Precision Medicine and Imaging

Clinical Cancer Research

Clinical and Pathological Characteristics of *KEAP1*- and *NFE2L2*-Mutated Non–Small Cell Lung Carcinoma (NSCLC)

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Abstract

Purpose: *KEAP1* and *NFE2L2* mutations are associated with impaired prognosis in a variety of cancers and with squamous cell carcinoma formation in non–small cell lung cancer (NSCLC). However, little is known about frequency, histology dependence, molecular and clinical presentation as well as response to systemic treatment in NSCLC.

Experimental Design: Tumor tissue of 1,391 patients with NSCLC was analyzed using next-generation sequencing (NGS). Clinical and pathologic characteristics, survival, and treatment outcome of patients with *KEAP1* or *NFE2L2* mutations were assessed.

Results: *KEAP1* mutations occurred with a frequency of 11.3% ($n = 157$) and *NFE2L2* mutations with a frequency of 3.5% ($n = 49$) in NSCLC patients. In the vast majority of patients, both mutations did not occur simultaneously. *KEAP1* mutations were found mainly in adenocarcinoma (AD; 72%), while *NFE2L2* mutations were more common in squamous cell carcinoma (LSCC; 59%). *KEAP1* mutations were spread over the whole protein, whereas *NFE2L2* mutations were clustered in specific hotspot regions. In over 80% of the patients both mutations co-occurred with other cancer-related mutations, among them also targetable aberrations like activating *EGFR* mutations or *MET* amplification. Both patient groups showed different patterns of metastases, stage distribution and performance state. No patient with *KEAP1* mutation had a response on systemic treatment in first-, second-, or third-line setting. Of *NFE2L2*-mutated patients, none responded to second- or third-line therapy.

Conclusions: *KEAP1*- and *NFE2L2*-mutated NSCLC patients represent a highly heterogeneous patient cohort. Both are associated with different histologies and usually are found together with other cancer-related, partly targetable, genetic aberrations. In addition, both markers seem to be predictive for chemotherapy resistance. *Clin Cancer Res; 24(13); 3087–96. ©2018 AACR.*

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Introduction

Systemic therapies targeting oncogenic aberrations in non–small cell lung cancer (NSCLC) have dramatically improved the outcome of genetically defined subgroups. Exemplarily, NSCLC patients with activating *EGFR* mutations, *ALK* or *ROS1* rearrangements benefit from tyrosine kinase inhibitor (TKI) treatment in terms of response and survival (1–6). It thus is a critical need to further identify and characterize genomic aberrations in NSCLC, which could either act as therapeutic targets themselves or modify response to targeted treatment.

The KEAP1–NRF2 (protein encoded by the *NFE2L2* gene) pathway plays a critical role in oxidative stress response by triggering antioxidant and anti-inflammatory effects (7). In healthy tissue KEAP1 counteracts NRF2 by leading to its degradation (7–11). Being exposed to oxidative stress, KEAP1 is inactivated and no longer able to bind and control NRF2, which is subsequently stabilized and translocates into the nucleus (7, 9, 12). There, KEAP1 promotes transcription of genes encoding detoxifying enzymes and antioxidative stress proteins (13–15).

Mutations in the KEAP1/NRF2-pathway are known to be involved in malignant transformation in various cancer types (16–24). Somatic loss-of-function mutations of *KEAP1* lead to

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Translational Relevance

In the present study, we show that *KEAP1* and *NFE2L2* mutations represent a heterogeneous NSCLC subgroup. Despite preclinical models showing a close interaction of these mutations in transformation, they occur nearly mutually exclusive in NSCLC and are associated with different histologies. Their frequent co-occurrence with other cancer-related mutations in line with the clinical heterogeneity argues against a role as "driver" mutations and stimulates further experiments investigating a modifier role, particular in tumors carrying already established drivers. In addition, our results suggest using NGS-based molecular multiplex diagnostics in clinical research to cover not only already established driver mutations but also potentially modifying co-occurring mutations. Finally, this work provides further evidence for a role of both *KEAP1* and *NFE2L2* mutations in chemoresistance.

an increase of NRF2 in the nucleus (16, 19, 25). Somatic gain-of-function mutations of *NFE2L2* are found near or within pivotal binding motifs (17) and interrupt binding of NRF2 to KEAP1 dimers (26). This leads to an increase of (i) intracellular NRF2, (ii) the synthesis of antioxidant and detoxification enzymes, and (iii) the production of drug efflux pumps in cancer cells (16, 19, 21, 26).

KEAP1 or *NFE2L2* mutations promote cell proliferation in tumors and may also participate in causing resistance to chemotherapy (19, 21, 27). Downregulation of *NFE2L2* or overexpression of *KEAP1* both triggered chemotherapy sensitivity (21, 26–29). In a squamous-cell carcinoma lung cancer (LSCC) mouse model, *KEAP1* mutations were associated with carcinoma formation and tumor aggressiveness, and both *KEAP1* and *NFE2L2* mutations promoted resistance against radiotherapy (RT; ref. 30).

