ORIGINAL ARTICLE

Comprehensive Analysis of *TP53* and *KEAP1* Mutations and Their Impact on Survival in Localized- and Advanced-Stage NSCLC

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ABSTRACT

Introduction: *TP53* and *KEAP1* are frequently mutated in NSCLC, but their prognostic value is ambiguous, particularly in localized stage tumors.

Methods: This retrospective cohort study included a total of 6297 patients with NSCLC who were diagnosed between November 1998 and February 2020. The primary end point was overall survival. Patients were diagnosed in a central pathology laboratory as part of the Network Genomic Medicine collaboration, encompassing more than 300 lung cancer-treating oncology centers in Germany. All patients underwent molecular testing, including targeted next-generation panel sequencing and in situ hybridization.

Results: A total of 6297 patients with NSCLC were analyzed. In 1518 surgically treated patients (Union for International Cancer Control [UICC] I–IIIA), truncating *TP53* mutations and *KEAP1* mutations were independent negative prognostic markers in multivariable analysis (hazard ratio $[HR]_{TP53truncating} = 1.43$, 95% confidence interval [CI]: 1.07–1.91, $p = 0.015$; $HR_{KEAP1mut} = 1.68$, 95% CI:1.24–2.26, $p = 0.001$). Consistently, these mutations were associated with shorter disease-free survival. In 4779 patients with advanced-stage (UICC IIIB–IV) tumors, *TP53* mutations did not predict outcome in univariable analysis. In contrast, *KEAP1* mutations remained a negative prognostic factor ($HR_{KEAP1mut} = 1.40$, 95% CI: 1.23–1.61, $p < 0.001$) in patients with advanced-stage tumors. Furthermore, those with *KEAP1*-mutant tumors with co-occurring *TP53* missense mutations had longer overall survival than those with *KEAP1*-mutant tumors with wild-type or truncating *TP53* mutations.

Conclusions: This study found that *TP53* and *KEAP1* mutations were prognostic for localized and advanced-stage NSCLC. The increased relative hazard of harboring *TP53* or *KEAP1* mutations was comparable to an increase in one UICC stage. Our data suggest that molecular stratification on the basis of *TP53* and *KEAP1* mutation status should be implemented for localized and advanced-stage NSCLC to optimize and modify clinical decision-making.

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Drs. Saleh and Scheffler contributed equally to this work.

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Introduction

Lung cancer remains the leading cause of cancer-related deaths worldwide.[1] Nowadays, therapeutic decisions are heavily reliant on molecular subtyping, and the number of approved targeted treatments continues to grow.[2]

The *TP53* gene encodes the p53 tumor-suppressor protein, which is a master regulator of cell cycle and cell death. On various biological, chemical, and physical stressors, p53 promotes the transcription of genes responsible for either damage repair or apoptosis.[3] Given the vulnerable role of p53, it is not surprising that *TP53* is the most frequently mutated gene in human malignancies.[4–6] In NSCLC, 39% of lung adenocarcinomas and up to 51% of lung squamous cell carcinomas (LSCCs) harbor somatic *TP53* mutations.[7] Unlike most other tumor-suppressor genes, *TP53* is mainly altered by missense rather than truncating mutations. In general, frameshift and nonsense mutations lead to a truncated protein structure and generally loss of expression, whereas missense mutations do not interfere with translation and oftentimes cause overexpression of the mutant protein.[8,9] The large selective pressure on *TP53* missense mutations rather than gene deletions indicates an oncogenic-like effect of mutant p53. In fact, the mutated p53 protein elicits a variety of dysregulations, ranging from total loss-of-function to gain-of-function mutations, in which p53 can have oncogenic-like effects.[10–12] Recently, cell culture experiments have systematically evaluated the effect of different mutations in cell cultures and model organisms.[8,13,14] Nevertheless, tumor environments are much more complex and include various biological variables other than mere cellular replication rate, such as cell metabolism, immune regulation, and inflammation. Given this fragile ground of translational knowledge, the difficulty of using the *TP53* mutational status as a prognostic biomarker becomes evident.

A plethora of retrospective studies tried to answer the question of the effects of *TP53* mutation on the survival of patients. For lung cancer, the results are mixed.[15–21] A previously published meta-analysis on the prognostic impact of *TP53* mutations concludes that *TP53* mutations lead to shorter overall survival (OS), particularly in localized stage (I–IIIA) NSCLC and in adenocarcinoma.[16] Moreover, *TP53*-mutated NSCLC is reported to display more aggressive tumor progression and higher resistance to chemotherapy compared with wild-type *TP53*.[17,21]

For survival analysis, different *TP53* classification systems have been widely used. Nevertheless, the sample sizes were small and the approaches were unstandardized.[16,20,22] Poeta et al.[23] suggested a classification of the missense mutation group into “disruptive” and “nondisruptive” mutations. No matter how, given the variety of effects caused by different types of *TP53* mutations, it lies at hand that a dichotomous classification as “wildtype” and “mutant” underestimates the complexity and leads to low-resolution results.

KEAP1 mutation status represents a more recent and less ambiguous prognostic marker in NSCLC. Under homeostatic conditions, KEAP1 binds and negatively regulates NRF2 by recruiting the CUL3 ubiquitin ligase. This leads to proteasomal degradation of NRF2, holding NRF2 at low cellular levels. When confronted with oxidative stress, NRF2 is released from KEAP1 and then translocates into the nucleus and promotes the transcription of various genes related to protection from reactive oxygene species (ROS).[24] *KEAP1* mutations lead to a derepression of NRF2 and consequently improved oxidative stress responses. Several studies reveal that *KEAP1* mutations are associated with shorter OS. This is explained owing to improved resistance to radiotherapy in patients with *KEAP1*-mutated NSCLC.[24] Recent studies suggest that one of five patients with advanced-stage NSCLC harbors a *KEAP1* mutation.[25–27] For localized stage NSCLC, considerably fewer studies exist.

We here hypothesize that both KEAP1 and p53 play a major role in oxidative stress response.[28] Goeman et al.[29] and our research group have analyzed the associations of *KEAP1* and *TP53* mutations in NSCLC. Goeman et al.[29] suggest no strong correlation between KEAP1 and TP53 mutations and that they might promote two distinct cancer-related pathways.[26] Nonetheless, other studies suggest that mutant p53 plays an important modifying role in the Nrf2 pathway, which is shared with KEAP1.[30–32] Large studies investigating the effects of co-occurring TP53 and KEAP1 mutations on patient survival could help to translate the molecular findings into clinical practice.

In this study, we provide a comprehensive analysis of a large patient cohort with NSCLC diagnosed and treated within the Network Genomic Medicine.[33] Our analysis includes patients with both localized and advanced-stage NSCLC. We compared *KEAP1* mutation status and different *TP53* classification systems to establish an easy-to-use prognostic classification with emphasis on therapeutic decisions. Furthermore, for

the first time, we systematically analyzed the impact of different *TP53* mutations in *KEAP1*-mutated tumors on OS.

