Original Article

TP53 co-mutations as an independent prognostic factor in 2nd and further line therapy—*EGFR* mutated non-small cell lung cancer IV patients treated with osimertinib

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Background: The negative prognostic and predictive value of *TP53* co-mutations (*TP53* mt+) in *EGFR* mutated (*EGFR* mt+) non-small cell lung cancer (NSCLC) is increasingly being acknowledged. Data consistently show that *TP53* mt+ impact negatively on 1st line objective response rate (ORR), progression free survival (PFS) and overall survival (OS) with 1st and 2nd generation tyrosine kinase inhibitors (TKI). However, a negative predictive impact has not been shown for the 3rd generation TKI Osimertinib. Therefore, we investigated the impact of *TP53* mt+ in *EGFR* mt+ NSCLC carrying a T790M resistance mutation and treated in 2nd/further lines with Osimertinib.

Methods: A total of 77 *EGFR* mt+ NSCLC IV patients carrying a T790M resistance mutation from two institutions were analyzed for *TP53* mt+. Clinical data including sex, age, presence of CNS metastases, etc., as well as types of *EGFR* and *TP53* mt+ were captured. PFS and OS were calculated from the start of Osimertinib.

Results: *TP53* mt+ were found in 32/77 patients (42%). *TP53* mt+ was a statistically significant independent negative predictive factor for PFS and OS. PFS for *TP53* mt+ patients were 9 months *vs.* 14 months for patients with *TP53* wild-type (*TP53*WT) (P<0.008). OS for *TP53* mt+ patients was 16 months *vs.* 24 months patients with *TP53*WT (P<0.025).

Conclusions: *TP53* mt+ have a negative impact on PFS and OS in a group of patients carrying a sensitizing *EGFR* mt+ and a T790M resistance mutation treated with Osimertinib. These data, together with the data for 1st/2nd generation TKI in 1st line treatment call for additional therapeutic and management concepts for this subgroup of patients.

Keywords: Non-small cell lung cancer (NSCLC); metastatic; EGFR; TP53 co-mutation; Osimertinib

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Introduction

TP53 mutations are observed in wild-type (WT) non-small-cell lung cancer (NSCLC) with an incidence of about 50% and have been associated with smoking status (1). In *EGFR* mutated (*EGFR* mt+) NSCLC patients, the frequency of *TP53* co-mutations (*TP53* mt+) ranges from 25.9% (2) to 49% (3-5) depending on the method of detection. *TP53* encoding p53 regulates cellular response to stress signals such as chemotherapy, radiation therapy and tyrosine kinase inhibition by inducing cell cycle arrest, senescence and/or apoptosis. Disruption of *TP53*'s normal function can lead to malignant transformation. Since most chemotherapeutics and most likely tyrosine kinase inhibitors (TKI) induce DNA-damage and consequently activate p53, mutations in the *TP53* gene might negatively affect response to cytotoxic treatment and thus represent a negative predictive factor (6-8). Also, *TP53* mt+ might be a negative prognostic factor in lung cancer (9-12), as in other tumor types (13,14).

The groups of Rosell, Crino and others have shown that *TP53* mt+ are negative predictors for chemotherapy or 1st line TKI in *EGFR* mt+ NSCLC. These analyses were hampered by inhomogeneous treatment or small numbers or the inclusion of different stages (2-4,15). We have recently shown in a group of *EGFR* mt+ NSCLC homogeneously treated in 1st line therapy with 1st or 2nd generation TKI that *TP53* mt+ have a negative impact on the objective response rate (ORR), the progression free survival (PFS) and the overall survival (OS). We could show that *TP53* mt+ were not associated with specific clinical characteristics with one exception, i.e., the risk of developing central nervous system (CNS) metastases during the course of the disease. Also, *TP53* mt+ seemed to be stable during the course of the disease, as no new *TP53* mt+ were acquired at the time of 1st or 2nd generation *EGFR* TKI resistance (5).

With the FLAURA trial being positive for both PFS and OS in comparison to 1st generation TKI, the standard of therapy for *EGFR* mt+ NSCLC has quickly changed to using 3rd generation TKI in 1st line therapy (16). Until now data on the potential impact of *TP53* mt+ in *EGFR* mt+ NSCLC treated with Osimertinib are lacking. Therefore, we investigated the role of *TP53* mt+ in *EGFR* mt+ NSCLC carrying a T790M resistance mutation and being treated with Osimertinib. To this end a multicentric cohort of NSCLC patients treated at the University Hospitals of Oldenburg and Heidelberg was employed. As in 1st and 2nd generation TKIs, we demonstrated an important and statistically significant negative impact of *TP53* mt+ on PFS and OS.