It has recently been demonstrated that NSCLC patients with *KEAP1* mutation in addition to an activating *KRAS* mutation have a worse prognosis compared with *KRAS*-mutated patients without *KEAP1* mutation (31). The co-occurrence of *KEAP1* mutations generally describes a biologically unique subtype of *KRAS*-mutated NSCLCs (32). In adenocarcinoma of the lung (AD), patients with a high mutational load and benefit from anti-PD1 treatment also showed a high prevalence of *KEAP1* mutations (33). Furthermore, *NFE2L2* mutations were found to be associated with high PD-L1 expression in LSCC (34). It thus suggests a link between these mutations and a possible benefit from immune-checkpoint inhibition.

So far, however, little is known about the clinical presentation of NSCLC patients harboring mutations in either *KEAP1*, *NFE2L2*, or both as well as on their impact on systemic NSCLC treatment. In the present study, we describe and analyze the clinical and genetic characteristics of NSCLC patients with *KEAP1* or *NFE2L2* mutations and compare both groups with each other. We further evaluated the survival of these patients and their responses on systemic treatments.

Materials and Methods

Patients

Patient data was analyzed consecutively within the Network Genomic Medicine (NGM) Lung Cancer. NGM is a German health care provider network where next-generation sequencing (NGS)-based molecular diagnostics of lung cancer is performed centrally for about 280 hospitals and private-practices-based oncologists (www.ngm-cancer.com). Incoming formalin-fixed paraffin-embedded (FFPE) lung cancer samples were analyzed from 2011 to 2013 and from Mai 2015 to August 2015 at the Institute of Pathology, University Hospital of Cologne (Cologne, Germany). Screening procedures and data assessment were performed in accordance with local standards. Data assessment was approved by the responsible ethics committee (ref. number 10-242), and all patients consented for data analysis.

The cohorts consisted of both an establishment cohort and a validation cohort for the implementation of NGS in routine lung cancer diagnostics (35). Patients of both cohorts were not preselected regarding smoking history, age, stage, or sex.

Samples and immunohistochemistry

Histopathologic diagnostics was performed centrally per local standard operating procedures. The histopathologic differentiation between AD and SQCC was based on immunohistochemical staining [CK5/6, CK7, p40, and thyroid transcription factor 1 (TTF1)] as previously published (36).

Next-generation sequencing (NGS) and FISH diagnostics

NGS was performed using a MiSeq benchtop sequencer as described previously (Illumina; ref. 35; Supplementary Table S1). We used an in-house algorithm to call for genomic variants of the targeted sequences (37). The variants were then stored in a FileMaker (Filemaker GmbH, Germany) database for further analyses. After reporting, we used COSMIC (http://cancer.sanger.ac.uk/cosmic), OncoKB (http://oncokb.org), and CancerHotspots (http://cancerhotspots.org) databases for further annotation. *In silico* evaluation on the impact of the detected mutations was made with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2).

In a subset of patients, FISH were carried out to determine rearrangements and/or amplifications (Supplementary Table S6). *MET* and *FGFR1* amplifications were tested and classified as reported (38, 39). *FGFR1* was analyzed routinely in all LSCC patients, whereas *MET* amplification testing by FISH was routinely introduced for AD in June 2014.

Clinical characteristics

We assessed age, sex, tumor stage (UICC), TNM status, smoking status (qualitatively following CDC reporting; ref. 40, and in pack-years), exposure to other known pulmonary carcinogens, histology, metastases and treatment modalities and their outcome, if available. Outcome was provided by the network partners and followed Response Evaluation Criteria in Solid Tumors V1.0 (RECIST), proposing that stable disease (SD) and responses [partial response (PR), complete response (CR)] need to be confirmed by a CT scan not earlier than 4 weeks after the initial response assessment. If restaging was not performed or available, we asked for investigators' assessments. Staging and restaging procedures were performed in accordance with local standards from each partner, usually including CT scans of the involved areas and MRI scans for brain metastases in all patients.

Statistics and software

Qualitative variables (e.g., sex or smoking status) were summarized by count and percentage, quantitative variables by mean

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and standard deviation or median and range. Distribution of time-to-event was described by the Kaplan–Meier estimator. Follow-up was assessed using "reversed" Kaplan–Meier statistics. Association between categorical variables was assessed using χ^2 tests or Fisher exact tests, if appropriate. Statistical analyses were performed using SPSS v23.0 (IBM Corp.). Plots were generated using GraphPad Prism 6 (GraphPad Software Inc.). For the "lollipop charts," we used cBioPortal for Cancer Genomics' MutationMapper (http://www.cbioportal.org/mutation_mapper.jsp).

Results

KEAP1 mutations

Tumor tissue of 1,391 NSCLC patients was analyzed using NGS (Fig. 1; Supplementary Table S1). *KEAP1* mutations were detected in 157 patients (11.3%). Up to 134 different mutations were identified (Supplementary Table S2). They are spread over the whole *KEAP1* length (Fig. 3A). The most common mutations were Q620 deletions ($n = 5$), R320L ($n = 4$), R320W ($n = 4$), and V369A ($n = 3$). No hotspot mutations could be identified (Fig. 3A).