Methods

Patients

This real-world retrospective cohort study included patients with NSCLC who were diagnosed in a single central pathology laboratory as part of the Network Genomic Medicine collaboration, which encompasses more than 300 lung cancer-treating oncology centers in Germany.[33] Patients with localized stages (Union for International Cancer Control [UICC] stages I–IIIA) and advanced stages (UICC stages IIIB–IV) were analyzed separately. The following data were obtained: age (<55, 55–65, >65 y), sex, smoking status, histological type (lung adenocarcinomas or LSCCs), and TNM and UICC stage (as defined by the TNM eighth classification). For the exclusion criteria, see Supplementary Figure 1. All patients were treated in accordance with the national and international guidelines. Specific data on individual treatments and responses were not available. The study was reviewed and approved by the Ethics Committee of the University of Cologne. All patients consented in writing to analysis of their clinical data. OS time was determined by means of either medical records or requests to local registry offices and defined as the time from the date of first diagnosis until death. Patients who were alive at the data cutoff or who were lost to follow-up were censored. Disease-free survival (DFS) was defined as the time from date of first diagnosis until occurrence of cancer relapse.

Next-Generation Sequencing

All patients were routinely screened for molecular alterations in accordance with the national and international recommendations. Of three different customized lung cancer panels covering the regions of interest in 14 to 19 lung cancer-related genes (LUN3, LUN4, LUN5; Supplementary Fig. 2), one was used. Multiplex polymerase chain reaction- or hybrid capture-based target enrichment was performed as described previously.[34] Depending on DNA concentration, isolated DNA was amplified with either an Ion AmpliSeq Custom DNA Panel (Thermo Fisher Scientific, Waltham, MA) and the Ion AmpliSeq Library Kit 2.0 (Thermo Fisher Scientific) according to the Ion AmpliSeq Library Preparation User Guide (Thermo Fisher Scientific) or a GeneRead DNAseq Targeted Panel V2 (Qiagen, Hilden, Germany) and the GeneRead DNAseq Panel Polymerase Chain Reaction Kit V2 (Qiagen) according to the GeneRead DNASeq Gene Panel Handbook[35] (Qiagen). Alignment and annotation were done using a modified version of a previously described method.[36] The cutoff for variant calls was set to 5%, and the results were only interpreted if the coverage was greater than or equal to 200 reads.

Fluorescence In Situ Hybridization

ALK and *ROS1* rearrangements were diagnosed using fluorescence break-apart hybridization.[33] Indication of the analysis was in accordance with the national and international guidelines. Patients with LSCC are considered wild type with no further analysis.

TP53 Mutation Classification

Two classification systems for *TP53* survival analysis were compared, which are as follows: (1) mutational type and (2) "Poeta rules."[23] The first regards the "technical" type of mutation, separating frameshift and nonsense mutations (termed "TP53truncating"), from all other mutations, including missense, synonymous, and in-frame mutations (termed "TP53others"). Poeta et al.[23] distinguish between "disruptive" and "nondisruptive" *TP53* mutations, as previously defined. For *KEAP1* mutation analysis, we concentrated on mutation status with no further subdivision. For visualization of TP53 and KEAP1 mutations, MutationMapper was used.[37,38]

Statistics

Statistical analyses were performed by Statistical Package for the Social Sciences (IBM SPSS Statistics 26). Distribution of time to event was analyzed using Kaplan-Meier statistics and compared between groups by log-rank test. Association of qualitative variables was tested for by chi-square or Fisher's exact test, depending on distributional assumptions. Landmark analyses have been calculated with GraphPad Prism (GraphPad Prism, version 9). Hazard ratios (HRs) and confidence intervals (CIs) were calculated by means of univariable analyses or multivariable Cox proportional hazard model. For multivariable analysis, we included the following variables: sex, age, histological type, UICC stage, and mutant status of targetable driver comutations (*EGFR* and *BRAF V600E* mutations, *ALK* or *ROS* translocations). Missing data strategy: Only a subset of patients received *KEAP1* analysis (3022 of 6297 cases). Therefore, to compensate for different group sizes, separate multivariable analyses for *TP53* and for *KEAP1* were performed. Data were missing completely at random, making complete case analysis suitable.

Results

Patients

OS was analyzed in 6297 patients with NSCLC. The follow-up period ranged from 30 to 7203 days, with a median follow-up time for censored patients of 642 days (calculated by means of Schemper method[39]). Of 6297 patients, 3416 (54.4%) were censored.

KEAP1 and TP53 Mutation Status

More than half of the tumors (3245 of 6297, 51.5%) had *TP53* mutations, and one of six (524 of 3022, 17.3%) had *KEAP1* mutations (Fig. 1*A* and *B*). For *TP53*, most mutations were missense mutations (2334), followed by truncating (640), in-frame (57), and other mutations, including synonymous and uncodified mutations (214) (Fig. 1*A*). Missense mutations were clustered in known hotspots, the most frequent being codons 157, 158, 179, 245, 248, 249, and 273. For *KEAP1*, a comparable mutational distribution was found: most were missense mutations (380), followed by truncating (97), and other (47) mutations. There were no distinct hotspots (Fig. 1*B*).

Patients With Localized Disease (Stages I-IIIA)

Table 1 summarizes the demographics and characteristics of the patients with localized stage disease. Most of the patients were of male sex (60.1%) and had adenocarcinoma (70.4%). More than half of the patients with localized stage disease (50.6%) had no lymph node involvement. T stage ranged between 13.8% for T4 and 37.7% for T2. Most patients were diagnosed with having UICC stage IIIA (42.5%) at first diagnosis, followed by stage I (29.8%), and stage II (27.7%). Clinically relevant comutations were distributed as follows: 8.9% had *EGFR* mutations, 1.5% *BRAF* V600E mutations, 1.8% *ALK* or *ROS* translocations, 28.5% *KRAS* mutations, 17.0% *KEAP1* mutations, and 5.5% *NFE2L2* mutations.

Mutations in *TP53* were significantly associated with male sex, squamous differentiation, wild-type status for *EGFR*, *KRAS*, and *ALK* or *ROS*, and mutant *NFE2L2*. Truncating *TP53* mutations were significantly associated with squamous differentiation, higher UICC stage at first diagnosis, and wild-type status for *KRAS*. *KEAP1* mutations were significantly associated with male sex and wild-type status for *EGFR* and *NFE2L2* (Supplementary Table 1).