We present the following article in accordance with the REMARK reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-21-754/rc).

Methods

Patients

To be included in this retrospective cohort study, patients had to be at least 18 years old and had to provide written consent to analyze their data for this project. All patients had to be tested for *TP53* mt+ at first diagnosis of *EGFR* positive NSCLC IV. Included patients were required to have a T790M resistance mutation against 1st or 2nd generation TKI, and have been treated with Osimertinib in the 2nd or further therapy lines. Patients were excluded if they did not fulfill the inclusion criteria.

A total of 77 *EGFR* mt+ NSCLC IV patients (76 Caucasians, 1 Asian) from two certified lung cancer centers from Germany (Pius Hospital Oldenburg; Thoraxklinik Heidelberg) were included in the study. All patients were *EGFR* 3rd generation TKI naïve when they started Osimertinib; 70/77 (91%) patients were treated with TKI on 1st line therapy and 7/77 (9%) with chemotherapy. In particular, patients who were treated with osimertinib in the further lines were initially treated with chemotherapy or had to be switched from one to another *EGFR* TKI due to toxicities.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committees of the University of Oldenburg (No. 2014-I) and the University of Heidelberg (No. S-145/2017). Written informed consent was obtained from all the participants.

Specimen characteristics

Microtome sections (5 µM) were prepared from FFPE-tissue of NSCLC samples and one object slide was HE stained for tumor evaluation by a pathologist. Tumor tissue was gained from the remaining slides by manual microdissection, or in case material was limited, enriched by Laser Capture Microdissection (Leica CTR6500). DNA extraction was performed either manually (Macharey Nagel) or semi-automated (Maxwell MDx, Promega). The library preparation for the samples was performed using the Agilent SureSelect XT Kit as per the manufacturers' recommendations. Specimens were processed and stored at either Hematopathology Hamburg or at the Department of General Pathology and Pathological Anatomy, University Hospital Heidelberg.

Assay methods

Mutational analyses were performed by standard methods with hybrid capture assays as previously described (5). The Heidelberg cohort investigated for mutations by next-generation sequencing (NGS), as previously described (17). In all patients sufficient tumor material was available to analyze the *TP53* status. No patient dropped out of the study. *TP53* mt+ were classified according to different algorithms as previously described (5). Further information is provided in the supplementary data. The interested reader can find them in a supplementary appendix online (Appendix 1).

Study design

The entire retrospective study data were obtained from the daily clinical setting, for this purpose the study does not require randomization, blinding of patients or coordinators. The 77 patients were first diagnosed at one of the two hospitals between 2011 and 2019. We followed up with patients until February 2021, median follow up calculated from start of Osimertinib was 21 months. In order to account for the influence of subsequent therapy after Osimertinib on OS, we captured the therapy after stop of Osimertinib: 4/77 (5%) patients were treated after Osimertinib failure with I/O therapy (n=1), chemotherapy (n=3) or TKI therapy (n=3). Thus, the influence of subsequent therapy on OS is limited in this cohort.

Clinical and molecular data of the patients including sex, age, histology, smoking status, Eastern Cooperative Oncology Group (ECOG) status, metastases at first diagnosis, presence of CNS metastases at baseline and during course of disease and comorbidities [Charlson Comorbidity Index (CCI)], as well as types of *EGFR* and *TP53* mt+ were captured. All clinical outcome parameters were calculated from the start of 3rd generation TKI (ORR, PFS and OS).

Statistical analysis

The clinical characteristics of the 77 *EGFR* mt+ NSCLC patients were descriptively evaluated.

Patient and tumor characteristics were compared using chi-square test or *t*-test of independence or Fisher exact test. To estimate the risk differences related to the *TP53* status and the response to Osimertinib in 2nd or further line therapy the odds ratio (OR) was calculated. For this purpose, a confidence interval of 95% was used, which corresponds to a significance level of $P<0.05$. The correlations between patient characteristics and clinical outcome parameters PFS and OS are explored by non-parametric survival analyses using Kaplan-Meier calculations. The Kaplan-Meier method considered the different points of first diagnosis and the different observation periods of the patients during the analysis (18). In this retrospective analysis, bivariate dependencies of OS and PFS were shown with Kaplan-Meier curves and the log rank test for the calculation of P values. Furthermore, we constructed Cox regression models to predict the median PFS and the median OS separately in multivariate analyses. In the Cox regression models we used following covariates: histology, CNS at first diagnosis, CNS in front of Osimertinib, CNS after Osimertinib, CNS in total, age, sex, CCI, ECOG status, *TP53* status, smoking status and *EGFR* exon status. Subsequently, the covariates were excluded step by step using the reverse procedure *Forest*. The results are presented as hazard ratio (HR) with 95% confidence intervals and P values.