NFE2L2 mutations

Forty-nine patients (3.5%) had 30 different *NFE2L2* mutations (Supplementary Table S3). The most common mutations were W24R ($n = 5$), R34Q ($n = 5$), W24C ($n = 4$), E79K ($n = 4$), and R34G ($n = 3$). Clusters of mutations were observed in region E79 ($n = 5$), R34 ($n = 11$), and W24 ($n = 9$; Fig. 3A). R34 and E79 have been described as hotspot regions in human cancer, whereas W24 has not (41). These hotspot regions are near or within the ETGE and DLG motifs, which are the KEAP1 binding domains of NFE2L2 (ref. 17; Fig. 3A).

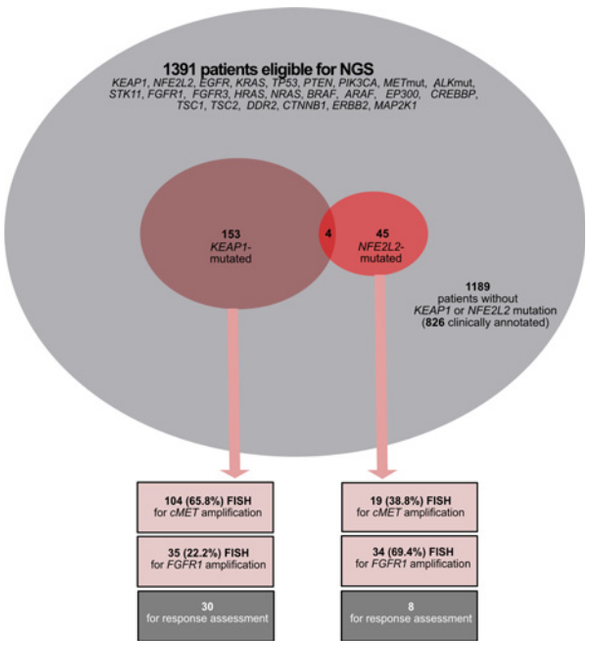

Figure 1.
Analytical flow sheet of the study. Euler diagram showing the proportions of mutated patients compared with the total cohort.

Clinical characteristics of patients with KEAP1 and NFE2L2 mutations

Detailed clinical characteristics of the cohort are provided in Table 1. A complete listing of the control group is provided in Supplementary Table S8. In both patients with *KEAP1* mutation (*KEAP1* group) and in patients with *NFE2L2* mutation (*NFE2L2* group), more males were affected. The same was true for the control group (Fig. 2A). There was no gender bias in this dataset ($P = 0.51$). The median age at diagnosis was 63.7 years (range, 40–84) for the *KEAP1* group and 66.6 years (range, 30–81) for the *NFE2L2* group.

Smoking status was assessable in 112 patients with *KEAP1* mutation and 35 patients with *NFE2L2* mutation. Both groups were characterized by strong smoking exposure, with only 6 never smokers in the *KEAP1* group and three in the *NFE2L2* group. In contrast, the control group consisted of 19.4% never-smokers (Fig. 2B). Median quantity of pack-years was 35 for *KEAP1* (range, 0–100) and 40 for *NFE2L2* (range, 0–120). Three patients in the *NFE2L2* group had a history of asbestos exposure and one had been exposed to quartz dust, while there was not a comparable exposure documented within the *KEAP1* group.

Patients with *KEAP1* mutation had predominantly AD (72.2%), comparable to the control group with 73.8% AD. In contrast, LSCC was the most frequent histology subtype with 59.2% in the *NFE2L2* group, followed by AD (32.7%; Fig. 2C, $P < 0.01$). In both groups, most patients had metastasized disease (stage IV) at first diagnosis, significantly more frequent in the *KEAP1* group (71.0% vs. 51.2%, $P = 0.03$).

Eastern Cooperative Oncology Group's Performance state (ECOG) was documented in 105 patients in the *KEAP1* group and 26 in the *NFE2L2* group. While ECOG 1 and ECOG 2 had a similar distribution pattern in both groups, there were differences for ECOG 0 (21.9% vs. 26.9%), ECOG 3 (7.6% vs. 3.8%), and ECOG 4 (1.9% vs. 0%). The difference in ECOG 3 plus 4 in both groups was not significant ($P = 0.46$). In the control group, nearly half of the patients presented with ECOG 0 (Fig. 2D).

In the *KEAP1* group, a high frequency of distant metastases affecting bones, brain, liver, skin, and spleen could be seen in stage IV patients, whereas patients in the *NFE2L2* mutation more frequently suffered from local spread (i.e., lung, pleura, mediastinal lymph nodes; Fig. 2E and F).

There were 4 patients with both a *KEAP1* mutation and a *NFE2L2* mutation. All four patients had a history of smoking and three patients had LSCC, while only 1 patient had AD. There were no further similarities regarding clinical characteristics and co-occurring aberrations between those patients. We performed a Fisher exact test, which revealed a z-pooled $P > 2.2^{-16}$ for mutually exclusivity.

