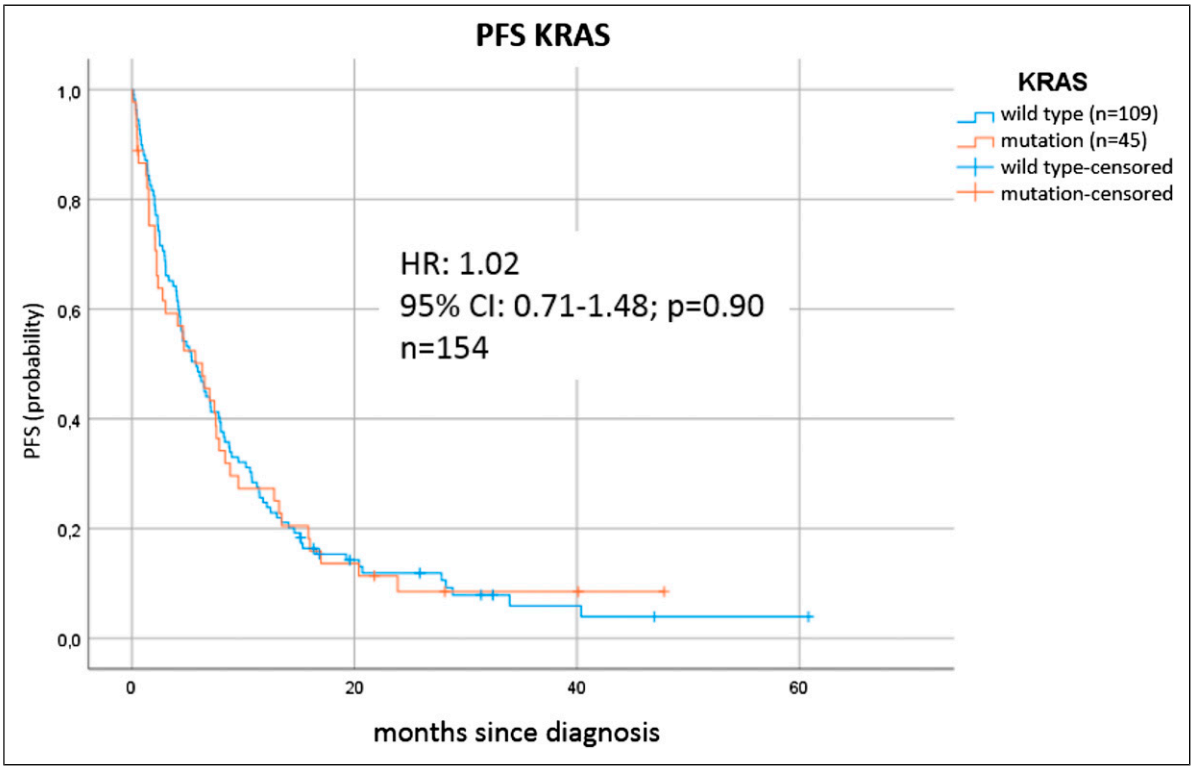

Figure 1. PFS of patients with a *KRAS* mutation and all patients carrying the wildtype gene without *EGFR*, *ALK*, *ROS1* and *BRAF* alteration.