Results

Data

Baseline characteristics of the 77 *EGFR* mt+ NSCLC IV patients are summarized in *Table 1*. All 77 (100%) patients presented a common primary driver mutation, either del19 (n=58/77; 75%) or L858R (n=19/77; 25%). The presence of *EGFR* mt+ was associated with female sex (n=50/77; 65%) and never/light smoking status (n=54/77; 71.4%).

Table 1 Clinical characteristics of the *EGFR* mt+ NSCLC IV patients (n=77)

Variable	*TP53* mt+, n=32	*TP53*WT, n=45	Total, n=77	P value
Age (years), median (range)	59 (28–82)	66 (35–92)	64 (28–92)	0.042
Sex				0.554
Male	10 (31%)	17 (38%)	27 (35%)	
Female	22 (69%)	28 (62%)	50 (65%)	
ECOG status				0.500
0	17 (53%)	24 (53%)	41 (53%)	
1	12 (39%)	17 (38%)	29 (38%)	
≥2	0 (0%)	1 (2%)	1 (1%)	
Histology				0.396
Adenocarcinoma	32 (100%)	44 (98%)	76 (99%)	
Squamous cell carcinoma	0 (0%)	1 (2%)	1 (1%)	
Metastases at first diagnosis				0.679
Brain	7 (22%)	8 (18%)	15 (19%)	
Liver	6 (19%)	6 (13%)	12 (16%)	
Other	19 (59%)	31 (69%)	50 (65%)	
Smoking status				0.077
Never smoker	20 (40.8%)	29 (59.2%)	49 (63.6%)	
Light smoker	2 (33.3%)	4 (66.7%)	6 (7.8%)	
Current smoker	5 (29.4%)	12 (70.6%)	17 (22.1%)	
Ex heavy smoker	4 (100%)	0 (0%)	4 (5.2%)	
Exon status				0.631
Del19	25 (78%)	33 (73%)	58 (75%)	
L858r	7 (22%)	12 (27%)	19 (25%)	
CNS metastasis				
At first diagnosis	10 (31%)	12 (27%)	22 (29%)	0.661
Before Osimertinib	15 (47%)	25 (56%)	40 (52%)	0.538
After Osimertinib	18 (56%)	26 (58%)	44 (57%)	0.980
CCI, mean (range)	2.43 (0–9)	2.40 (0–5)	2.38 (0–9)	0.890
CCI groups				0.691
0–2	20 (63%)	26 (58%)	46 (60%)	
3–4	8 (25%)	15 (33%)	23 (30%)	
≥5	4 (12%)	4 (9%)	8 (10%)	

In five patients no ECOG status was available; in one patient no smoking status was available, in one patient no CNS metastasis status could be detected before or after Osimertinib. *EGFR* mt+, epidermal growth factor receptor mutation; NSCLC, non-small cell lung cancer; *TP53* mt+, tumor suppressor gene mutation; ECOG, Eastern Cooperative Oncology Groups; del19, deletion 19; L858R, exon 21 L858R mutation; CNS, central nervous system; CCI, Charlson Comorbidity Score.

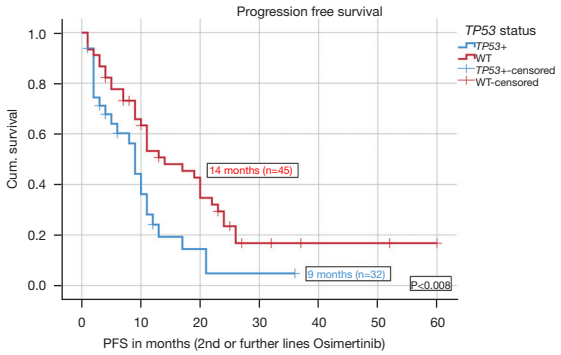

Figure 1 PFS of Osimertinib in 2nd/further lines depending on *TP53* status. Kaplan-Meier curve on median PFS of Osimertinib in 2nd or further line therapy for patients with *TP53* mt+ compared to patients with *TP53*WT; median PFS in months; for the calculation of the P value the log rank test was used. PFS, progression free survival; *TP53*, status tumor suppressor gene mutation status; WT, wild-type; cum., survival cumulative survival.

