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ORIGINAL ARTICLE

Impact of *TP53* mutation status on systemic treatment outcome in *ALK*-rearranged non-small-cell lung cancer

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Background: We analyzed whether co-occurring mutations influence the outcome of systemic therapy in *ALK*-rearranged non-small-cell lung cancer (NSCLC).

Patients and methods: *ALK*-rearranged stage IIIB/IV NSCLC patients were analyzed with next-generation sequencing and fluorescence *in situ* hybridization analyses on a centralized diagnostic platform. Median progression-free survival (PFS) and overall survival (OS) were determined in the total cohort and in treatment-related sub-cohorts. Cox regression analyses were carried out to exclude confounders.

Results: Among 216 patients with *ALK*-rearranged NSCLC, the frequency of pathogenic *TP53* mutations was 23.8%, while other co-occurring mutations were rare events. In *ALK/TP53* co-mutated patients, median PFS and OS were significantly lower compared with *TP53* wildtype patients [PFS 3.9 months (95% CI: 2.4–5.6) versus 10.3 months (95% CI: 8.6–12.0), $P < 0.001$; OS 15.0 months (95% CI: 5.0–24.9) versus 50.0 months (95% CI: 22.9–77.1), $P = 0.002$]. This difference was confirmed in all treatment-related subgroups including chemotherapy only [PFS first-line chemotherapy 2.6 months (95% CI: 1.3–4.1) versus 6.2 months (95% CI: 1.8–10.5), $P = 0.021$; OS 2.0 months (95% CI: 0.0–4.6) versus 9.0 months (95% CI: 6.1–11.9), $P = 0.035$], crizotinib plus chemotherapy [PFS crizotinib 5.0 months (95% CI: 2.9–7.2) versus 14.0 months (95% CI: 8.0–20.1), $P < 0.001$; OS 17.0 months (95% CI: 6.7–27.3) versus not reached, $P = 0.049$] and crizotinib followed by next-generation ALK-inhibitor [PFS next-generation inhibitor 5.4 months (95% CI: 0.1–10.7) versus 9.9 months (95% CI: 6.4–13.5), $P = 0.039$; OS 7.0 months versus 50.0 months (95% CI: not reached), $P = 0.001$].

Conclusions: In *ALK*-rearranged NSCLC co-occurring *TP53* mutations predict an unfavorable outcome of systemic therapy. Our observations encourage future research to understand the underlying molecular mechanisms and to improve treatment outcome of the *ALK/TP53* co-mutated subgroup.

Key words: ALK-rearranged NSCLC, sequential ALK-inhibitor therapy, TP53 mutation status

Introduction

ALK-positive non-small-cell lung cancer (NSCLC) is characterized by *ALK* gene rearrangements and an association with acinar histology, younger age and never-smoking status [1]. *ALK* rearrangements lead to constitutive activation of the encoded tyrosine kinase and downstream transforming signaling pathways [2]. Crizotinib, the first approved ALK-inhibitor, is superior to chemotherapy regarding overall response rate, progression-free survival (PFS), toxicity profile [3, 4] and overall survival (OS) [5–7]. Next-generation inhibitors with activity against *ALK* resistance mutations are in clinical evaluation and partly already approved [8–11]. An impressive OS was reported for sequential ALK-inhibitor therapy ranging from 45 to 89.6 months [12–14].

There are considerable differences in the clinical course of *ALK*-positive NSCLC patients treated with chemotherapy or ALK inhibitors [3, 4, 15, 16]. Genetic heterogeneity of *ALK*-positive tumors could explain this observation. We have molecularly analyzed 216 *ALK*-positive patients with advanced disease and hypothesized that co-occurring mutations might underlie these differences.