Survival of Patients With Localized Stage

Impaired survival of the patients with localized stage disease was associated with "conventional" prognostic factors, as expected,[40] including male sex, higher age, squamous differentiation, and higher UICC stage at first diagnosis. The patients had longer survival when tumors

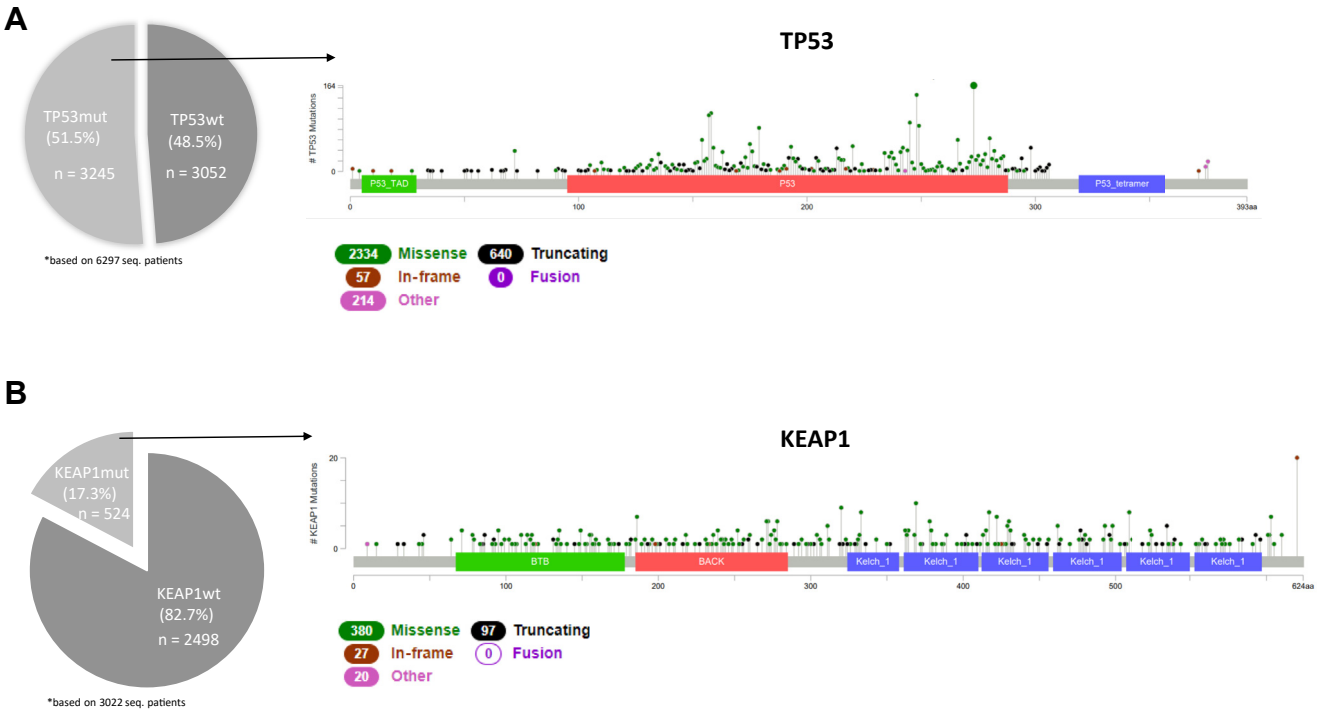

Figure 1. Mutation mapping for TP53 and KEAP1. Percentage of patients with (*A*) TP53 or (*B*) KEAP1 mutations from all analyzed patients. Note: Only a subgroup received KEAP1 sequencing (n = 3022), whereas all patients received TP53 sequencing (n = 6297). Mutational analysis, graph created with MutationMapper.[37,38] aa, amino acid; KEAP1mut, KEAP1 mutated; KEAP1wt, KEAP1 wild type; seq., sequencing; TP53mut, TP53 mutated; TP53wt, TP53 wild type.

Table 1. Characteristics for Patients With TP53wt and TP53mut Tumors (Localized Stage; I-IIIA)

Variants	Total, No. 1518	Patients With TP53wt, No. (%) 706	Patients With TP53mut, No. (%) 812	*p* Value (Chi-Square)	Patients With TP53 Truncating Mutations, No. (%) 168	Patients With TP53 "Other Mutations," No. (%) 646	*p* Value (Chi-Square)
Age at study entry (y)				0.175			0.971
≤50	63 (4.2)	24 (3.4)	39 (4.8)		8 (4.8)	31 (4.8)	
51–60	355 (23.4)	156 (22.1)	199 (24.5)		40 (23.8)	159 (24.7)	
>60	1100 (72.5)	526 (74.5)	574 (70.7)		120 (71.4)	454 (70.5)	
Sex				<0.001			0.193
Male	912 (60.1)	391 (55.4)	521 (64.2)		115 (68.5)	406 (62.8)	
Female	606 (39.9)	315 (44.6)	291 (38.8)		53 (31.5)	240 (37.2)	
Histological type				<0.001			0.005
LUAD	1068 (70.4)	594 (84.1)	474 (58.4)		82 (48.8)	392 (60.9)	
LSCC	450 (27.6)	112 (15.9)	338 (41.6)		86 (51.2)	252 (39.1)	
Smoking				0.002			0.493
Current	141 (36.3)	64 (31.4)	77 (41.8)		14 (33.3)	63 (44.3)	
Former	182 (46.9)	98 (48.0)	84 (45.7)		22 (52.4)	62 (43.7)	
Never	65 (16.8)	42 (20.6)	23 (12.5)		6 (14.3)	17 (12.0)	
Unknown	1130	502	628		126	502	
N stage				0.662			0.087
N0	769 (52.7)	354 (52.1)	415 (53.2)		76 (47.2)	339 (54.8)	
N1–N3	691 (47.3)	326 (47.9)	365 (46.8)		85 (52.8)	280 (45.2)	
Unknown	58	26	32		7	27	
T stage				0.416			0.144
T1	414 (28.0)	191 (27.6)	223 (28.3)		35 (21.5)	188 (30.0)	
T2	559 (37.7)	276 (39.9)	283 (35.9)		61 (37.4)	222 (35.5)	
T3	304 (20.5)	135 (19.5)	171 (21.4)		42 (25.8)	127 (20.3)	
T4	204 (13.8)	90 (13.0)	114 (14.4)		25 (15.3)	89 (14.2)	
Unknown	37	14	23		5	18	
UICC stage				0.577			0.041
I	452 (29.8)	209 (29.6)	243 (29.9)		38 (22.6)	206 (31.9)	
II	421 (27.7)	188 (26.6)	233 (28.7)		58 (34.5)	176 (27.2)	
IIIA	645 (42.5)	309 (43.8)	336 (41.4)		72 (42.9)	264 (40.9)	
EGFR				0.002			0.131
Wild type	1383 (91.1)	626 (88.9)	757 (93.2)		161 (95.8)	596 (92.5)	
Mutant	135 (8.9)	80 (11.1)	55 (6.8)		7 (4.2)	48 (7.5)	
BRAF V600E				0.164			0.909
Wild type	1495 (98.5)	692 (98.0)	803 (98.9)		166 (98.8)	639 (98.9)	
Mutant	23 (1.5)	14 (2.0)	9 (1.1)		2 (1.2)	7 (1.1)	
ALK or ROS				<0.001			0.252
Wild type	1490 (98.2)	683 (96.7)	807 (99.1)		168 (100.0)	639 (99.2)	
Transl.	28 (1.8)	23 (3.3)	5 (0.9)		0 (0.0)	5 (0.8)	
KEAP1				0.522			0.119
Wild type	547 (83.0)	277 (83.9)	270 (82.1)		57 (76.0)	213 (83.9)	
Mutant	112 (17.0)	53 (16.1)	59 (17.9)		18 (24.0)	41 (16.1)	
Unknown	859	376	483		93	39	
NFE2L2				0.016			0.729
Wild type	623 (94.5)	319 (96.7)	304 (92.4)		70 (93.3)	234 (92.1)	
Mutant	36 (5.5)	11 (3.3)	25 (7.6)		5 (6.7)	20 (7.9)	
Unknown	859	376	483		93	392	
KRAS				<0.001			0.029
Wild type	1086 (75.5)	428 (60.6)	658 (81.0)		146 (86.9)	512 (79.5)	
Mutant	432 (28.5)	278 (39.4)	154 (19.0)		22 (13.1)	132 (20.5)	