Median age was 64 years (range, 28–92 years). The vast majority of patients presented an ECOG status 0 or 1 (n=70/77; 91%). The average of the CCI was 2.38 (range, 0–9). A proportion of 28.6% (n=22/77) of patients had CNS metastases at primary diagnosis. Before start of Osimertinib, 18 patients had developed CNS metastases yielding to 40/77 (52%) of patients with brain metastases. At progression of Osimertinib and discontinuation of Osimertinib, only four additional patients developed brain metastases resulting in a total of 44/77 (57%). Thirty-seven/77 (48%) of the patients received Afatinib, 34/77 (44.1%) Erlotinib and 6/77 (7.8%) Gefitinib before start of Osimertinib. Seven patients (n=7/77, 9.1%) had received chemotherapy in 1st line therapy before being switched to 1st or 2nd generation TKI. All patients (n=77) were treated with Osimertinib in 2nd (n=51) or in further line therapy (n=26) at occurrence of T790M.

All patients were studied for *TP53* mt+ at first diagnosis and 42% (n=32/77) showed a *TP53* mt+. On TKI resistance (1st or 2nd generation TKI), all patients were rebiopsied (all with tissue). All patients had a T790M. In the Oldenburg cohort, all 11 patients with a *TP53* mt+ were retested for *TP53* status. In 2/11 (18%) there were missing data for the *TP53* status. In 3/9 (33%) patients, the *TP53* reanalysis was not successful. In 6/6 (100%) successfully retested patients, *TP53* configuration was stable in comparison to the test before start of 1st line TKI. In the Heidelberg cohort, the retest strategy for *TP53* was different with comparison to the Oldenburg cohort. In Heidelberg, only patients with *TP53*WT were reevaluated at acquired resistance for *TP53* status. Of the 38 *TP53*WT patients, 34 (89%) were reevaluated at progression for *TP53*; 28/34 (82%) patients were successfully retested for *TP53*. In 5/28 patients *TP53* status changed at progression from WT to mutation.

Clinical characteristics were analyzed depending on the *TP53* status and with the exception of age (P=0.042), no significant dependence as to any clinical characteristic was observed, including smoking status, type of *EGFR* mt+ or CNS metastasis status. *Table 1* shows the clinical characteristics of the patient cohort in total and stratified after the *TP53* status.

Analysis and presentation

ORR

ORR was available in 72/77 patients. ORR on Osimertinib in 2nd or further lines was 45% (n=32/72), 39% (n=28/72) had a stable disease (SD) and 16.7% (n=12/72) of the patients had primary progression.

Stratified according to *TP53* status, 38% (n=11/29) of patients with a *TP53* mt+ and 49% (n=21/43) of patients with a *TP53*WT achieved an ORR [complete response (CR)/partial response (PR)] on Osimertinib in 2nd or further line therapy. Patients with a *TP53*WT constellation had a numerically higher chance to have an objective response to Osimertinib than patients with a *TP53* mt+ (OR 1.5620; P=0.362) however this was not significantly different.

PFS

The PFS of 1st line therapy with 1st gen or 2nd generation TKI was 13 months (n=31) for patients with *TP53* mt+ and patients with *TP53*WT had an PFS of 17 months (n=39) (P=0.249). In a homogenously TKI treated patient population in our center published in Oncotarget, there was a significant difference in ORR, PFS and OS in the *EGFR* mt+ group dependent on *TP53* status.

Patients with *EGFR* del19 mutations had a similar median PFS on Osimertinib in 2nd and further line therapy [10 months (n=53)] as patients with *EGFR* L858R mutations [11 months (n=19); P=0.957].

Stratified according to *TP53* status, the median PFS on Osimertinib in 2nd or further lines was significantly shorter (9 months, n=32) for *TP53* mt+ patients compared to 14 months (n=45) for *TP53*WT patients (P=0.008). The HR for PFS on Osimertinib in 2nd and further lines was 0.502 with a significant P value of 0.012. *Figure 1* shows the median PFS on Osimertinib in 2nd or further line therapy

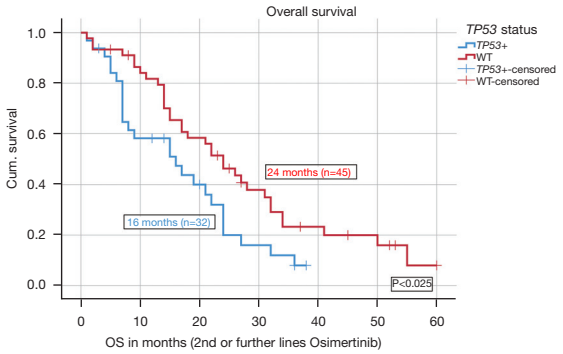

Figure 2 OS of Osimertinib in 2nd/further lines depending on *TP53* status. Kaplan-Meier curve on median OS of Osimertinib in 2nd or further line therapy for patients with *TP53* mt+ compared to patients with *TP53*WT; median OS in months; for the calculation of the P value the log rank test was used. OS, overall survival; *TP53*, status tumor suppressor gene mutation status; WT, wild-type; cum., survival cumulative survival.