Co-occurring aberrations

We found additional genomic aberrations in 87.3% of the *KEAP1* group and 83.7% of the *NFE2L2* group (Fig. 3B; Supplementary Tables S4 and S5). *TP53* mutations were the most common co-occurring mutations in both groups (44.9%, KEAP1; 40.8%, NFE2L2). *EGFR* mutations also showed a similar distribution pattern occurring in 6.3% (KEAP1) and 6.1% (NFE2L2). In both patient groups *MET* amplifications occurred at high frequency (18.3%, KEAP1; 26.3%, NFE2L2; Supplementary Table S6). Fitting to the correspondent histological distributions, we detected differences regarding the co-occurrence of *KRAS*

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Table 1. Comparison of clinical characteristics of patients with *KEAP1* mutations versus patients with *NFE2L2* mutations

Characteristics	Patients (%) *KEAP1*		Patients (%) *NFE2L2*		Patients (%) Control	
Number of patients	157 (100)		49 (100)		826 (100)	
Age at diagnosis						
Median		63.7		66.6		67.0
Range		40–84		30–81		23–93
Sex						
Male	95 (60.5)		27 (55.1)		471 (57.0)	
Female	62 (39.5)		22 (44.9)		355 (43.0)	
ECOG						
0	23 (14.6)		7 (14.3)		293 (35.4)	
1	55 (35.0)		14 (28.6)		211 (25.5)	
2	17 (10.8)		4 (8.2)		54 (6.5)	
3	8 (5.1)		1 (2.0)		39 (4.7)	
4	2 (1.3)		0 (0.0)		3 (0.3)	
5	0 (0.0)		0 (0.0)		3 (0.3)	
NA	52 (33.1)		23 (46.9)		233 (28.2)	
Smoking history						
Never smoker	6 (3.8)		3 (6.1)		75 (9.1)	
Former smoker	37 (23.6)		8 (16.3)		153 (18.5)	
Current smoker	69 (43.9)		24 (49.0)		159 (19.2)	
N/A	45 (28.7)		14 (28.6)		439 (53.1)	
Pack years	86		28			
Median (range)		35 (0–100)		40 (0–120)		
Histology	158		49		826	
Squamous cell carcinoma	28 (17.7)		29 (59.2)		140 (16.9)	
Adenocarcinoma	114 (72.2)		16 (32.7)		610 (73.8)	
Adenosquamous carcinoma	5 (3.2)		1 (2.0)		4 (0.5)	
Others/NA	11 (7.0)		3 (6.1)		72 (8.7)	
Stage at diagnosis	158		49		826	
I	10 (6.3)		8 (16.3)		20 (2.4)	
II	6 (3.8)		4 (8.2)		15 (1.8)	
III	24 (15.2)		9 (18.4)		104 (12.6)	
IV	98 (62.0)		22 (44.9)		451 (54.6)	
N/A	20 (12.7)		6 (12.2)		236 (28.6)	
T stage (TNM)	158		49			
T1	20 (12.7)		5 (10.2)			
T2	34 (21.5)		11 (22.4)			
T3	22 (13.9)		8 (16.3)			
T4	43 (27.2)		17 (34.7)			
N/A	39 (24.7)		8 (16.3)			
N stage (TNM)	158		49			
N0	28 (17.7)		12 (24.5)			
N1	16 (10.1)		6 (12.2)			
N2	41 (25.9)		9 (18.4)			
N3	33 (20.9)		13 (26.5)			
N/A	40 (25.3)		9 (18.4)			
Metastases localization (stage IV), thereof	98		22			
Bones	27 (27.6)		2 (9.1)			
Brain	19 (19.4)		2 (9.1)			
Lung	20 (20.4)		9 40.9)			
Pleura	12 (12.2)		3 (13.6)			
Distant lymph nodes	9 (9.2)		3 (13.6)			
Adrenal gland	7 (7.1)		4 (18.2)			
Liver	11 (11.2)		1 (4.5)			
Skin	3 (3.1)		0 (0.0)			
Spleen	1 (1.0)		0 (0.0)			
Stomach	2 (2.0)		0 (0.0)			
Pancreas	0 (0.0)		1 (4.5)			

($P = 0.03$), *PTEN* (n.s.), *PIK3CA* (n.s.) mutations and *FGFR1* amplifications (Supplementary Table S6; Fig. 3B). Out of the reported mutations, we identified only one *TP53* polymorphism (P72R) in both groups, six proposed *MET* polymorphisms (T1010I), and one *EGFR* P848L. The differences in mutational profile is strongly supported by data on gene expression differences and mutational data (42, 43).