LSCC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; TP53mut, TP53 mutated; TP53wt, TP53 wild type; Trans, translocation; UICC, Union for International Cancer Control.

harbored *KRAS* mutations (Fig. 2*A* and Supplementary Table 2).

Median OS was 1181 days (95% CI: 818–1543 d) in the TP53truncating cohort, compared with 1474 days (95% CI: 1232–1751 d) in the TP53 wild-type cohort (TP53wt) and 1486 days (95% CI: 1133–1838 d) in the TP53other cohort ($HR_{TP53truncating} = 1.61$, 95% CI: 1.22–2.12, $p = 0.001$) (Fig. 3*A*). After 2 years, approximately 40% of the patients with truncating *TP53* tumors died, compared with approximately 25% in both the other groups (for 1-, 2-, and 5-y landmark analyses; Supplementary Fig. 3). Furthermore, DFS was significantly shorter in patients with *TP53* mutations compared with TP53wt tumors, regardless of the type of mutation (791 d in TP53wt versus 630 d in TP53other versus 657 d in TP53truncating; numbers in mean) (Fig. 3*B*). Although statistically significant, the shorter survival was less robust when using the *TP53* classification suggested by Poeta et al.[23] ($HR_{TP53disruptive} = 1.37$, 95% CI: 1.10–1.72, $p = 0.006$) (Supplementary Fig. 4*A*).

KEAP1 mutations were associated with a striking drop in patient survival: Median OS was 755 days (95% CI: 500–1010 d) in the *KEAP1*-mutated (*KEAP1*mut) cohort, compared with 1264 days (95% CI: 1116–1412 d) in the *KEAP1* wild-type (*KEAP1*wt) cohort ($HR_{KEAP1mut} = 1.74$, 95% CI: 1.30–2.33, $p < 0.001$) (Fig. 3*C*). Similar to *TP53*, the *KEAP1*mut cohort had significantly shorter DFS compared with the *KEAP1*wt cohort (732 d versus 473 d; numbers in mean) (Fig. 3*D*). Further stratification of the *KEAP1*mut cohort on the basis of the *TP53* mutation status revealed no significant stratification of the prognostic groups (Supplementary Fig. 5).

In multivariable analysis, after adjustment of all covariates, impaired survival of truncating TP53 or *KEAP1* mutations remained significant ($HR_{TP53truncating} = 1.43$, 95% CI: 1.07–1.91, $p = 0.015$; $HR_{KEAP1mut} = 1.68$, 95% CI: 1.24–2.26, $p = 0.001$, respectively) (Fig. 2*B*, Supplementary Tables 3.1 and 3.2). The increased relative hazard of harboring truncating *TP53* mutations or *KEAP1* mutations was comparable with the increased relative hazard of UICC stage I versus stage II (Fig. 2*B*). Thus, truncating *TP53* mutations and *KEAP1* mutations are two independent prognostic factors of worse OS and DFS in patients with localized stage NSCLC.

Patients With Advanced Stage (UICC Stages IIIB-IV)

Most patients with advanced-stage disease were of male sex (56.2%) and had adenocarcinoma (81.5%).