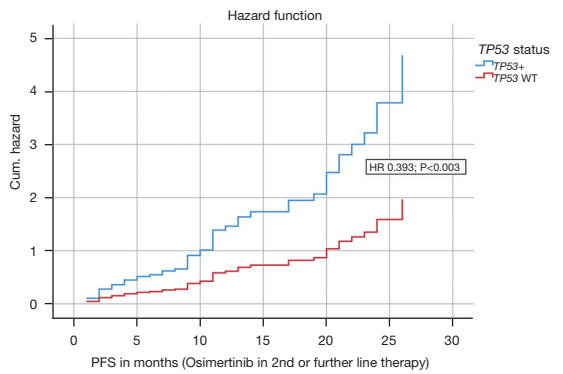

Figure 3 Hazard curve *TP53* mt+ *vs.* *TP53*WT on PFS (Osimertinib in 2nd/further lines). The figure shows hazard curve for the *TP53* status on median PFS on Osimertinib in 2nd or further line therapy. In the cox regression model for PFS patients with a *TP53*WT had a 60.7% lower hazard of progression to Osimertinib in 2nd or further line therapy than patients with a *TP53* mt+. PFS, progression free survival; *TP53*, status tumor suppressor gene mutation status; WT, wild-type; cum. hazard, cumulative hazard; HR, hazard ratio.

in detail. More results including PFS data from different *TP53* classifications and stratified after treatment lines are given in the Appendix 1. There were significant differences in PFS based on disruptive and nondisruptive, pathogenic and non-pathogenic mutations. Exon 8 was not significantly predictive due to low number (Tables S1,S2).

OS

The median OS for the entire cohort from the initial diagnosis was 46 months. Patients with *EGFR* del 19 mutations had a numerically longer median OS, calculated from the start of Osimertinib treatment in 2nd and further line therapy [22 months (n=53)] compared to patients with L858R mutations [15 months (n=19); P=0.223], however the difference was not significant.

When stratified according to *TP53* status, the median OS on Osimertinib in 2nd or further lines was significantly shorter (16 months, n=32) for *TP53* mt+ patients compared to 24 months for *TP53*WT patients (n=45) (P=0.025). The HR for OS on Osimertinib in 2nd and further lines was 0.561 with a significant P value of 0.031. *Figure 2* shows the median OS of Osimertinib in 2nd or further line therapy in detail. More results including OS data from different *TP53* classifications and stratified after treatment lines are given in the Appendix 1. There were no significant differences in the OS based on disruptive and non-disruptive mt+, pathogenic and non-pathogenic mt+ as well as exon 8 and non-exon 8 mt+, most likely due to low numbers (Tables S3,S4).

Multivariate analysis

PFS

In a multivariate analysis for the endpoint PFS on Osimertinib in 2nd or further line therapy the following covariates were included: CNS metastases at first diagnosis, CNS metastases after Osimertinib, CNS metastases in total, sex, CCI, ECOG status, *TP53* status, smoking status, *EGFR* exon status. The model with the 9 variables was statistically significant {likelihood =380.694; Chi-square[9] =17.386; P=0.043}. The covariates *TP53* status (HR 0.393; P=0.003) and ECOG status (HR 0.174; P=0.045) contributed significantly to the survival time model. *Figure 3* shows the hazard curve of the covariate *TP53* status and its negative impact on PFS (Osimertinib in 2nd or further line therapy). *Table 2* presents the survival time model and the covariates in detail.

OS

In a multivariate analysis on the endpoint OS on Osimertinib in 2nd or further line therapy the following covariates were included: histology, CNS metastases at first diagnosis, CNS metastases before Osimertinib, CNS metastases after Osimertinib, CNS metastases in total, age, sex, CCI, ECOG status, *TP53* status, smoking status, *EGFR* exon status. The model with the 12 covariates was