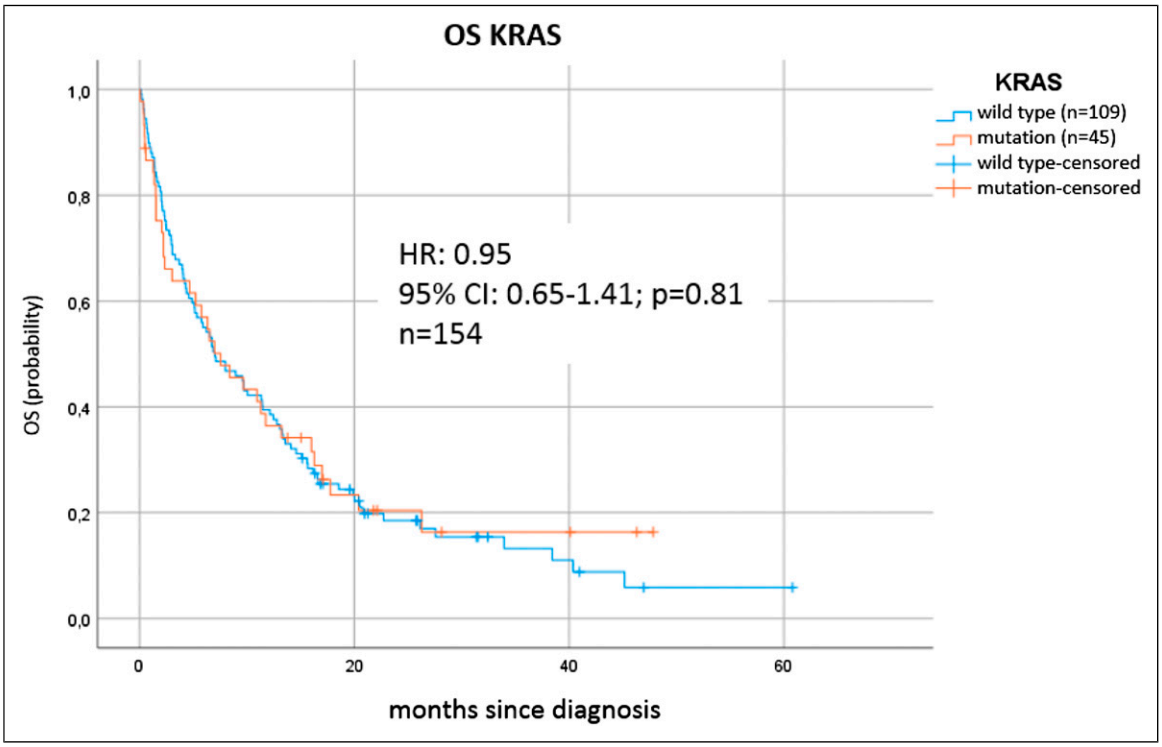

Figure 2. OS of patients with a *KRAS* mutation and all patients carrying the wildtype gene without *EGFR*, *ALK*, *ROS1*, and *BRAF* alteration.

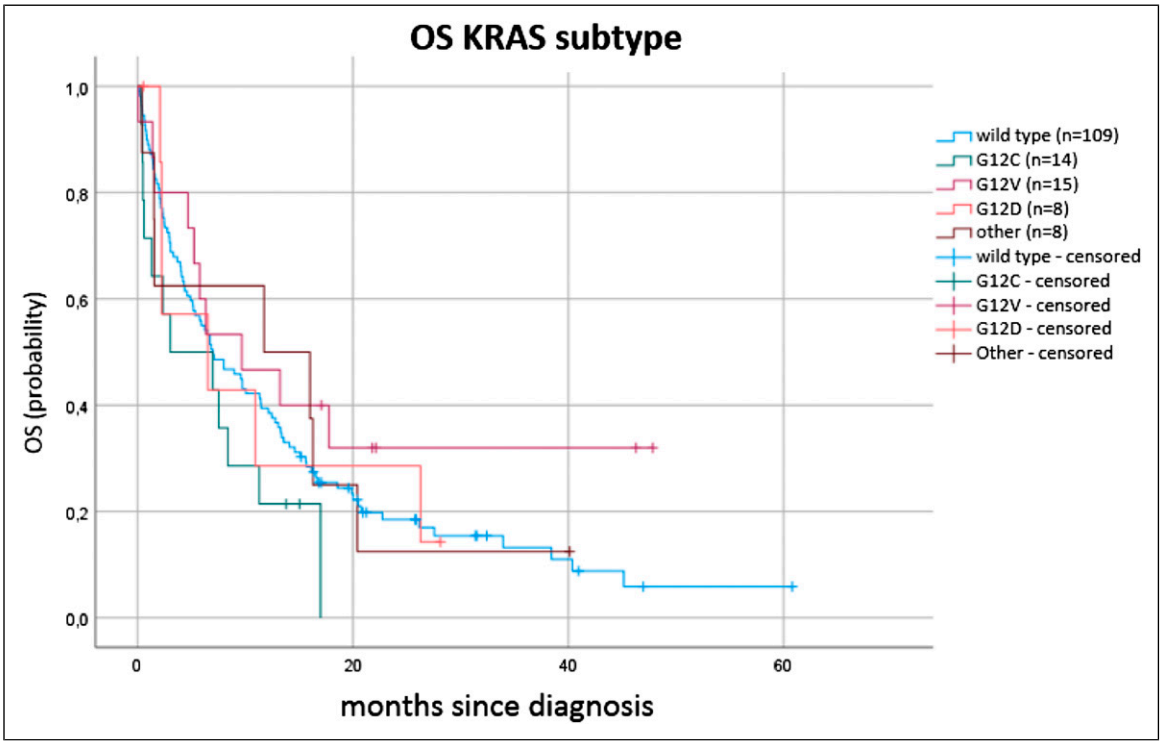

Figure 3. OS with respect to *KRAS* subtype.