Patients and methods

Patients and samples

The study was carried out within the Network Genomic Medicine [17], which offers centralized molecular diagnostics at the University Hospital of Cologne for patients with lung cancer from 300 participating partners. The study was conducted in concordance with local ethical guidelines. Patients were treated with crizotinib, ceritinib, alectinib or brigatinib according to national guidelines or within clinical trials [PROFILE1005 (NCT00932451); PROFILE1007 (NCT00932893); CLDK378X2101 (NCT-1283516); ASCEND-5 (NCT01828112); ALTA (AP26113) (NCT02094573); ACCALIA (NCT01801111)].

Fluorescence *in situ* hybridization

ALK, *RET* and *ROS1* rearrangements were diagnosed using break-apart fluorescence *in situ* hybridization (FISH) [17]. *MET* and *ERBB2* were tested for amplification as reported [18]. Details are described in supplementary Table S1, available at *Annals of Oncology* online.

Next-generation sequencing

Samples were analyzed with either a validated gene panel using AmpliSeq chemistry (Thermofisher, LUN3) comprising 102 amplicons of 14 different genes or a validated gene panel using GeneRead chemistry (Qiagen, LUN4), comprising 17 genes [19]. Details are described in supplementary Table S6, available at *Annals of Oncology* online. *ALK* variants were determined using the Archer® FusionPlex® Lung Kit and Archer Molecular Barcode (MBC) Adapters (both for Illumina) according to the manufacturer's instructions.

Programmed death-ligand 1 immunohistochemistry

Programmed death-ligand 1 (PD-L1) immunohistochemistry was carried out on the Leica Bond platform using primary antibody clone 28-8 (Abcam, Cambridge, UK). Interpretation was done according to the Dako PD-L1 22C3 pharmDx guidelines, results were reported based on an integrated proportion score [20, 21].

Data collection

The Network Genomic Medicine database covers molecular diagnostics and basic demographic and clinical data. For treatment outcome medical records were reviewed. PFS was determined based on RECIST v1.1. Time of death was determined either via medical records or requests to local registry offices. OS was defined as the time from first diagnosis of stage IIIB/IV until death. For subjects alive at completion of this analysis, time to death was censored at the time of last contact.

Statistical analyses

Statistical analyses were carried out using IBM SPSS software 24 (IBM, Armonk, NY). Chi-squared and two-sided Fischer's exact tests were used for analyzing qualitative variable characteristics in different groups. The Kaplan–Meier estimator was used to calculate OS and PFS. Two-sided log-rank tests were applied to compare differences between treatment groups. Cox proportional hazards model was used to adjust for potential confounders. P values <0.05 were considered statistically significant.

Results

Patient characteristics

Between January 2011 and December 2016, 423 *ALK*-positive patients were identified using FISH. From 289 patients with written informed consent, 53 had no stage IIIB/IV and 20 were lost to follow up. About 216 patients were eligible (Figure 1A). Median age, distribution of sex and histology are in line with earlier reports (Table 1) [3, 4]. Median follow-up was 34 months.

From 147 (68%) patients' tumors were analyzed by next-generation sequencing [LUN3 panel: 90 patients (61%); LUN4 panel: 57 patients (39%)]. Fifty patients (23%) were tested by additional single gene sequencing. Thirty-four (17%) of 197 patients were tested for PD-L1 expression, 135 (69%) received further FISH analyses. In 34 of 216 *ALK*-positive patients (16%) distribution of *ALK* variants was assessed by RNA sequencing (Figure 1A).

For 175 patients (81%) follow-up data for OS were available including 7 patients (3.2%) treated with best supportive care. Thus, 168 patients (77.8%) were subdivided (Figure 1B) into cohort A including 42 patients (19.4%) treated with chemotherapy only, cohort B including 71 patients (33%) with crizotinib and chemotherapy, cohort C including 18 patients (8.3%) with first-line crizotinib and cohort D including 37 patients (17.1%) with ceritinib after crizotinib with or without chemotherapy. Supplementary Figure S2, available at *Annals of Oncology* online shows treatment sequences in cohort D.

From 41 patients (19%, cohort Z) no complete therapy data until death or final follow-up were available including 5 patients treated with alectinib and 2 with brigatinib.