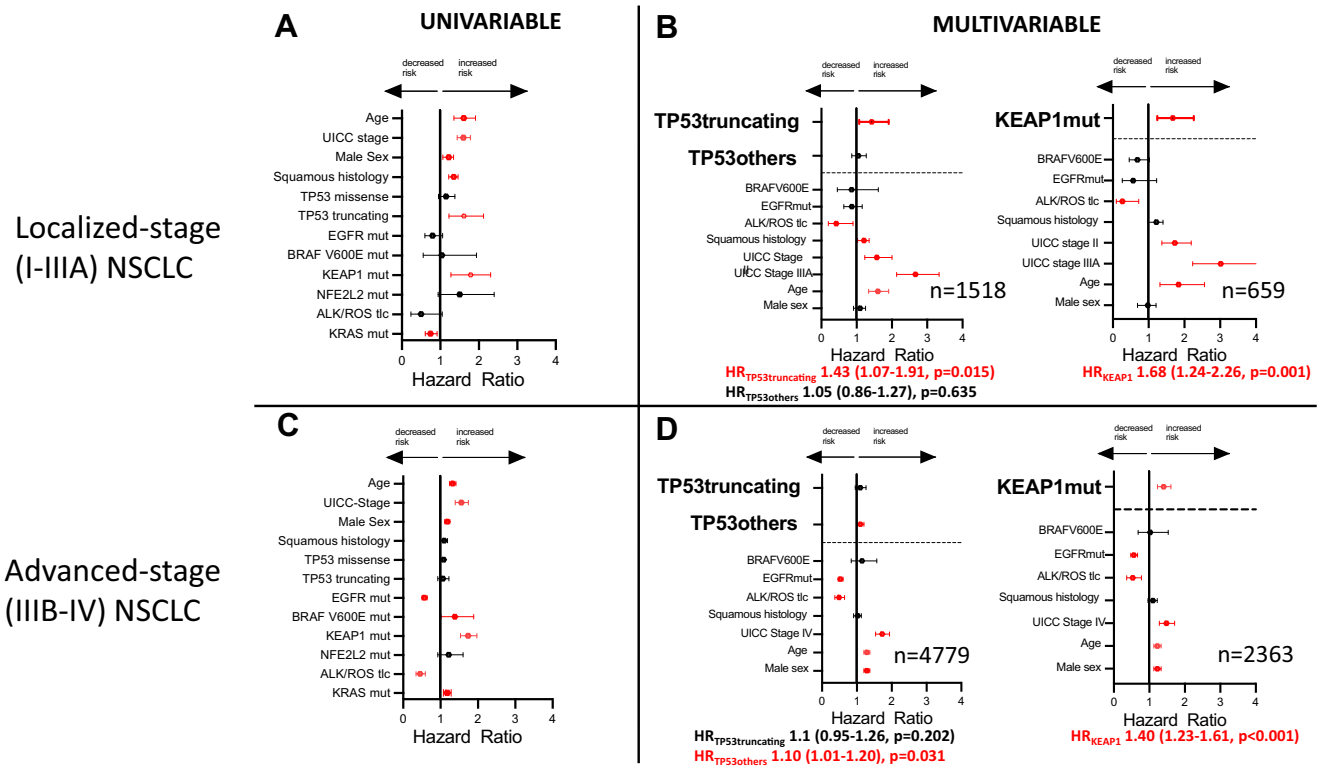

Figure 2. Forest plot for prognostic factors in patients with (*A*, *B*) localized stage and (*C*, *D*) advanced-stage diseases by (*A*, *C*) univariable and (*B*, *D*) multivariable analyses. Multivariable analyses for TP53 and KEAP1 were performed separately (see the Methods section). The forest plot reveals the HRs and 95% confidence intervals of the prognostic factors. Red color indicates significant *p* values. HR, hazard ratio; mut, mutation; tlc, translocation; UICC, Union for International Cancer Control.

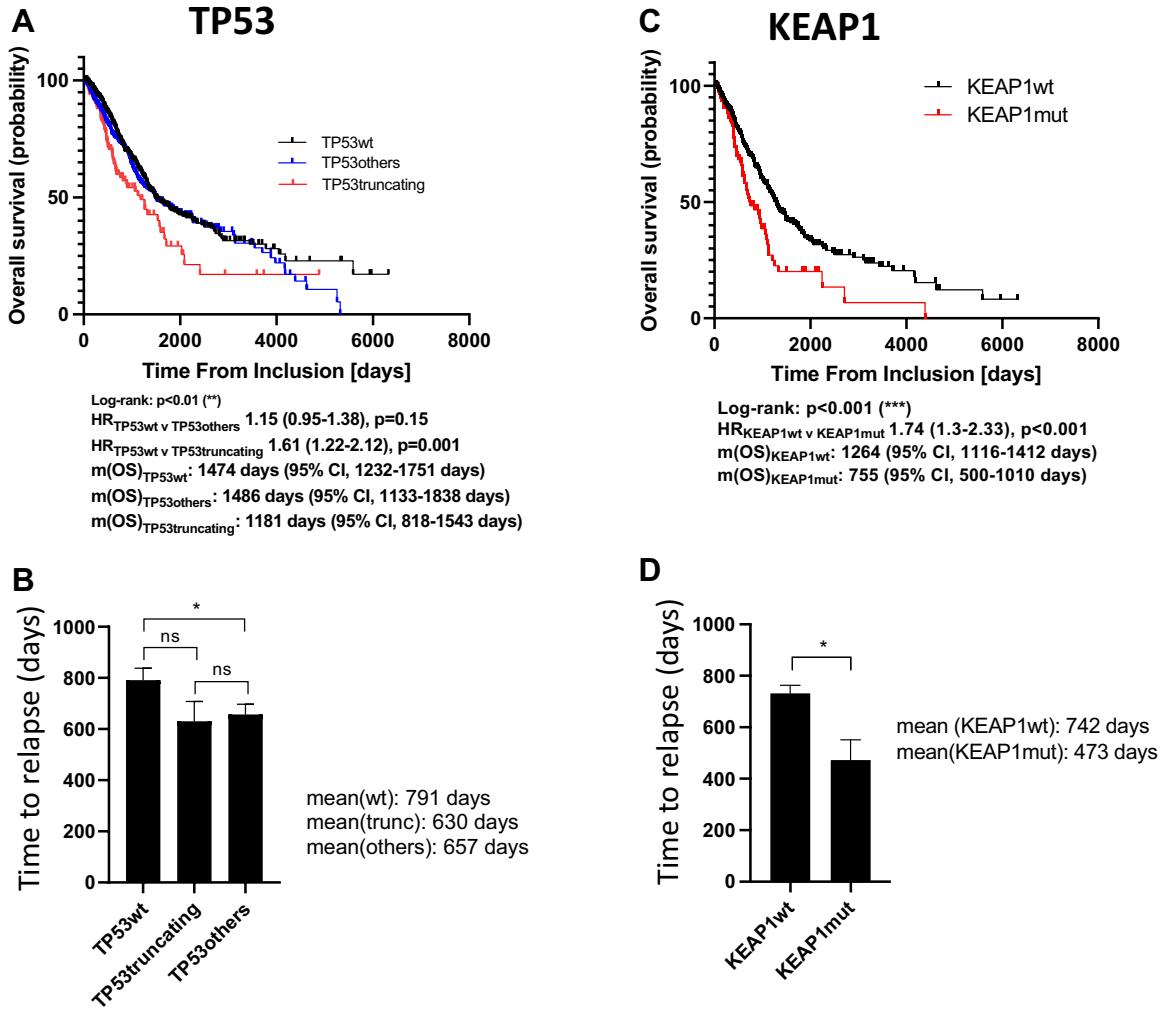

Figure 3. (*A*, *B*) OS for localized stage NSCLC according to (*A*) TP53 or (*C*) KEAP1 mutation status, respectively. TP53 classification by means of mutational type (see the Methods section). (*B*, *D*) Time to cancer relapse; means are indicated next to the graph. CI, confidence interval; HR, hazard ratio; KEAP1mut, KEAP1 mutated; KEAP1wt, KEAP1 wild type; m(OS), median overall survival; ns, not significant; OS, overall survival; TP53wt, TP53 wild type; trunc, truncating.

Nearly three-fourth had lymph node metastases (72.5%). Furthermore, most of them were diagnosed with having UICC stage IV (81.5%). Clinically relevant comutations were distributed as follows: 12.7% had *EGFR* mutations, 1.6% *BRAF* V600E mutations, 3.2% *ALK* or *ROS* translocations, 31.6% *KRAS* mutations, 17.4% *KEAP1* mutations, and 3.4% *NFE2L2* mutations (Table 2).