Table 5. OS by *KRAS* subtype.

	P value (HR)	HR vs WT	95% CI (HR)	Median OS	95% CI (median)
KRAS wildtype				7.000	3.590-10.410
G12 C	.155	1.553	.847-2.849	3.033	.0-11.528
G12 V	.195	.648	.337-1.248	9.667	.241-19.092
G12D	.974	.986	.431-2.257	6.533	.0-17.568
Other	.851	.929	.430-2.006	11.733	0-.0-31.783

becomes even clearer. In the group exposed twice, *KRAS* mutations occurred more than 3 times as frequently. The odds ratio was 6.43 (95% CI, 1.42-29.08; P = .01; Fisher 0.017).

PFS and OS were analyzed according to *KRAS* status (Figures 1 and 2). Because of the known significantly better prognosis of patients with driver alterations that can be treated as first-line treatment, patients with *EGFR*, *ALK*, *ROS1* and *BRAF* alterations were excluded. In total, this corresponded to 154 patients, of whom 45 patients were *KRAS* mutated.

There were no differences in PFS between patients with or without the *KRAS* mutation. The median of patients carrying a *KRAS* mutation progressed at 5.8 months (95% CI, 4.00-7.53) vs 6.3 months (95% CI, 3.24-9.43) for patients carrying with wildtype gene. Even in multivariable regression, no prognostic influence of *KRAS* could be concluded from the values of this sample.

In terms of OS, there was also no difference between the patients carrying the mutated and wildtype *KRAS* gene. The median survival times were almost identical at 7.5 months (*KRAS* mutation, 95% CI, 3.32-11.74) and 7.0 months (wildtype, 95% CI, 3.59-10.41) for patients with the wildtype *KRAS*.

Finally, OS was analyzed with regard to *KRAS* subtypes (Figure 3, Table 5). All patients were also included, with the exception of those with *EGFR*, *ALK*, *ROS1* and *BRAF* alterations. Among them, there were 109, 15, 14, and 8 patients with the wildtype *KRAS*, a *G12 V* mutation, *G12 C* and *G12D* mutation, respectively. We combined the remaining 8 mutations in 1 study arm, since these groups would otherwise be too small for analysis.

Again, no significant differences between the groups could be determined. However, an interesting trend has emerged. Comparing patients with the *G12 C* mutation to those with the *G12 V* mutation yields an approximately twice as high risk of death for patients with *G12 C* mutation compared to those with *G12 V* mutation.