Co-occurring mutations, PD-L1 status and *ALK* variants

Mutations in *TP53* were the most frequent co-occurring mutations with 23.8% (34/143) of the tested patients. Among 36 *TP53* mutations 34 were classified as nonfunctional [22], 2 were of unknown functional significance (supplementary Table S5, available at *Annals of Oncology* online). All other co-alterations occurred rarely with frequencies between 0.6% for *BRAF* (1/171), 0.6% for *KRAS* (1/174) and 3.6% (4/112) for low-level *MET* amplification (Figure 2A and

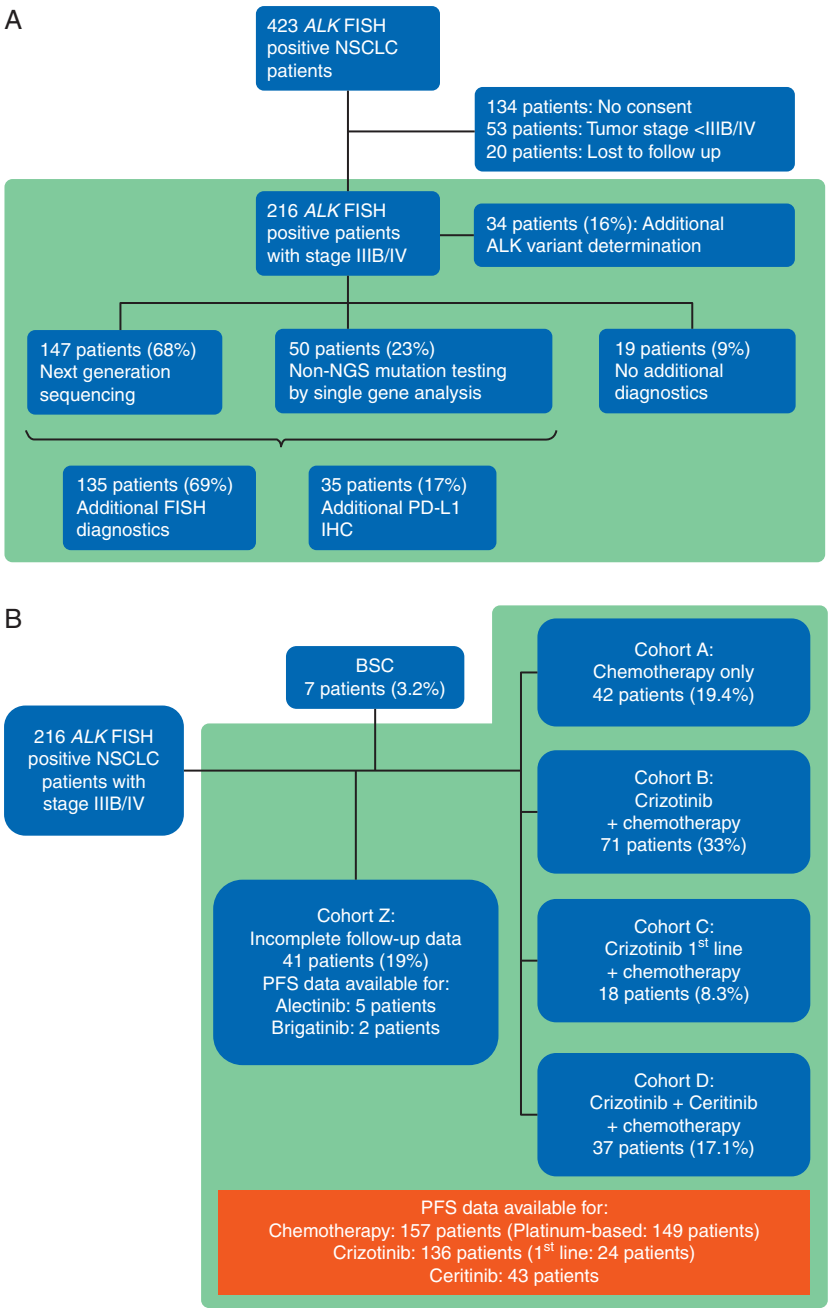

Figure 1. (A) Flowsheet of molecular diagnostics. NGS, next-generation sequencing; FISH, fluorescence *in situ* hybridization; IHC, immunohistochemistry. (B) Allocation of patients to cohorts for evaluation of treatment-related OS. BSC, best supportive care; PFS, progression-free survival; OS, overall survival.

supplementary Table S4, available at *Annals of Oncology* online). Four patients showed more than 1 co-occurring alteration (supplementary Figure S1, available at *Annals of Oncology* online).