Survival

Median follow-up for stage IV patients was only 1.8 months (95% CI, 0.0–3.7 months) for the *KEAP1* group ($n = 80$) and 5.3 months (95% CI, 2.1–8.5 months) for the *NFE2L2* group ($n = 21$). Thus, we abstained from further survival analyses. The median OS of these patients with *KEAP1* mutation was 19.1 months (95% CI, 1.8–36.3 months) and 14.0 months

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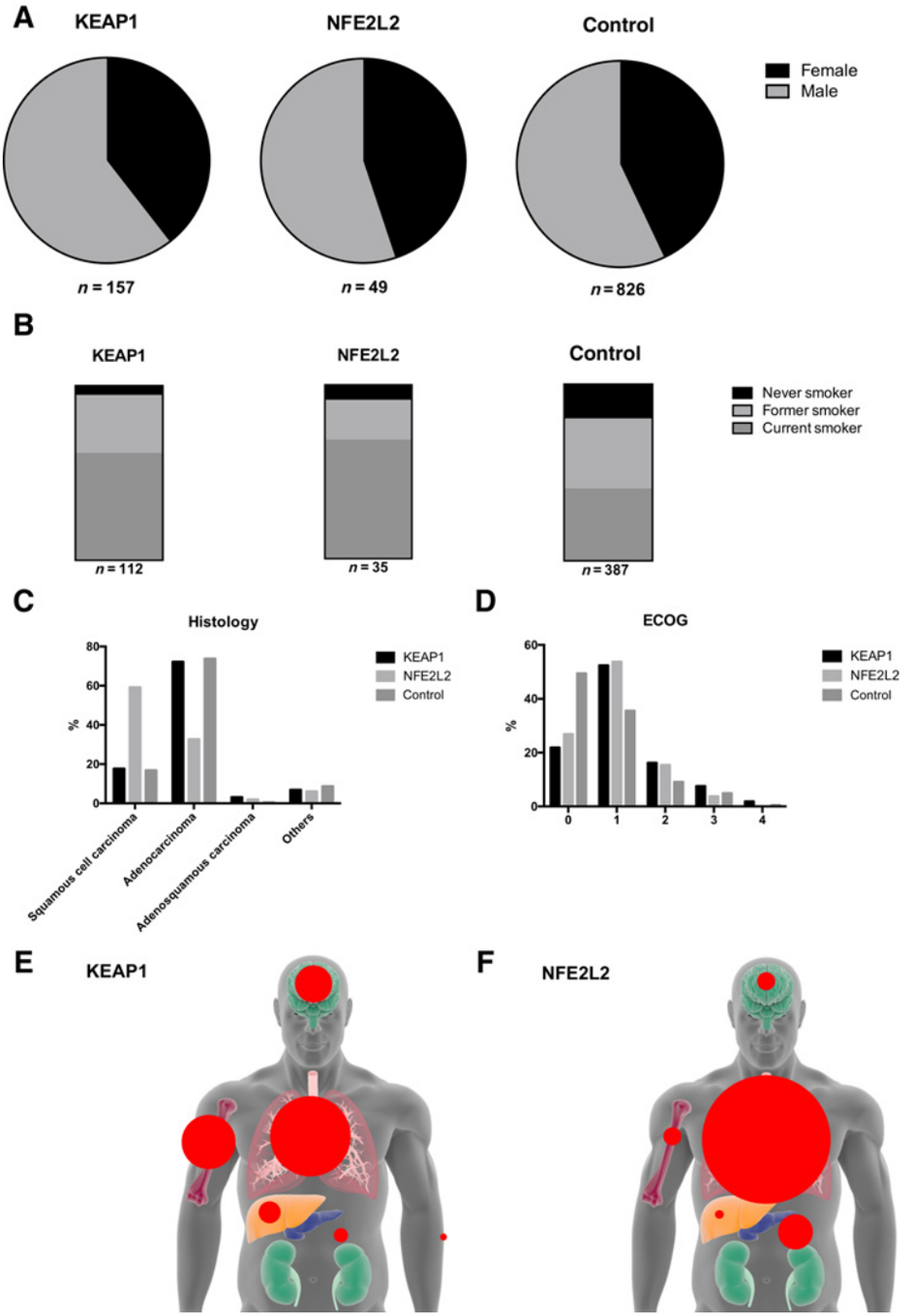

Figure 2.
Clinical characteristics of patients with *KEAP1* and *NFE2L2* mutations. **A,** Gender distribution. **B,** Smoking history. **C,** Histologic subtypes. **D,** Eastern Cooperative Oncology Group's Performance state (ECOG) at diagnosis. **E** and **F,** Distribution of metastatic sites in stage IV patients. Body and organ pictures taken from the Library of Science & Medical Illustrations under the CC BY-NC-SA 4.0 license.

(95% CI, 5.6–22.3 months) for patients with *NFE2L2* mutation (Fig. 3C).

Response assessment

For 30 patients with *KEAP1* mutation, details about systemic therapy were assessed. Twenty-seven patients had additional aberrations (Supplementary Table S7). These patients received between one and three lines of chemotherapy or TKIs as systemic therapy. Most patients (28) did not respond to first-line chemotherapy [progressive disease (PD)]. Both two patients with SD had an activating *EGFR* mutation and one of them received erlotinib and bevacizumab, the other had SD under carboplatin/paclitaxel, representing the only non-PD *KEAP1* patient treated with conventional chemotherapy. Due to the short duration of first-line treatment, 17 patients (56.7%) received systemic first-line therapy. Outcome analysis was available for 8 patients, which all developed PD regardless of therapy regimen. In third-line, 3 patients received therapy. Two of them had a PD but the outcome of the other patient is not known (Supplementary Table S7). There was no responder in none of the therapy lines.