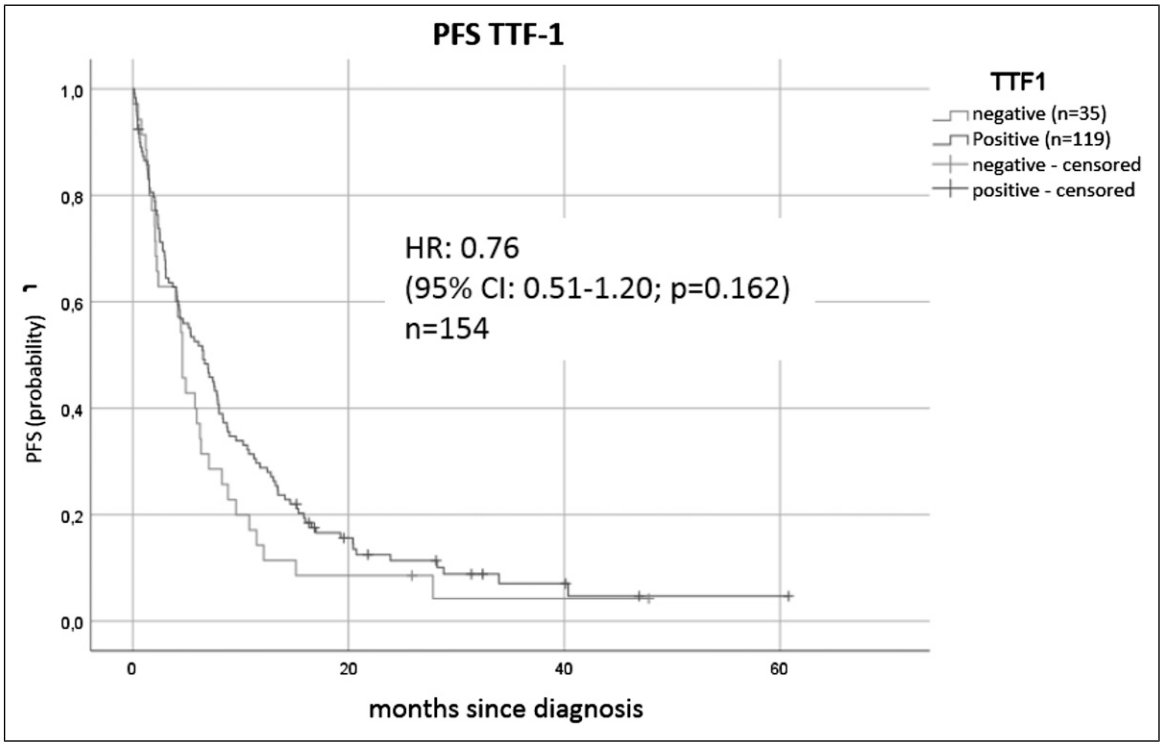

Figure 4. PFS with respect to *TTF1* status excluding patients with *EGFR*, *ALK*, *ROS1*, or *BRAF* alteration.

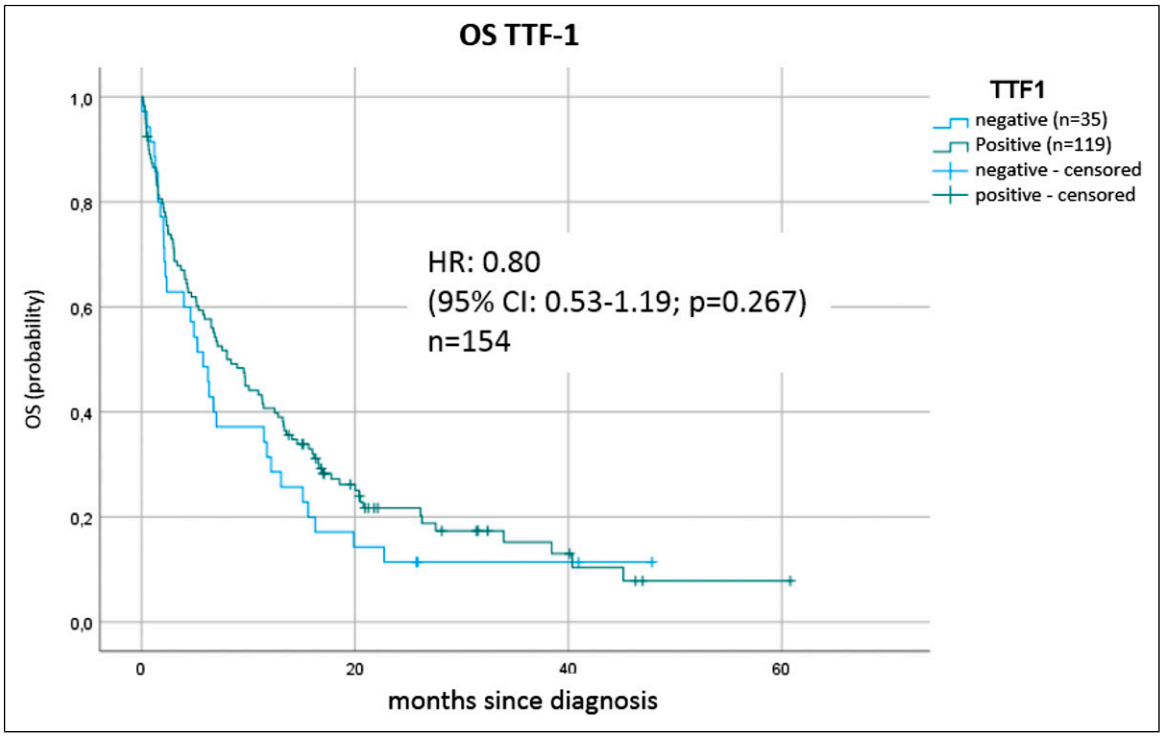

Figure 5. OS with respect to *TTF1* status excluding patients with *EGFR*, *ALK*, *ROS1*, or *BRAF* alteration.