PD-L1 expression of tumor cells was assessed in 34 patients (supplementary Table S4, available at *Annals of Oncology* online). In eight patients (23.5%) the PD-L1 score [21] was 5, i.e. more than 50% of the tumor cells expressed PD-L1. PD-L1 positivity was significantly correlated with *TP53* mutations [*TP53* wildtype (wt): 41% PD-L1 positive/*TP53* mutated: 90% PD-L1 positive; $P = 0.009$]. No significant difference was observed for high PD-L1 positivity (score 5) between wt and mutated *TP53* (Figure 2B).

In 18 of 34 patients (53%) *ALK* variant 1 was found, in 14 patients (41%) variant 3a/b and in 2 patients (6%) variant 2 (supplementary Table S8, available at *Annals of Oncology* online).

PFS dependent on therapy and *TP53* mutations

PFS was assessed in 157 patients with first-line chemotherapy (140 patients cohorts A–D plus 17 patients cohort Z, see Figure 1B), thereof 149 patients with platinum-based chemotherapy (109 resp. 103 PD at data cutoff), for crizotinib after chemotherapy in 112 patients (cohorts B–D and partly Z; 73 PD at data cutoff), for

Table 1. Baseline patient characteristics ($n = 216$)

	n	%
Sex	216	
Male	111	51.4
Female	105	48.6
Age at diagnosis (years)		
Mean	58.09	
Standard deviation	14.52	
Median	58 (19–89)	
Histology		
AD	210	97.2
Adenosquamous	3	1.4
w/o differentiation	3	1.4
Smoking history		
Never	86	46.3
Former	62	33.3
Current	38	20.4
n/a	30	
ECOG performance status		
0	63	43.4
1	63	43.4
2	16	11.1
3	3	2.1
n/a	71	
Tumor stage at diagnosis		
I	4	1.9
II	9	4.2
IIIA	14	6.5
IIIB	23	10.6
IV	166	76.8

w/o, without differentiation; n/a, not available; AD, adenocarcinoma.