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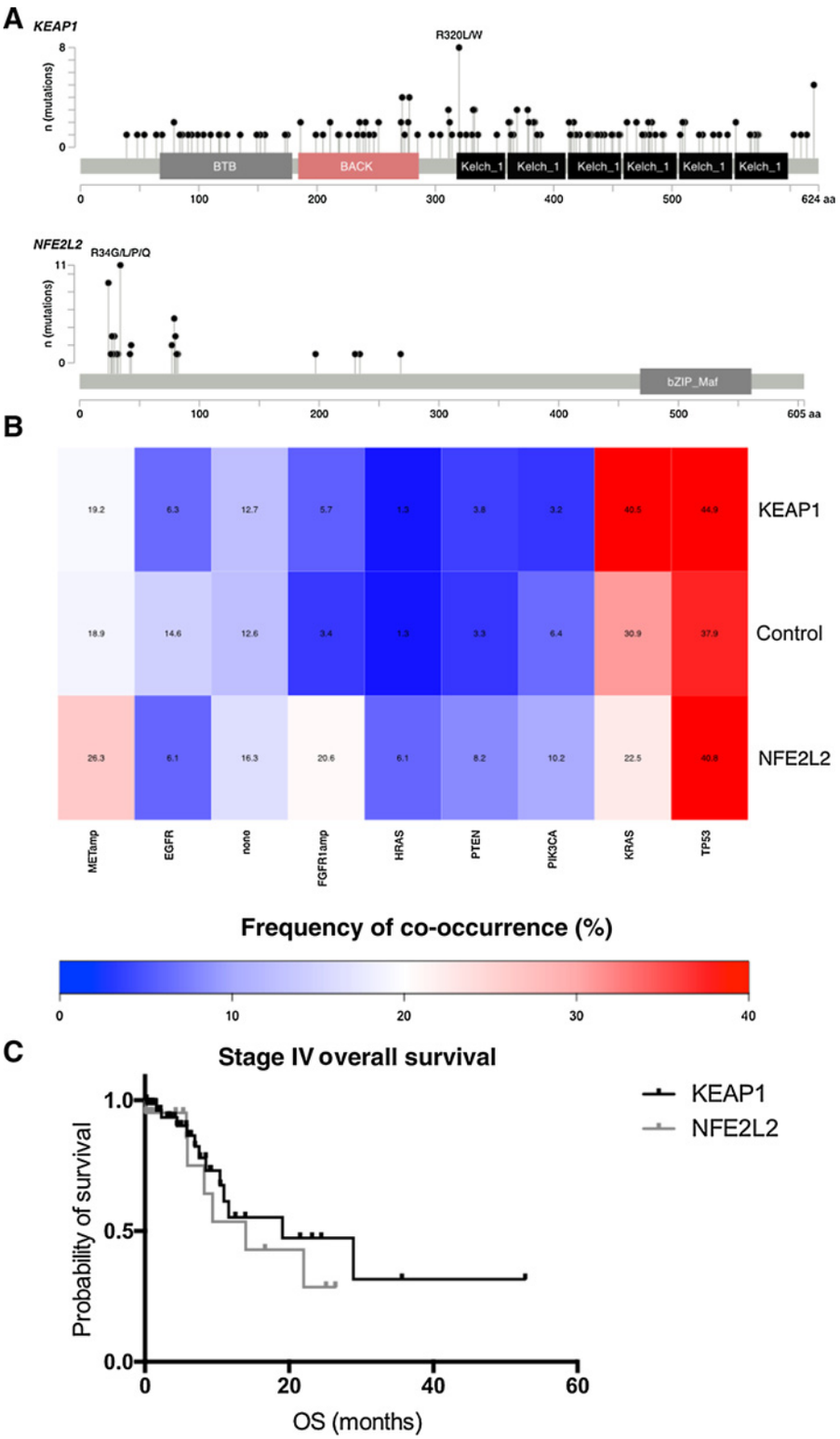

Figure 3.
Results of sequencing analyses and survival analyses. **A,** Distribution of *KEAP1* mutations and *NFE2L2* mutations among the genomic position. **B,** Frequency of co-occurring mutations in percentage (only mutations with ≥5% in one of the subgroups). **C,** Overall survival (Kaplan–Meier curve) in stage IV patients with *KEAP1* mutations and *NFE2L2* mutations ($n = 101$).

Eight patients with *NFE2L2* mutation receiving systemic therapy were evaluable for outcome analysis. Only one patient did not present an additional aberration. Four patients developed PD during first-line therapy, leading to a significantly better outcome regarding non-PD first-line as compared with the *KEAP1* group ($P = 0.01$, Fig. 4B). Two patients had a SD as best outcome