Table 2 Multivariate analysis on PFS and OS

Covariate	HR	95% CI	P value
PFS			
TP53 mt+ (*vs.* wild-type)	0.393	0.210–0.735	0.003
ECOG status	0.174	0.032–0.962	0.045
CNS at first diagnosis	0.486	0.229–1.033	0.061
CNS after Osimertinib	0.674	0.372–1.219	0.192
CNS in total	0.928	0.443–1.944	0.844
Sex	0.551	0.295–1.031	0.062
CCI	0.960	0.797–1.157	0.667
Smoking status	0.550	0.250–1.210	0.137
EGFR exon status	0.863	0.433–1.718	0.674
OS			
TP53 mt+ (*vs.* wild-type)	0.352	0.180–0.687	0.002
ECOG status	0.098	0.016–0.601	0.012
Histology	0.135	0.16–1.153	0.067
Age	1.003	0.963–1.045	0.877
Sex	0.678	0.352–1.305	0.244
CNS at first diagnosis	0.236	0.098–0.571	0.001
CNS before Osimertinib	0.870	0.224–3.373	0.840
CNS after Osimertinib	0.720	0.186–2.785	0.634
CNS in total	0.474	0.208–1.080	0.076
CCI	0.964	0.727–1.280	0.800
Smoking status	0.758	0.326–1.764	0.520
EGFR exon status	1.075	0.561–2.062	0.827

PFS, progression free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; *TP53* mt+, tumor suppressor gene; ECOG, Eastern Cooperative Oncology Groups; CNS, central nervous system; CCI, Charlson Comorbidity Score; EGFR, epidermal growth factor receptor.

statistically significant {likelihood =388.239; Chi-square[12] =23.144; P=0.027}. The covariates *TP53* status (HR 0.352; P=0.002), ECOG status (HR 0.098; P=0.012) and CNS at first diagnosis (HR 0.236; P=0.001) contributed significantly to the survival time model and were independent negative factors. *Figure 4* shows the hazard curve of the covariate *TP53* status and its negative impact on OS (Osimertinib in 2nd or further line therapy). *Table 2* presents the survival time model and the other covariates in detail.

Discussion

Previous studies showed that *TP53* mt+ have a negative impact on ORR, PFS and OS in *EGFR* mt+ NSCLC treated in 1st line with 1st or 2nd generation *EGFR* TKI (1-5,14,15-19). The current study addressed the question whether *TP53* mt+ influences the outcome when patients are treated with Osimertinib, a 3rd generation *EGFR* TKI that was initially approved for 2nd line treatment of *EGFR* mt+ NSCLC patients carrying a T790M resistance mutation.

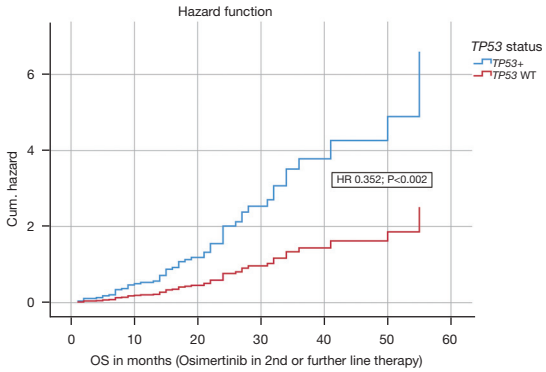

Figure 4 Hazard curve *TP53* mt+ *vs.* *TP53*WT on OS (Osimertinib in 2nd/further lines). The figure shows the hazard curve for the TP53 status on median OS on Osimertinib in 2nd or further line therapy. In the cox regression model for OS patients with a *TP53*WT had a 64.8% lower hazard of progression to Osimertinib in 2nd or further line therapy than patients with a *TP53* mt+. OS, overall survival; *TP53*, status tumor suppressor gene mutation status; WT, wild-type; cum., hazard cumulative hazard; HR, hazard ratio.

Our data show that the presence of *TP53* mt+ impact ORR (not significant) and PFS and OS significantly. Overall, the low response rate of 45% for Osimertinib in our cohort and the significant difference in OS between del19 and L858R (Table S3) is somewhat different to the results of the AURA3 trial (19,20). As our study is a retrospective study there is a possibility of group selection bias due to the small sample size. Therefore, there might be a limitation as to the association between *TP53* mt+ and the effect of TKIs in our analysis.

The prevalence/frequency of *TP53* mt+ was similar in this group of homogeneously *EGFR* T790M positive patients compared to a group of patients analyzed before the start of 1st line therapy, underlining the fact that *TP53* mt+ seem to be stable throughout the course of the disease. Notably, with the exception of a weak association with age, *TP53* mt+ was not associated with any clinical parameters such as sex and smoking status. There was a slightly higher incidence of CNS metastases at baseline (before the start of Osimertinib) in the *TP53* mt+ subgroup compared to the *TP53*WT group, consistent with the finding that *TP53* mt+ confer a higher risk of progression in the CNS on 1st or 2nd generation TKI (5). Importantly CNS progression on Osimertinib was not different in the *TP53* mt+ compared to the *TP53*WT group consistent with the high CNS efficacy of Osimertinib in the brain.