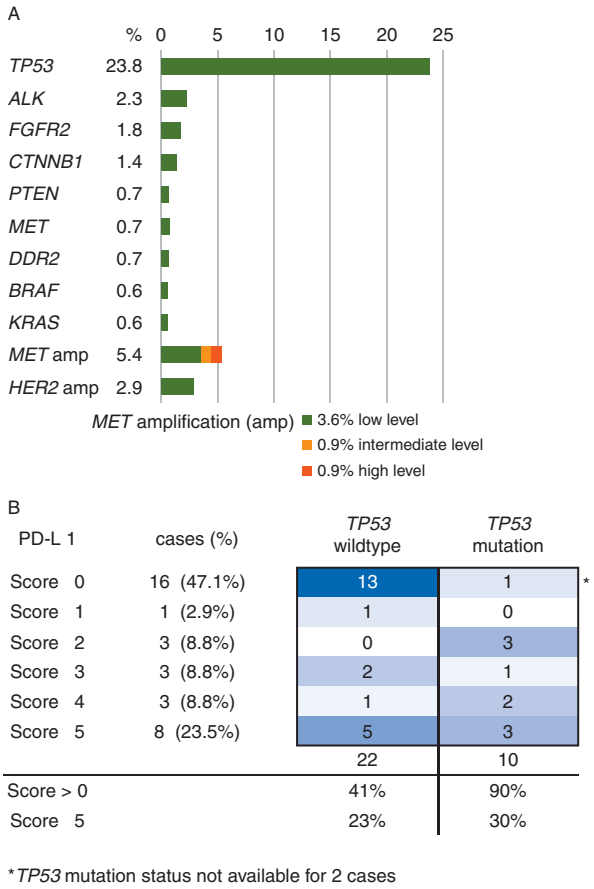

Figure 2. (A) Frequencies of co-occurring genetic aberrations in *ALK*-positive NSCLC patients. Results of NGS, single gene sequencing and FISH analysis in 197 *ALK* FISH-positive patients. (B) Correlation between PD-L1 positivity (expression score) and *TP53* mutation status in 34 *ALK*-positive patients.

crizotinib first line in 24 patients (cohorts C and D and partly Z; 12 PD at data cutoff) and for ceritinib after crizotinib with or without chemotherapy in 43 patients (cohorts D and partly Z; 28 PD at data cutoff). PFS of next-generation ALK inhibitors was calculated in 50 patients combined for ceritinib, alectinib (5 patients, 4 PD at data cutoff) and brigatinib (2 patients, 1 PD at data cutoff) because of the small patient number. PFS for first-line chemotherapy [5.4 months (95% CI: 3.7–7.1)] and for the subgroup of first-line platinum-based chemotherapy [5.5 months (95% CI: 3.8–7.2)] was inferior to PFS for first-line crizotinib [12.3 months (95% CI: 0.0–34.9); $P = 0.001$] and for crizotinib after chemotherapy [9.4 months (95% CI: 6.4–12.4); $P < 0.001$]. PFS for next-generation ALK inhibitors as sequential therapy after crizotinib was 7.0 months [(95% CI: 5.4–8.6); $P = 0.449$].

TP53 mutations were a negative prognostic factor for PFS regardless of systemic therapy. Median PFS with first-line chemotherapy was 2.6 months (95% CI: 1.3–4.1) with mutated *TP53* ($n = 27$) and 6.2 months (95% CI: 1.8–10.5) with *TP53* wt ($n = 75$) ($P = 0.021$). For crizotinib first-line median PFS was 5.5 months only (95% CI: 0.0–10.9) with mutated *TP53* ($n = 3$) versus 29.9 months (95% CI: 0.0–63.9) with *TP53* wt ($n = 15$) ($P = 0.007$). Similarly, for crizotinib after chemotherapy median PFS was 5.0 months (95% CI: 2.3–7.8) with mutated *TP53* ($n = 19$) versus 14.0 months (95% CI: 9.5–18.6) with *TP53* wt ($n = 56$) ($P = 0.004$). Regardless of treatment line, *TP53* mutation status segregated the median PFS of crizotinib-treated patients in an unfavorable *TP53*-mutated group [$n = 22$; 5.0 months (95% CI: 2.9–7.2)] and a favorable *TP53* wt group ($n = 71$; 14.0 months (95% CI: 8.0–20.1); $P < 0.001$]. Also, median PFS with next-generation ALK inhibitors after crizotinib was worse in patients with mutated *TP53* [$n = 11$; 5.4 months (95% CI: 0.1–10.7)] compared with *TP53* wt [$n = 22$, 9.9 months (95% CI: 6.4–13.5); $P = 0.039$]. In total, PFS of *TP53* co-mutated patients was 3.9 months [$n = 60$ (95% CI: 2.4–5.6)] and 10.3 months in *TP53* wt patients [$n = 168$ (95% CI: 8.6–12.0)] regardless of treatment ($P < 0.001$) (Figure 3A and supplementary Table S2, available at *Annals of Oncology* online).