The second part of the evaluation involved analysis of *TTF1* expression. A total of 146 (80.7%) of patients showed weak *TTF1* expression in the nuclei. Among the patients with *TTF1* expression, the proportion of women was higher at 35.6% (52 out of 146) than among the those who were negative for *TTF1* at 25.7% (9 out of 35). At least 85.2% of all women tested (52 out of 61) were *TTF1* positive, but only 78.3% of all men tested were positive (94 out of 120). When tested for the association between sex and *TTF1* expression, P = .266 (Chi^2), Fisher 0.322. Among non-smokers, the *TTF1* rate slightly increased to 85.3% (P = .448; Fisher 0.630). The *KRAS*-mutated group showed slightly lower *TTF1* expression (73.3% vs 83.1%; P = .151; Fisher 0.191). Among the 19 *EGFR*-mutated subjects tested, all subjects had *TTF1* expression (P = .024; Fisher 0.027).

For the analysis of PFS and OS of patients expressing *TTF1* compared to those not expressing *TTF1*, we included all patients except for those with *EGFR*, *ALK*, *ROS1* and *BRAF* alterations (Figures 4 and 5). Patients who do not express *TTF1* progressed after a median of 4.6 months (95% CI, 3.75-5.45) compared to 6.5 months (95% CI, 4.52-8.54) for patients with proven *TTF1* expression. The hazard ratio in this case was .76 (95% CI, .51-1.20) with a *P* value of .162.

While individuals without *TTF1* expression died after a median of 5.8 months (95% CI, 3.76-7.78), those with expression did not die until after 8.4 months (95% CI, 5.77-11.03). According to the HR, patients expressing *TTF1* showed only .80-fold (95% CI, .53-1.19; P = .267) higher risk of death.

Discussion

In this analysis, the mutation frequency of *KRAS*, at just under 25%, was slightly lower than that observed in other samples, which is most likely due to demographic and geographical differences.[16,17] A clear association between the occurrence of a *KRAS* mutation and current or former nicotine consumption and exposure to asbestos could be shown. This has also been described in other studies in the past.[18,19] The subtype analysis showed the *G12 V* mutation to be the most common subtype with a third of all *KRAS* mutations, closely followed by the *G12 C* mutation with a good 31%. All other subtypes occurred with a significantly lower frequency. Overall, 95% of all mutations were in codon 12 and almost 5% in codon 13. While the distribution between the codons corresponds to that of other studies, this sample shows a lower frequency of the *G12 C* mutation in favor of the *G12 V* mutation in comparison with previous work.[20,21] Similarly, this could most likely be due to demographic and geographical differences, but for our geographical region, there is no study that has analyzed the *KRAS* subtypes to a relevant extend. Based on our data, no conclusion can be drawn between nicotine use or asbestos exposure and the subtypes of the *KRAS* mutation.

When considering the PFS and OS of the patients with and without *KRAS* mutation, any relevant difference between the groups cannot be determined. Older studies have shown an increased risk of death for patients with a *KRAS* mutation. For example, in the study by Huncharek et al., patients with a *KRAS* mutation had a 2.35-fold increased risk of death compared to those carrying the wildtype *KRAS*.[10] However, in this study, there was no adjustment with the tumor stage, while in 1999, there were no further therapy options, such as immunotherapy. In addition, Meng et al. found a worse prognosis for patients with *KRAS* mutation in a meta-analysis in 2013 with approximately 7,000 patients (HR, 1.39; 95% CI, 1.24-1.55) [11]; however, no immunotherapy was used either.

There is already evidence that patients with the *KRAS* mutation respond better to immunotherapy than those with the wildtype *KRAS*.[12,13] In our analysis, approximately 64% of all patients with *KRAS* mutation had either first- or second-line immunotherapy either in combination with chemotherapy or as monotherapy. This can thus explain the lack of differences in PFS and OS in patients carrying the *KRAS* mutants compared to the wildtype gene. Further analyzes could be performed in the future, comparing patients with a *KRAS* mutation with and without immunotherapy with regard to PFS and OS.

Subtype analysis showed a slightly increased risk of death in patients with a *G12 C* mutation. The hazard ratio was 1.553 (95% CI, .847-2.849, P = .155), and the median survival was only approximately 3 months compared to 7 months. This shows a clear trend that should be verified using a larger number of cases. With a hazard ratio of .648 (95% CI, .337-1.248, P = .19), *G12 V* was also a subtype that could be associated with a different prognosis, this time with a risk reduction. Comparing these 2 mutations, the hazard ratio was 2.02 (95% CI, .84-4.87; P = .115), which was approximately twice the risk of death for patients with *G12 C* compared to those with *G12 V*.