Among the 4818 patients with advanced-stage disease, 50.9% had *TP53* mutations. Patients in the *TP53*-mutated cohort were significantly more likely to be of male sex and having squamous differentiation, lower UICC stage at first diagnosis, larger tumor size, and wild-type status for *KEAP1*, *NFEL2*, and *ALK* or *ROS*. Truncating *TP53* mutations were significantly associated with *KEAP1* mutations. *KEAP1* mutations were significantly associated with higher age, adenomatous differentiation, male sex, larger tumor size, and wild-type status for *EGFR*, *ALK* or *ROS*, *NFE2L2*, and *TP53* (Supplementary Table 4).

Univariable analysis revealed a significantly shorter survival for patients with high age, male sex, and higher UICC stage at first diagnosis. Patients lived significantly longer with mutated *EGFR* and translocated *ALK* or *ROS*, which is explained by the availability of potent targeted treatment (Fig. 2*C* and Supplementary Table 5).

In contrast to the patient cohort with localized stage disease, those with advanced-stage disease had no shorter OS for the TP53truncating cohort. Median OS was 448 days (95% CI: 375–520 d) for the TP53truncating cohort, compared with 506 days (95% CI: 463–549 d) for the TP53wt cohort and 436 days (95% CI: 392–480 d) for the TP53other cohort ($HR_{TP53truncating}$ = 1.06, 95% CI: 0.93–1.22, p = 0.379) (Fig. 4*A*). When classifying *TP53* mutations by the "Poeta rules," the TP53nondisruptive cohort had slightly shorter OS compared with those with TP53wt, as hinted in previous

Table 2. Characteristics for Patients With TP53wt and TP53mut Tumors (Adv. Stage IIIB–IV)

Variants	Total, No. 4779	Patients With TP53wt, No. (%) 2346	Patients With TP53mut, No. (%) 2433	*p* Value (Chi-Square)	Patients With TP53 Truncating Mutations, No. (%) 509	Patients With TP53 "Other Mutations," No. (%) 1934	*p* Value (Chi-Square)
Age at study entry (y)				0.054			0.565
≤50	416 (8.7)	205 (8.7)	211 (8.7)		50 (9.8)	161 (8.4)	
51–60	1212 (25.4)	559 (23.8)	653 (26.8)		133 (26.2)	520 (27.0)	
>60	3151 (65.9)	1582 (67.4)	1569 (64.5)		325 (64.0)	1244 (64.6)	
Sex				<0.001			0.926
Male	2688 (56.2)	1246 (53.1)	1442 (59.3)		303 (59.4)	1140 (59.2)	
Female	2091 (43.8)	1100 (46.9)	991 (40.7)		206 (40.6)	785 (40.8)	
Histological type				<0.001			0.192
LUAD	3895 (81.5)	2092 (89.2)	1803 (74.1)		365 (71.9)	1438 (74.7)	
LSCC	884 (18.5)	254 (10.8)	630 (25.9)		143 (28.1)	487 (25.3)	
Smoking				<0.001			0.665
Current	786 (45.3)	364 (42.5)	422 (48.1)		91 (50.0)	331 (47.6)	
Recent	684 (39.4)	328 (38.3)	356 (40.5)		75 (41.2)	281 (40.4)	
Never	265 (15.3)	165 (19.2)	100 (11.4)		16 (8.8)	84 (12.0)	
Unknown	3044	1489	1555		326	1229	
N stage				0.167			0.627
N0	584 (14.4)	300 (15.2)	284 (13.7)		64 (14.4)	220 (13.5)	
N1–N3	3466 (85.6)	1673 (84.8)	1793 (86.3)		381 (85.6)	1411 (86.5)	
Unknown	730	373	357		64	293	
T stage				0.012			0.904
T1	453 (11.0)	209 (10.4)	244 (11.6)		49 (10.9)	195 (11.8)	
T2	984 (23.9)	522 (25.9)	462 (21.9)		98 (21.8)	364 (22.0)	
T3	895 (21.7)	413 (20.5)	482 (22.9)		108 (24.0)	374 (22.6)	
T4	1792 (43.5)	873 (43.3)	919 (43.6)		195 (43.3)	724 (43.7)	
Unknown	655	329	326		59	277	
UICC TNM stage				<0.001			0.195
IIIB	884 (18.5)	365 (15.6)	519 (21.3)		119 (23.4)	400 (20.8)	
IV	3895 (81.5)	1981 (84.4)	1914 (78.7)		389 (76.6)	1525 (79.2)	
EGFR				0.726			0.375
Wild type	4170 (87.3)	2043 (87.1)	2127 (87.4)		450 (88.6)	1677 (87.1)	
Mutant	609 (12.7)	303 (12.9)	306 (12.6)		58 (11.4)	248 (12.9)	
BRAF V600E				0.228			0.363
Wild type	4704 (98.4)	2304 (98.2)	2400 (98.6)		499 (98.2)	1901 (98.8)	
Mutant	75 (1.6)	42 (1.8)	33 (1.4)		9 (1.8)	24 (1.2)	
ALK or ROS				<0.001			0.497
Wild type	4624 (96.8)	2235 (95.3)	2389 (98.2)		498 (97.8)	1901 (98.3)	
Transl.	155 (3.2)	111 (4.7)	44 (1.8)		11 (2.2)	33 (1.7)	
KEAP1				0.037			0.013
Wild type	1951 (82.6)	955 (80.9)	996 (84.2)		183 (78.9)	813 (85.5)	
Mutant	412 (17.4)	225 (19.1)	187 (15.8)		49 (21.1)	138 (14.5)	
Unknown	2416	1166	1250		277	983	
NFE2L2				<0.001			0.084
Wild type	2283 (96.6)	1156 (98.0)	1127 (95.3)		216 (93.1)	911 (95.8)	
Mutant	80 (3.4)	24 (2.0)	56 (4.7)		16 (6.9)	40 (4.2)	
Unknown	2416	1166	1250		277	983	
KRAS				<0.001			0.045
Wild type	3268 (68.4)	1415 (60.3)	1853 (76.2)		404 (79.5)	1449 (75.3)	
Mutant	1511 (31.6)	931 (39.7)	580 (23.8)		104 (20.5)	476 (24.7)	

Adv., advanced; LSCC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; TP53mut, TP53 mutated; TP53wt, TP53 wild type; Trans, translocation; UICC, Union for International Cancer Control.

reports20 ($HR_{TP53nondisruptive}$ = 1.11, 95% CI: 1.02–1.220, $p = 0.021$) (Supplementary Fig. 4*B*).