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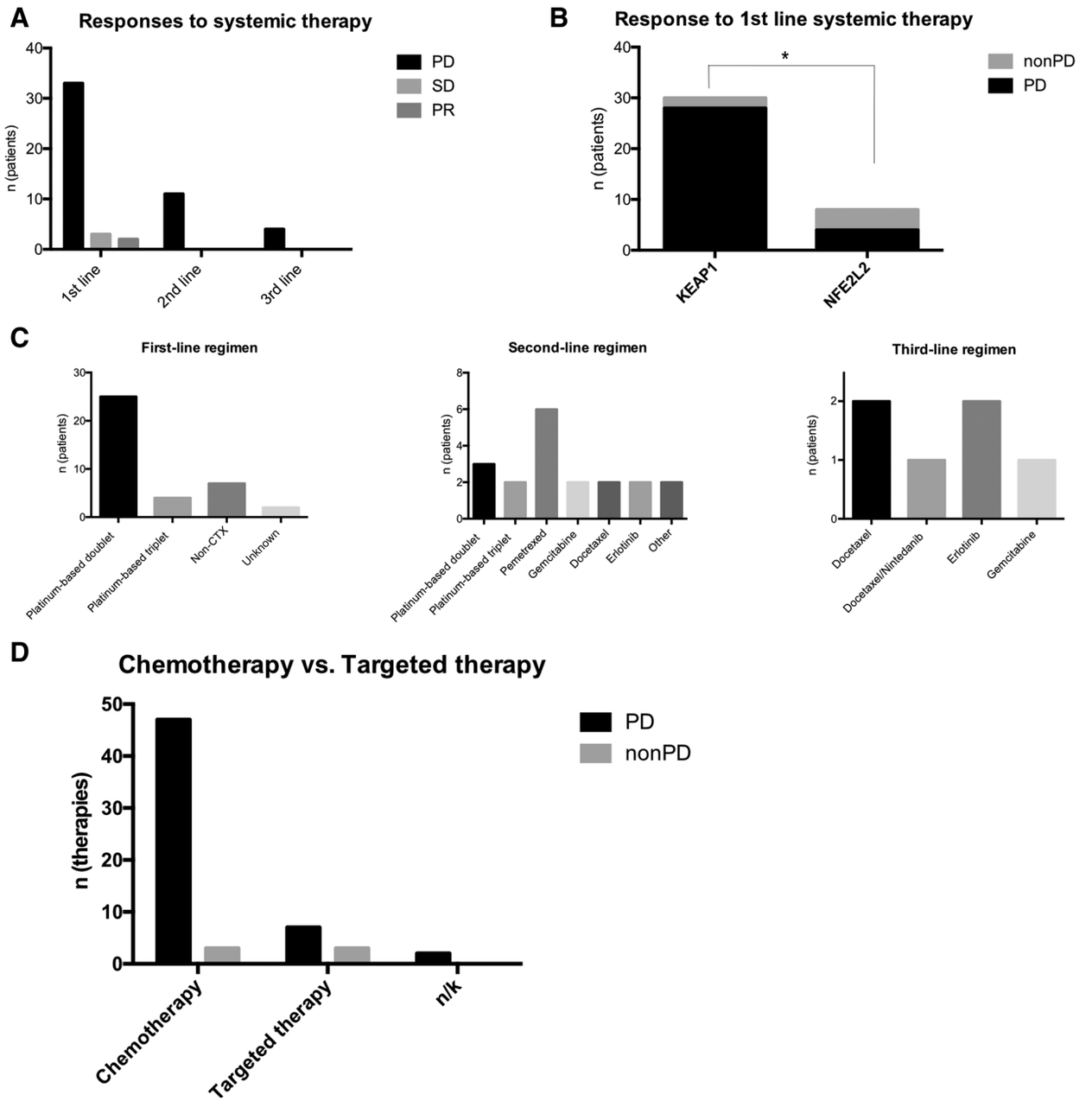

Figure 4.

Treatment outcome. **A,** Responses to systemic therapy according to the therapy line. **B,** PD vs. non-PD in first-line systemic therapy. **C,** Regimen used in first-line therapy, second-line therapy, and third-line therapy. **D,** Progressive disease (PD) versus non-progressive disease (non-PD) of chemotherapy-based and targeted therapy-based therapies regardless of therapy line.

and two patients had a partial response (PR). Of these 4 patients with benefit from therapy, 2 patients received erlotinib and bevacizumab and 1 of these 2 patients had an activating *EGFR* mutation and responded. The third patient harbored an additional *STK11* mutation and received carboplatin and etoposide resulting in a PR, representing the only responder to a platinum-based regimen in the cohort. The fourth patient did not harbor a detectable comutation and received cisplatin, pemetrexed, and bevacizumab, developing a SD. Two patients received both second- and third-line treatments without response (Fig. 4A).

Out of the 38 patients with an outcome analysis 16 patients harbored an additional *TP53* mutation. Twelve out of 32 patients (37.5%) with a PD during first-line therapy had a *TP53* mutation. In contrast, also 4 out of 6 patients (66.6%) with a non-PD during first-line therapy had *TP53* mutations. We therefore conclude that

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chemoresistance in our cohort is not biased by the co-occurrence of *TP53* mutations.

Comparing treatment with regard to the therapy used regardless of therapy line, 62 regimens could be identified in 38 patients, whereof 50 were chemotherapy treatments and 10 erlotinib-based treatments (± bevacizumab). Two lines could not be identified retrospectively. In each group, we found one PR and two SD, respectively (Fig. 4D).