An association between *G12 C* and poorer survival was reported in 2014 by Nadal et al. who examined 179 resected AC with known *KRAS* status in a retrospective study of OS. According to them, *G12 C* is a negative prognostic factor with a strong risk increase in OS (HR, 2.35; 95% CI, 1.35-4.10, P = .003) compared to the wild type, but also compared to other *KRAS* mutations.[22] In addition, Svaton *et al.* described a poorer prognosis for patients carrying a *KRAS* mutation, especially *G12 C*, compared to the wildtype gene.[23] In contrast, Cui *et al.* found no difference between the 65 *G12 C* mutants and 79 other patients carrying a *KRAS* mutation in their study of 346 patients with NSCLC (HR, 1.19; 95% CI, .78-1.80, P = 0, 39).[24] Spira et al. found no survival disadvantage for *G12 C* in a retrospective study of over 7,000 patients with NSCLC. A somewhat longer OS has been observed in this subgroup.[25] Cai et al. found a poorer prognosis for *G12D* than for *G12 C* and *G12 V* (P < .0001). They had 20, 24, and 16 patients at their disposal, respectively.[26]

The current data situation is very contradictory, and the individual studies are partly based on a small number of cases, since the respective subtypes comprise only a small proportion

of patients. According to our data, there is currently no reliable difference in OS by *KRAS* subtype, although the trend mentioned above exists for the KRAS-G12 C and *G12 V* mutations.

Looking at *TTF1* expression as the second part of our study, 80% of the patients had at least weak nuclear *TTF1* expression, whereby our values correspond to the results of other studies.[5,7] It is striking, however, that among the *EGFR* mutants, no patient tested negative for *TTF1* and the rate of the patients tested positive was therefore significantly higher than among those with *EGFR*-wildtype gene, in which only 78% showed nuclear *TTF1* expression (P = .018). This connection has been described by other authors.[7,27,28] For example, Somaiah et al. found a high negative predictive value of over 96% in the absence of an *EGFR* mutation in the case of negativity for *TTF1*.[27]

Regarding PFS and OS, the overall trend was that *TTF1*-negative patients had a poorer prognosis. In the case of OS, the Kaplan-Meier curve showed a separation of the 2 graphs after a few months, with a higher proportion of survival among the positive patients. This accounts for the largest difference in median OS of 8.4 months (95% CI, 5.77-11.03, positives) vs 5.8 months (95% CI, 3.76-7.78, negatives). However, the curves overlapped and almost matched at the end of the observation period. According to the hazard ratio, positive patients were at a .8-fold (95% CI, .53-1.19; P = .267) lower risk of death. The same trends were observed in the PFS (6.5 vs 4.6 month; HR, .76; 95% CI, .51-1.20; P = .162), but statistically more reliable due to the smaller number of censored cases. The graphs in the Kaplan-Meier curves did not overlap and were separated from each other at the end of the observation period, with a larger proportion of patients with progression-free disease among those who were expressing the *TTF1* gene.

A possible cause could be the poor response of patients with missing *TTF1* expression to pemetrexed-containing chemotherapy.[8] Almost 63% of *TTF1*-negative patients in our analysis were treated with a pemetrexed-containing regimen as first-line therapy. Over the course of time, based on the above-mentioned findings, pemetrexed therapy was already dispensed in our institution in this type of patient, and other regimens, such as a combination with a taxane, were selected instead. This is most likely the reason why, although there is a trend towards a poorer prognosis in *TTF1*-negative patients, it is not statistically relevant.

In summary, our analysis does not show a worse prognosis for patients with *KRAS* mutation or for those with missing *TTF1* expression, which is most likely related to new therapeutic options. As a result of the addition of immunotherapy in patients with *KRAS* mutation and the change from a regimen containing pemetrexed to a regimen containing no pemetrexed in patients with missing *TTF1* expression, the corresponding patients no longer seem to have a worse prognosis. This observation should be verified in larger samples in the future.

Abbreviations

AC	adenocarcinoma
ALK	anaplastic lymphoma kinase
BRAF	rapidly accelerated fibrosarcoma isoform B
CISH	chromogenic hybridizations
EGFR	epidermal growth factor receptor
KRAS	Kirsten rat sarcoma
OE	occupational exposure
OS	overall survival
PCR	polymerase chain reaction
PFS	progression-free survival
ROS1	proto-oncogene tyrosine-protein kinase -1
TTF1	thyroid transcription factor 1

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Declaration of conflicting interests

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Ethical Approval

A positive ethics vote for this retrospective study is available under number 2020-199 from the local ethics committee of Martin Luther University Halle-Wittenberg.

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