TP53 mt+ were significantly associated with inferior outcome both in univariate as well as multivariate analyses. Therefore, the impact of *TP53* mt+ seems to be consistent independently of the generation of *EGFR* TKI. It is difficult to imagine why Osimertinib would overcome the effect of *TP53* mt+ in *EGFR* mt+ NSCLC, as the mechanism of action is dependent on the inhibitor of the *EGFR* pathway as with 1st and 2nd generation TKI.

Similarly, in *ALK* translocated NSCLC, it is well known that *TP53* mt+ impact PFS and OS, an effect which is independent of the *ALK* fusion variant (21,22). In a recent analysis with the 3rd generation *ALK* inhibitor Lorlatinib, it was shown that *TP53* mt+ impact significantly on PFS and OS therefore underlining the overriding effect of *TP53* mt+ on the effectiveness of the 3rd generation *ALK* inhibitor (23).

Given the negative impact of *TP53* mt+ irrespective of the line of treatment and irrespective of the generation of *EGFR* TKI, it is tempting to speculate that 1st line Osimertinib might also be less efficacious in *EGFR* mt+ patients carrying a *TP53* mt+. An analysis of the *TP53* mt+ analysis within the FLAURA trial and their impact on PFS and OS are eagerly awaited.

In our opinion, it is clear that these data should have an impact on the design of future trials, i.e., that future *EGFR* mt+ trials should be stratified for the presence of *TP53* mt+. This strategy would help to dissect the impact of *TP53* mt+ on ORR, PFS and OS in a controlled trial scenario. Second, with the revival of TKI and chemotherapy combinations, it would be of interest to investigate whether the influence of *TP53* mt+ might be overcome by the adding chemotherapy to *EGFR* TKI. Third, in routine practice we would advocate for testing patients for *TP53* mt+ and for monitoring these patients more closely when treated with TKI therapy then patients with *TP53*WT.

Sallman *et al.* demonstrated that combination treatment with a *TP53* modulator, that restores *TP53* function when mutated or inactivated (APR-246) and chemotherapy (Azacytidine) was well-tolerated yielding high rates of clinical response (71%) and an OS of 10.8 months in patients with *TP53*-mutant myelodysplastic syndrome (MDS) and oligoblastic acute myeloid leukemia (AML) (24). Finally, as T*P53* modulators, such as APR-246 have been shown to be effective in hematologic disorders, one could envision to combine *EGFR* TKI and *TP53* modulators in NSCLC clinical trials.

Conclusions

As in 1st line therapy with 1st and 2nd generation TKI, *TP53* mt+ have a negative impact on ORR, PFS and OS in 2nd and further line with the 3rd generation TKI Osimertinib. Strategies should be developed to monitor *TP53* mt+ patients on *EGFR* TKI more closely than *TP53*WT patients. Clinical studies should stratify for the presence of *TP53* mt+ and should investigate *EGFR* TKI combinations, potentially with chemotherapy or with specific *TP53* modulators.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by ethics committees of the University of Oldenburg (No. 2014-I) and the University of Heidelberg (No. S-145/2017). Written informed consent was obtained from all the participants.

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Details on the detection of *TP53* co-mutations and the three different *TP53* classifications

Assay methods

Against the background of technological advances in recent years, *EGFR* exon 18–21 and *TP53* (exons 4–10) mutational analysis were performed by NGS-based methods. Alternatively, an amplicon-based NGS panel (Illumina platform) was used to detect mutations in 38-42 relevant genes, including *TP53*. Part of the samples were analyzed with a hybrid capture based target enrichment followed by massively parallel sequencing [Hybrid Capture NGS, NeoSelect, NEO New Oncology, IonTorrent (ThermoFisher Scientific)]. The library preparation for the samples was performed using the Agilent SureSelect XT Kit as per the manufacturers' recommendations (5,17).

TP53 mt+ were classified according to three different algorithms as previously described: (I) classification by Poeta *et al.* (14), (II) by an extended algorithm based on Poeta *et al.* (14) with additional parameters like structural prediction and GVDV biophysical analysis (25) and (III) based on exon 8 vs. non-exon 8 mutations (4).

In an effort to specify the functional significance of the respective mutations in further detail (14), we included additional parameters in order to modify differentiation into pathogenic vs. non-pathogenic *TP53* co-mutations (25). These mutations are likely to interfere with *TP53* function significantly. Also, if an Align-GVGD score of C65 was reached, mutations were classified as pathogenic. Specifically, DNA-contact-mutations R273C, R273G, R248Q were reclassified as pathogenic mutations, since functional impairment is likely (25). Mutation R280I is located within the LSH2- (loop-sheet-helix region 2), which is part of the DNA-binding core and was therefore re-categorized as pathogenic. Mutations H179R and C176S constitute Zn^{2+}-binding sites and were therefore also regarded as pathogenic upon review.