The *ALK* variant 3a/b subgroup (cohorts A–D, $n = 20$) showed a nonsignificant trend toward better PFS with 11.9 months (95% CI: 0.9–23.1) versus variant 1 ($n = 31$) with 7.9 months (95% CI: 1.6–14.4) ($P = 0.285$). *TP53* mutations were negative predictive in both variant subgroups ($n = 30$; $P = 0.001$) with a strong trend in variant 1 [2.6 month (95% CI: 0.0–10.9) versus 15.9 months (95% CI: 1.4–30.6); $P = 0.068$] and reaching statistical significance in variant 3a/b ($P = 0.022$). Cox regression suggested a negative impact of *TP53* mutations on PFS regardless of ALK

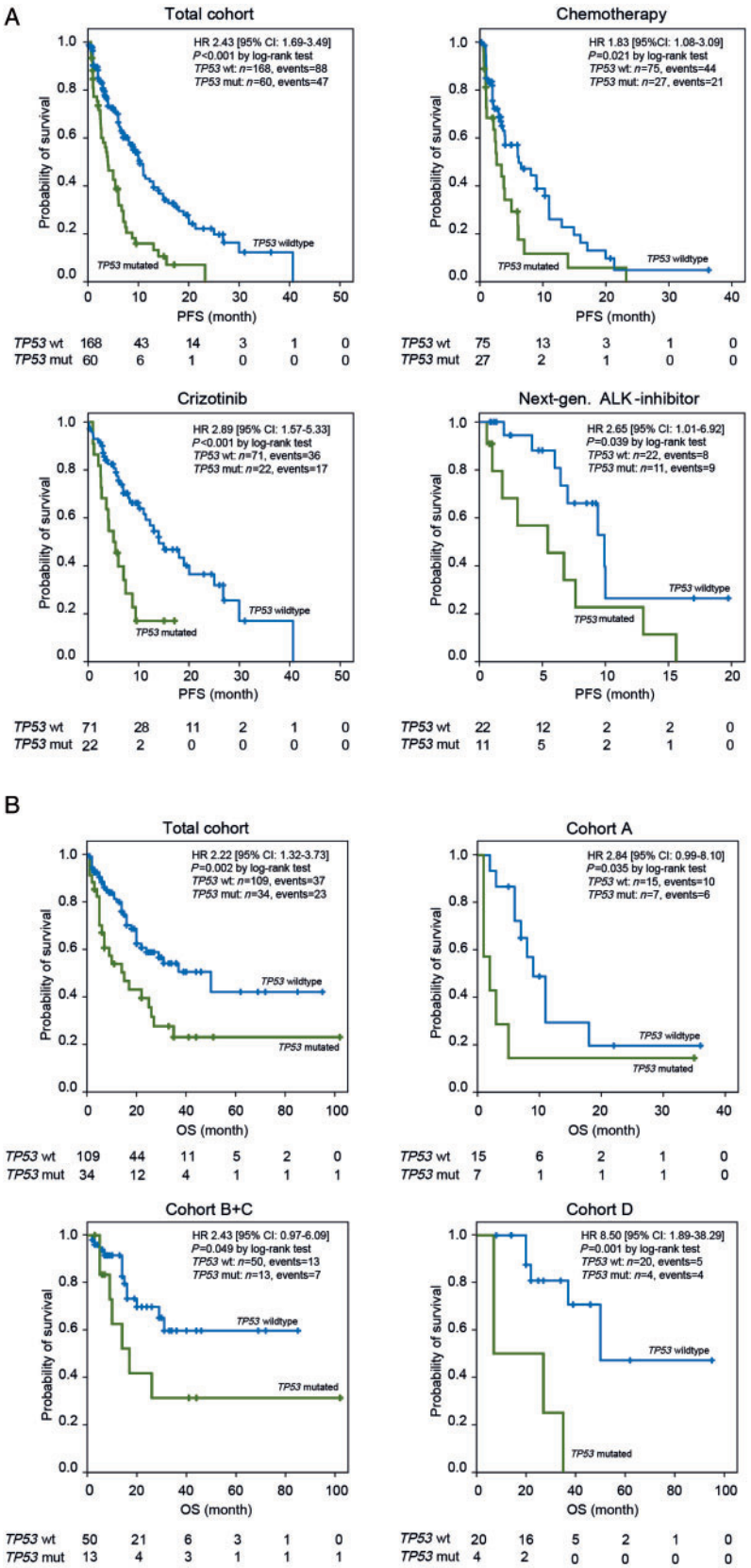

Figure 3. (A) PFS with different systemic treatments dependent on *TP53* mutation status. Kaplan–Meier blots for the total cohort ($n = 228$), for chemotherapy ($n = 102$), for crizotinib ($n = 93$) and for next-generation ALK inhibitors ($n = 33$). (B) OS in the treatment-related cohorts dependent on *TP53* mutation status. Kaplan–Meier blots for the total cohort ($n = 143$), for chemotherapy ($n = 22$), for crizotinib ($n = 63$) and for ceritinib ($n = 24$).

variants ($n = 30$; $P = 0.002$) (supplementary Tables S8 and S9, available at *Annals of Oncology* online).

OS dependent on therapy and *TP53* mutations

OS was assessed for 168 patients in cohorts A–D (Figure 1B). Median OS with chemotherapy only (cohort A, $n = 42$, 31 events at data cutoff) was with 9.0 months (95% CI: 5.0–12.9) inferior to all other cohorts treated with ALK inhibitors: cohort B ($n = 71$, 32 events) 31.0 months (95% CI: 0.4–61.6); $P < 0.001$, cohort C ($n = 18$, 2 events) median not reached ($P = 0.001$), cohort D ($n = 37$, 20 events) 45.0 months (95% CI: 32.3–57.7); $P < 0.001$. OS of patients treated with crizotinib starting from the first dose ($n = 89$; cohorts B + C) was 17.0 months (95% CI: 10.6–23.9).