Similar to those in the patients with localized stage disease, *KEAP1* mutations lead to a significant decrease in median OS, from 446 days (95% CI: 412–480 d) in the KEAP1wt cohort to 257 days (95% CI: 214–300 d) in the KEAP1mut cohort ($HR_{KEAP1mut}$ = 1.74, 95% CI: 1.53–1.97, $p < 0.001$) (Fig. 4*B*). Further stratification of *KEAP1*-mutated tumors on the basis of *TP53* mutation status reveals significantly longer survival when harboring co-occurring nontruncating *TP53* mutations (Fig. 4*C*). Median OS was 342 days (95% CI: 259–425 d) in KEAP1mut-TP53other cohort compared with 201 days (95% CI: 158–244 d) in KEAP1mut-TP53wt and 189 days (95% CI: 30–348 d) in KEAP1-TP53truncating cohort ($HR_{KEAP1mut-TP53other}$ = 0.65 [95% CI: 0.50–0.85], $p = 0.002$). Therefore, within the KEAP1mut cohort, co-occurring missense *TP53* mutations seem to level out the deleterious effects of the *KEAP1* mutation.

In multivariable analysis, *KEAP1* mutation remained significantly associated with shorter survival, thus representing an independent negative prognostic factor (KEAP1; $HR_{KEAP1mut}$ = 1.40, 95% CI: 1.27–1.61, $p < 0.001$) (Fig. 2*D*). The relative increase in hazard was of comparable magnitude to the increase of hazard from UICC stage IIIB to stage IV. Interestingly, in opposite to those in the patients with localized stage disease, missense *TP53* mutations turned to a weak negative prognostic factor in multivariable analysis ($HR_{TP53other}$ = 1.10, 95% CI: 1.01–1.20, $p = 0.031$) (Fig. 2*D*, Supplementary Tables 6.1 and 6.2).

Discussion

Here, a total of 6297 patients with NSCLC were analyzed in a period of two decades in a retrospective real-world cohort study. Patients with localized stage NSCLC with tumors harboring truncating *TP53* mutations or *KEAP1* mutations have significantly shorter OS and DFS compared with those with *TP53*wt and TP53others or *KEAP1*wt, respectively. In advanced-stage NSCLC, truncating *TP53* mutations lose their prognostic properties, whereas "TP53other" mutations become a weak negative prognostic marker in multivariable analysis, as reported previously.[20] These data point to different oncogenic effects of truncating versus other and missense mutations. KEAP1 mutations remain a negative prognostic marker in advanced-stage NSCLC. Furthermore, when KEAP1-mutant tumors harbor additional TP53other mutations, patients live significantly longer.

The prognostic role of *TP53* mutations in NSCLC has been controversial, which can be partly attributed to small sample sizes and unstandardized study approaches.[16] In our analysis, defining three subsets on the basis of mutational type was pivotal to identify a subgroup of patients with shorter OS. In localized stage NSCLC, truncating rather than missense *TP53* mutations lead to shortened survival. This implies that loss of TP53 expression, a typical consequence of truncating *TP53* mutations,[8] worsens the situation in patients with complete tumor resection. This observation seems not to be limited to NSCLC. Lindenbergh-van der Plas et al.[41] have analogously found that truncating but not missense *TP53* mutations lead to increased survival in resected head and neck tumors. One might therefore assume that in early carcinogenesis, mutant p53, typically resulted by missense mutations, still executes some of its tumor-suppressive functions. Possible explanations for differences in survival of patients with localized stage disease with truncating *TP53* mutations are higher presence of micrometastatic lesions, worse response to adjuvant chemotherapy, or more aggressive tumor progression.[22,42,43]

In patients with advanced-stage disease, *TP53* missense rather than truncating mutations seem to worsen the outcome. Patients with advanced-stage NSCLC typically receive a combination of chemotherapy combined with immunotherapy or molecular-based targeted treatment. It is therefore plausible to believe that gain-of-function effects of mutant p53 play a larger role in advanced multimodal treatment scenarios rather than in surgically resected tumors.[10,12,20]

An unresolved question is whether the large group of *TP53* missense mutations can be further stratified. In this study, "Hotspot," "gain-of-function" mutations, and the "Poeta rules" had minimal to no "separative" effect. Initial experiments with annotating so-called evolutionary action scores for *TP53* missense mutations, as successfully found in previous studies,[44] revealed no significant results. No matter how, a dichotomous analysis regarding TP53 mutation status is insufficient, leads to low-resolution results, and should be abandoned.

Most previous studies regarding *KEAP1* mutations in NSCLC concentrate on patients with advanced stage, and sample sizes were relatively small.[26,27,45–47] Our study is the largest study up to date investigating the prognostic effects of *KEAP1* mutations on NSCLC patient prognosis. This observation deserves special attention, because glutaminase inhibitors for patients with *KEAP1*mut are a promising treatment strategy and currently under clinical evaluation (ClinicalTrials.gov NCT04471415). KEAP1-mutant tumors are highly resistant to radiotherapy owing to improved oxidative stress signaling by means of the Nrf2 pathway. With our work, we delineate *KEAP1* mutation status as an important prognostic