The third classification was recently proposed by the group of Canale *et al.* (3). The authors characterized a cohort of *EGFR* mt+ patients that in 30.1% of cases carried additional *TP53* mt+ and these were categorized based on exons. *TP53* mt+ within exon 8 were associated with significantly lower DCR, and shorter PFS and OS. In addition to that, we showed similar results for *TP53* exon 8 co-mutations in our *EGFR* mt+ NSCLC IV cohort treated with 1st or 2nd generation TKI's.

Detailed results on each treatment line with Osimertinib and the three different *TP53* classifications.

Results

Analysis and presentation

PFS

Table S1 Median PFS in months on Osimertinib in 2nd line therapy

	n	PFS	P value
EGFR exon status			0.684
del19	33	10	
L858R	15	11	
TP53 status			0.033
TP53 mt+	24	13	
*TP53*WT	27	9	
TP53 status according to Poeta *et al.* (14)			0.100
TP53 disruptive mt+	15	8	
TP53 non-disruptive mt+	9	11	
*TP53*WT	27	13	
TP53 status according to Roeper *et al.* (25)			0.079
TP53 pathogenic mt+	17	8	
TP53 non-pathogenic mt+	7	12	
*TP53*WT	27	13	
TP53 status according to Canale *et al.* (3)			0.052
TP53 exon 8	4	10	
TP53 non-exon 8	20	8	
*TP53*WT	27	13	

PFS, progression free survival; *EGFR*, epidermal growth factor receptor; del19, deletion 19; L858R, exon 21 L858R mutation; *TP53*, status tumor suppressor gene status; *TP53* mt+, tumor suppressor gene mutation; WT, wild-type; mt+, mutation.

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Table S2 Median PFS in months on Osimertinib in 2nd and further line therapy

	n	PFS	P value
TP53 status according to Poeta *et al.* (14)			0.011
TP53 disruptive mt+	19	8	
TP53 non-disruptive mt+	13	11	
*TP53*WT	45	14	
TP53 status according to Roeper *et al.* (25)			0.030
TP53 pathogenic mt+	23	9	
TP53 non-pathogenic mt+	9	11	
*TP53*WT	45	14	
TP53 status according to Canale *et al.* (3)			0.017
TP53 exon 8	4	10	
TP53 non-exon 8	28	9	
*TP53*WT	45	14	

PFS, progression free survival; *TP53*, tumor suppressor gene status; mt+, mutation; *TP53* mt+, tumor suppressor gene mutation; WT, wild-type.

OS

Table S3 Median OS in months on Osimertinib in 2nd line therapy

	n	OS	P value
EGFR exon status			0.019
del19	33	24	
L858R	15	11	
TP53 status			0.135
TP53 mt+	24	16	
*TP53*WT	27	24	
TP53 status according to Poeta et al. (14)			0.287
TP53 disruptive mt+	15	21	
TP53 non-disruptive mt+	9	15	
*TP53*WT	27	24	
TP53 status according to Roeper et al. (25)			0.250
TP53 pathogenic mt+	17	21	
TP53 non-pathogenic mt+	7	15	
*TP53*WT	27	24	
TP53 status according to Canale et al. (3)			0.232
TP53 exon 8	4	27	
TP53 non-exon 8	20	15	
*TP53*WT	27	24	
*TP53*WT	27	13	

OS, overall survival; EGFR, epidermal growth factor receptor; del19, deletion 19; L858R, exon 21 L858R mutation; *TP53*, status tumor suppressor gene status; *TP53* mt+, tumor suppressor gene mutation; WT, wild-type; mt+, mutation.

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Table S4 Median OS in months on Osimertinib in 2nd and further line therapy

	n	OS	P value
TP53 status according to Poeta *et al.* (14)			0.081
TP53 disruptive mt+	19	16	
TP53 non-disruptive mt+	13	15	
*TP53*WT	45	24	
TP53 status according to Roeper *et al.* (25)			0.032
TP53 pathogenic mt+	23	16	
TP53 non-pathogenic mt+	9	15	
*TP53*WT	45	24	
TP53 status according to Canale *et al.* (3)			0.054
TP53 exon 8	4	27	
TP53 non-exon 8	28	15	
*TP53*WT	45	24	

OS, overall survival; *TP53*, status tumor suppressor gene status; *TP53* mt+, tumor suppressor gene mutation; mt+, mutation; WT, wild-type.

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