TP53 mutations were a strong negative predictor for median OS in all cohorts. Median OS of mutated *TP53* patients ($n = 34$) was 15.0 months (95% CI: 5.0–24.9) compared with 50.0 months (95% CI: 22.9–77.1) for *TP53* wt patients ($n = 109$) ($P = 0.002$). With chemotherapy only (cohort A), the median OS in *TP53*-mutated patients ($n = 7$) was 2.0 months (95% CI: 0.0–4.6) compared with 9.0 months (95% CI: 6.1–11.9) in *TP53* wt patients ($n = 15$) ($P = 0.035$). For crizotinib-treated patients (cohorts B + C), OS for *TP53*-mutated patients ($n = 13$) was 17.0 months (95% CI: 6.7–27.3) compared with *TP53* wt patients ($n = 50$) for whom the median OS was not reached ($P = 0.049$). Also for patients treated with ceritinib after crizotinib (cohort D), a striking difference in OS was observed with 7.0 months only (95% CI: not reached) for *TP53* mutated patients ($n = 4$) and 50.0 months (95% CI: not reached) ($P = 0.001$) for *TP53* wt patients ($n = 20$).

Within the *TP53*-mutated patient cohort, median OS with chemotherapy only ($n = 7$) was 2.0 months (95% CI: 0.0–4.6) and thus inferior to ALK-inhibitor treatment ($n = 15$) with 17.0 months (95% CI: 4.6–29.4) ($P = 0.025$). For *TP53* wt patients treated with chemotherapy only ($n = 15$), the median OS was 9.0 months (95% CI: 6.1–11.9) compared with a median OS of 50.0 months (95% CI: 22.3–77.7) ($P < 0.001$) for *TP53* wt patients treated with ALK inhibitors with or without chemotherapy ($n = 54$) (Figure 3B and supplementary Table S3, available at *Annals of Oncology* online).

In univariate analysis including age, sex, smoking history, current smoker status, Eastern Cooperative Oncology Group (ECOG) performance status, number of brain metastases, number of treatment lines before crizotinib or ceritinib and *TP53* mutation status only current smoker status and *TP53* mutations were significant negative prognostic factors for OS ($P = 0.016$ and $P = 0.002$, respectively). In multivariate Cox regression analysis only *TP53* mutation remained an independent negative prognostic factor ($P = 0.004$) (supplementary Table S7, available at *Annals of Oncology* online).