Discussion

To the best of our knowledge, this is the largest cohort of NSCLC patients with *KEAP1* and *NFE2L2* mutations analyzed so far regarding clinical and pathologic characteristics. Both mutations are discussed to play a pivotal role in cancer formation and maintenance. We detected *KEAP1* mutations with a frequency of 11.3% and *NFE2L2* mutations with a frequency of 3.5% among a European all-comer NSCLC cohort.

Surprisingly, even though both types of mutations affect the same pathway and show a close functional interaction, they only exceptionally co-occur within the same tumor. Thus, only 4 patients in our cohort harbored a *KEAP*1 and a *NFE2L2* mutation simultaneously. Moreover, both mutations are associated with different NSCLC histologies, as well as their known expression patterns (43). While most *KEAP1* mutations occurred in patients with AD, patients with *NFE2L2* mutations mostly presented with LSCC. On a molecular level, *KEAP1* mutations were not found in specific hotspot regions, but heterogeneously spread over the whole protein, whereas *NFE2L2* mutations were found in specific hotspot regions, in line with previous reports (17, 41). Regarding clinical presentation, *KEAP1* patients had a significantly higher frequency of stage IV at primary diagnosis than *NFE2L2* patients. Further, although not significantly, the *KEAP1* group presented in worse performance state and tended to present with a higher frequency of hematogenous spread.

In a small subset of our cohort in which reproducible response on systemic therapies was assessable, response on different lines and regimens was remarkably poor, especially in the *KEAP1* group. None of the patients in both groups responded to second-line or third-line therapy. The co-occurrence of *TP53* mutation did not bias the outcome of these patients. Given the broad spectrum of chemotherapy agents used, these data suggest that these mutations might contribute to chemotherapy resistance, as proposed in preclinical lung cancer models and in other tumor types (19, 21, 26–29). However, in view of the small number of patients and the short follow-up, further data are needed to confirm this finding.

For both mutations, the majority of patients had additional aberrations. In our cohort, in some contrast to recent work (26), even the co-occurrence of targetable *EGFR* mutations was detected in both groups and clinical benefit for patients treated with an EGFR-TKI was seen. Further, nearly half of the *NFE2L2* patients had amplifications of either *MET* (26.3%) or *FGFR1* (20.6%). *MET* amplifications were also frequent in *KEAP1*-mutated patients (18.3%), which, in addition, harbor *KRAS* mutations in nearly half of the cases. It remains to be established whether and how co-occurring *NFE2L2* or *KEAP1* mutations affect efficacy of specific targeted treatments against already established targets like activating *EGFR* mutations or against targets under clinical investigation like amplifications of *MET* and *FGFR1* or *KRAS* mutations and whether they themselves might represent targets for specific therapies. To date, there are efforts reported to overcome the proposed chemotherapy-resistance triggered by the KEAP1/NRF2 pathway using brusatol as an NRF2 inhibitor (44). This potential therapeutic option might be considered when reevaluating treatment approaches for *KEAP1*/*NFE2L2*-mutated NSCLC. Nevertheless, it still remains unclear how the mutations in *KEAP1* and *NFE2L2* interact with NRF2 inhibition.

We are aware of the main limitations of this study: Even though we conducted parts of this study prospectively, robust therapeutic data are retrospective and available only for a subset of patients. Further, due to the short follow-up period, we could not obtain robust survival data that would be needed to link genomic aberrations with a prognostic impact. The number of patients reported here with the analyzed mutations is also small due to the prevalences of both mutations in our cohort. Nevertheless, the prevalences were in line with data from the TCGA cohorts LUAD and LUSC (17, 45). Further, with the diagnostic setup we used here, it is not possible to make assumptions about *KEAP1* allelic losses, which have been shown to occur frequently in lung cancer (16).

In summary, our data provide a comprehensive description of *NFE2L2* and *KEAP1* mutations regarding frequency and clinical as well as molecular characteristics. The observed heterogeneity of NSCLC harboring these mutations as well as the co-occurrence of further cancer-related mutations in the majority of patients harboring *NFE2L2* or *KEAP1* strongly argue against a function as driver mutations and rather imply a role as modifiers. Our observations also suggest that both mutations may play a role in chemoresistance and advocate for the development of therapeutic approaches different than chemotherapy for these patients. In particular, the interaction of mutations in *KEAP* and *NFE2L2* with co-occurring targetable genetic aberrations needs to be better understood and underlines the need for NGS-based molecular multiplex diagnostics to cover co-occurring mutations at least in the setting of clinical research.

Disclosure of Potential Conflicts of Interest

R. Fischer is a consultant/advisory board member for and reports receiving speakers bureau honoraria from Lung Cancer. J. Fassunke reports receiving speakers bureau honoraria from AstraZeneca. C. Heydt reports receiving speakers bureau honoraria from AstraZeneca. M. Serke reports receiving speakers bureau honoraria from Bristol, Myers, Squibb; Roche; AstraZeneca; Boehringer; and Celgene, and is a consultant/advisory board member for Bristol, Myers, Squibb; MSD; Roche; Boehringer, and Abbvie. No potential conflicts of interest were disclosed by the other authors.

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