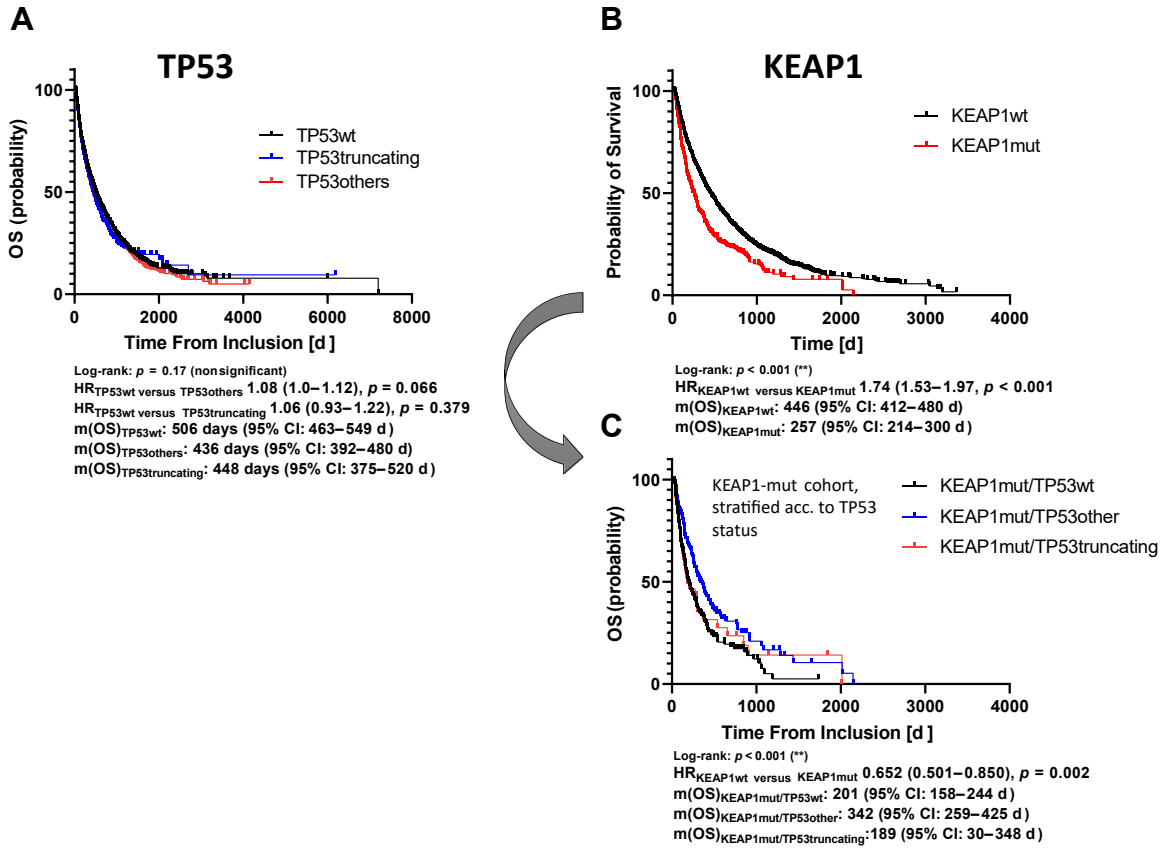

Figure 4. (*A*, *B*) OS according to (*A*) TP53 or (*B*) KEAP1 mutation status, respectively. TP53 classification by means of mutational type (see the Methods section). (*C*) All patients with KEAP1 mutations were further stratified according TP53 mutation status. acc., according; CI, confidence interval; HR, hazard ratio; KEAP1mut, KEAP1 mutated; KEAP1wt, KEAP1 wild type; m(OS), median overall survival; OS, overall survival; TP53wt, TP53 wild type.

marker in both localized and advanced-stage NSCLC. We therefore propose that treatment and diagnostic protocols should evaluate to expand *KEAP1* mutation detection to all NSCLC stages.

For the first time, the effects of various co-occurring *TP53* mutations in *KEAP1*-mutant tumors were evaluated. To our surprise, co-occurring *TP53* missense, rather than truncating, mutations in *KEAP1*-mutated tumors lead to a survival benefit. Previous reports already hinted that *TP53* mutations could lead to survival benefits in the context of *STK11* mutations.[48] Mechanistically, *KEAP1*-mutant tumor cells have hyperactivated NRF2[49] and thus improved resistance to oxidative stress. Mutant p53 is reported to stabilize and potentiate NRF2.[30–32] This seems counterintuitive to our results; however, mutant TP53 also has opposing effects to NRF2, for example, mutant p53 down-regulates HMOX-1, a target gene of NRF2.[50] Goeman et al.[29] have found that TP53 and KEAP1 mutations do not correlate. Our work, for the first time, hints that in advanced-stage NSCLC, complex interactions between mutant p53 and the Nrf2 pathway seem to substantially affect patient survival. Further research is needed to investigate the exact interactions of mutant p53 and NRF2 in the context of *KEAP1* mutations.

In the recent past, efforts have been made to increase the inclusion of molecular markers in clinical staging of NSCLC.[51,52] Patients with localized stage disease do not receive routine molecular analysis, and current recommendations limit next-generation sequencing (NGS) testing to those with advanced-stage NSCLC.[53,54] This leads to low-resolution risk stratification and missing out of high-risk patients who would profit from different treatment modalities. Platinum-based adjuvant treatment leads to an improvement of 4.1% for 5-year OS.[55,56] Because all treatment recommendations regarding the adjuvant setting are derived from a meta-analysis performed in the premolecular NSCLC era, it can be assumed that our findings could help to optimize NSCLC treatment for localized stages. This implies a substantial economical and organizational task, and one needs to weigh off the benefits and costs of such a decision. This is crucially dependent on the availability of treatment options. Targeting *TP53* or *KEAP1* remained

difficult; however, relevant progress is expected in the future.[49,57–59]

Limitations of our study are due to the nature of the analyzed data and biases of this analytical approach.[60–62] First, a minimal degree of informative censoring cannot be ruled out, possibly leading to overestimation of median survival times.[63] Nonetheless, hazard ratios should be unaffected, and the degree of informative censoring should be minimal owing to our stringent follow-up procedures. Furthermore, the treatment landscape for NSCLC has changed during the 20-year-long observational period, introducing secular trend bias. Nevertheless, this affects all of our analyzed groups equally, thus limiting its relevance. Second, some information is missing, including the "R-status," systemic treatment protocols and clinical responses, programmed death-ligand 1 immunostatus, and presence of other relevant prognostic markers, such as *STK11* mutations. Therefore, it is possible that some confounders were not included in our multivariable analysis. Nevertheless, we compensated for all conventional prognostic factors (age, sex, histological type) and included all highly prognostic targetable driver mutations (*EGFR*, BRAFV600E, ALK or *ROS* translocations). Third, NGS technology limits our analysis to the genome level. We have no information on other genetic alterations, such as large deletions or posttranscriptional changes, such as splicing. The existence of up to 12 different *TP53* isoforms has been described in the literature and reports hint to a biological effect of such isoforms.[64,65] Future studies might broaden their view on *TP53* isoforms to further delineate prognostic subgroups in the "wild-type" *TP53* cohorts. Fourth, recently used NGS panels revealed significantly longer survival rates, which can be attributed to (1) improved OS in general1,[66] and (2) higher percentage of patients with localized stage disease in recent panels. Fifth, our high censoring rate might have affected the measured survival times. Nonetheless, median follow-up time was between two and three years, which is in accordance with general guidelines and should be enough to detect most events.

In conclusion, the large sample size of our cohort provides unparalleled statistical power to the question of the prognostic properties of *KEAP1* and *TP53* mutations in NSCLC. Targeted NGS testing is standardized for advanced-stage NSCLC. Our data suggest that panels should include *TP53* and *KEAP1* and that testing should be broadened to include localized stage NSCLC. Identification of worse prognostic groups on the basis of *TP53* and *KEAP1* mutation status can help to modify and optimize treatment in both localized and advanced-stage NSCLC.

CRediT Authorship Contribution Statement

Mohamed Mahde Saleh: Data analysis, Statistical analysis, Writing of the manuscript.

Matthias Scheffler: Data analysis, Writing of the manuscript.

Sabine Merkelbach-Bruse: Molecular typing of lung cancers, Data analysis.

Andreas Hans Scheel: Data analysis, Tumor analysis.

Bastian Ulmer: Statistical analysis.

Jürgen Wolf: Treatment of patients, Data acquisition.

Reinhard Buettner: Design of the study, Molecular typing and diagnostics of lung cancer, Writing of the manuscript.

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Supplementary Data

Note: To access the supplementary material accompanying this article, visit the online version of the *Journal of Thoracic Oncology* at www.jto.org and at https://doi.org/10.1016/j.jtho.2021.08.764.

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