Patients with *ALK* variant 3a/b ($n = 14$) had a nonsignificant better OS of 50 months (95% CI: 0.0–108.9) compared with variant 1 ($n = 18$) with 29.0 months (95% CI: 9.4–48.6) ($P = 0.815$). *TP53* mutations were prognostic negative in both variant subgroups reaching statistical significance in variant 1 ($P = 0.032$) (supplementary Table S8, available at *Annals of Oncology* online).

Discussion

We show that in *ALK*-positive NSCLC *TP53* mutations separate roughly one quarter of patients with a substantially worse outcome. PFS and OS were inferior compared with *TP53* wt patients treated with chemotherapy and ALK inhibitors.

TP53 alterations may damage tumor suppressor functions as loss of function mutations or trigger inhibition of apoptosis and genomic instability as gain of function mutations [23]. Thus, a negative prognostic impact of *TP53* mutations in cancer has been postulated and preclinical observations support this hypothesis [24]. While in unselected NSCLC such a negative prognostic impact has not been proven unequivocally [25–29], it has been reported in numerous reports for *EGFR*-mutated NSCLC treated with *EGFR* inhibitors. These results, however, only partly reached statistical significance [30–34]. In *ALK*-positive lung cancer, *TP53* mutations so far have not been described as significant negative prognostic factors.

The outcome in our treatment-related subgroups independently of *TP53* status confirmed what has been described in clinical trials [3, 4, 16] and registry analyses [6, 7, 12–14, 35]: superiority of ALK inhibitors over chemotherapy in terms of PFS and superiority of sequential ALK-inhibitor therapy compared with crizotinib monotherapy in terms of OS. Our results additionally show that *TP53* mutations represent the by far most frequent co-occurring mutations in *ALK*-positive NSCLC. By comparison, we found other co-mutations with a frequency of below 4% only; among them rarely those with actionable mutations like *BRAFV600*, high-level *MET* amplification or activating *KRAS* mutation.

Most important, our results suggest that about one-fourth of *ALK*-positive patients do not substantially benefit from recent progress of targeted therapy. As a limitation, concerning the use of next-generation ALK inhibitors statistically valid OS data could be assessed only for ceritinib. Future studies will have to prove, whether our findings can be confirmed for other next-generation ALK inhibitors.

In many cancer types, *TP53* mutations were shown to be associated with higher genetic instability [24]. Accordingly, we could recently show that early *TP53* mutations can lead to chromosomal instability in *ALK*-positive NSCLC [36]. It is tempting to speculate that a higher mutational burden might lead to a better efficacy of immune checkpoint inhibitors. Of note, the proportion of patients with PD-L1 positive tumor cells is enriched in our *TP53*-mutated group, although the number of patients is rather small.

Recently, in post ALK-inhibitor treatment biopsies it was shown that the type of *ALK* variant influences the development of ALK-inhibitor resistance mutations. In particular, *EML4-ALK* variant 3 was correlated with the development of *ALK G1202R* resistance mutation and a better PFS under treatment with the third-generation ALK-inhibitor lorlatinib, but not with first- and second-generation ALK inhibitors [37]. Similarly, in our pretreatment biopsies we saw a nearly equal distribution between *ALK* variants 1 and 3a/b and no significant influence of first- and second-generation ALK inhibitors on PFS. *TP53* mutations were negative prognostic in terms of PFS and OS in both variant subgroups. Based on the low patient number, which limits our conclusions, significance was only partly reached. It remains to be elucidated whether *TP53* mutation status and *ALK* variant status are independent prognostic factors.

In summary, we here describe *TP53* mutations as the first pretreatment biomarker in *ALK*-positive NSCLC identifying

patients with a substantially worse outcome from therapy. In future clinical trials stratification of this patient subgroup should be considered and new treatment strategies investigated to improve the outcome of *ALK/TP53* co-mutated patients.

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Disclosure

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