Research Paper

TP53 co-mutations in EGFR mutated patients in NSCLC stage IV: A strong predictive factor of ORR, PFS and OS in EGFR mt+ NSCLC

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ABSTRACT

Introduction: The impact of TP53 co-mutations in EGFR mutated patients on PFS and OS is controversial. Different classifications of TP53 mutations with respect to functional and potential clinical impact have been published. Therefore, we retrospectively analyzed the impact of TP53 co-mutations on ORR, PFS and OS in a cohort of EGFR mutated NSCLC IV patients (UICC 7) using different classifications of TP53 mutations.

Methods: 75 EGFR mutated NSCLC IV patients homogeneously treated with 1st line EGFR TKI were analyzed for TP53 co-mutations. TP53 mutations were classified according to three different types of classifications. The endpoints ORR, PFS and OS were investigated.

Results: TP53 co-mutations were found in 29/59 patients (49.2%). TP53 co-mutations were a statistically significant independent negative predictive factor for ORR, PFS and OS. TP53 co-mutations were associated with inferior mPFS and mOS: mPFS/mOS 12 vs. 18/24 vs. 42 months for non-disruptive/disruptive mutations vs. WT ($p < 0.004$)/($p < 0.009$), 11 vs. 17/23 vs. 42 months for pathogenic vs. non-pathogenic/WT ($p < 0.001$)/($p < 0.001$), and 7 vs. 12 vs. 18/12 vs. 28 vs. 42 months for exon 8 vs. non-exon 8 vs. WT ($p < 0.001$)/($p < 0.002$).

Conclusions: TP53 co-mutations are frequent in EGFR mt+ NSCLC and have a strong negative impact on all clinical endpoints of TKI therapy.

INTRODUCTION

EGFR mutations have been identified as one of several driver mutations in NSCLC. EGFR mutations are associated with female sex, non-smoking status and non-squamous histology and occur in the Caucasian NSCLC population with an incidence of 12–15%. EGFR mutations are differentiated into common mutations (exon 19 deletions and exon 21 L858R mutations) and uncommon mutations. The standard of care of EGFR mutated patients is 1st line EGFR TKI treatment based on the results of a number of

phase III trials showing a statistically significant advantage of EGFR TKI regarding ORR (objective response rate), PFS (Progression Free Survival), toxicity and quality of life (QoL) as well as in some studies OS (Overall Survival) over chemotherapy [1, 2]. TP53 co-mutations are observed in wild type (WT) NSCLC with an incidence of about 50% and have been associated with smoking status [3].

In EGFR mt+ NSCLC patients the frequency of TP53 mutations ranges from 25.9% [4] and 41% [5] depending on the method of detection. TP53 protein 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 TKI's induce DNA-damage and consequently activate the TP53 protein, mutations in the TP53 gene might negatively affect response to cytotoxic treatment and thus represent a negative predictive factor [8–10]. Also, TP53 mutations might be a negative prognostic factor in lung cancer [11–14], as in other tumor types [15, 16]. However, the impact of TP53 mutations on treatment outcome has not been definitively analysed in EGFR mt+ NSCLC.

Different types of TP53 mutations might confer different prognostic outcome, as shown by Poeta et al. [16]. The autors showed a significantly inferior outcome for EGFRmt+ patients with disruptive TP53 mutations. Classification into disruptive and non-disruptive mutations was based on parameters like localization (i. e. DNA binding domain) and altered biochemical characteristics of the exchanged aminoacid. However, this classification yielded different results in head and neck cancer [16], where disruptive mutations were associated with superior outcomes. An extended functional analysis including additional biophysical parameters (align Grantham Variation Grantham Deviation - GVGD) and a structural analysis might predict more precisely the biologic impact of TP53 mutations [17]. Lastly a third classification that grouped TP53 mutations according to the exon position (exon 8 vs. non-exon 8 mutations) was proposed by Canale et al. [18], where a significant negative impact of exon 8 TP53 mutations on Disease Control Rate (DCR) and PFS in EGFR mt+ NSCLC was shown, however only for the EGFR exon 19 deletion positive cases. No information is available on the stability of TP53 mutations at primary diagnosis and at TKI resistance, and non information as to potential differences in clinical characteristics of EGFR mt+ patients with and without TP53 co-mutations is available.

Therefore, this manuscript addresses the following questions (a) what is the impact of TP53 co-mutations clinically relevant endpoints ORR, PFS and OS in EGFR mt+ positive patients homogeneously treated with a 1st line EGFR TKI (afatinib, erlotinib or gefitinib), (b) do different classifications have different impact on outcome parameters (c) are TP53 mutations associated with distinct clinical characteristics, and (d) is TP53 mutation status stable at acquired TKI resistance.

RESULTS

Patients

Overall, 74/75 patients received first line TKI and one patient died before initiation of treatment. First line treatment with erlotinib (33/74; 44.6%), gefitinib (22/74; 29.7%) and afatinib (19/74; 25.7%) and follow-up was performed according to standard guidelines. 69/75 (92%) patients presented with a common mutation, either del19 or L858R in EGFR. 6 patients carried an uncommon EGFR mutation (group I: $n = 4$/III: $n = 2$, according to Yang et al. [19]). The presence of EGFR mutations was associated with female sex (52/75; 69.3%) and never/light smoking status (51/75; 68%). Median age was 66 years. The vast majority of patients presented with an ECOG status 0 (43/75; 57.3%) or 1 (23/75; 30.7%). 38/75 (50.7%) of patients had CCI (Charlson Comorbidity Score) of 2 or 3 points. No dependence as to the type of EGFR mutation was found, as patients with an del19 EGFR mutation had TP53 mutations in 17/42 (40.5%) cases and patients with L858R mutation in 10/27 (37%) cases. For details see appendix (Supplementary Table 1).

TP53 analysis

In 59/75 (78.7%) cases TP53 mutation analysis was successful, 16 cases could not be tested because of insufficient tumor material. Figure 1 shows the classification of the different types of TP53 mutations. In 30/59 (50.8%) cases a TP53 WT configuration was observed. TP53 mutations were grouped according to Poeta et al. [16] into non-disruptive (13/59; 22%) and disruptive TP53 mutations (16/59; 27.1%). The structural/biopysical classification resulted in 7/59 (11.9%) patients with non-pathogenic and 22/59 (37.3%) patients with pathogenic TP53 mutation. 6/59 (10.2%) patients had a TP53 exon 8 mutation and 23/59 (39%) a TP53 non-exon 8 mutation.

With the exception of the risk of developing CNS metastases during the course of the disease, no dependence as to any clinical characteristic was observed, including smoking status or type of EGFR mutation. Patients with TP53 mutations (both Poeta et al. [16] and structural/biophysical classifications) developed CNS metastases during the course of the disease in 10/29 (34.5%) cases, in contrast to only 2/29 (6.9%) with TP53 WT ($p < 0.02$ Poeta et al. [16]; $p < 0.01$ structural/biophysical classification). Detailed information is shown in Table 1. Patients with a TP53 mutation have shown a 5 times higher risk of developing CNS metastases during the course of the disease compared to patients with a TP53 WT configuration (OR 5.17 [1.04 to 25.66]; $p < 0.04$). Table 1 shows the clinical characteristics in detail.

On TKI resistance (1st or 2nd generation TKI) 10/29 (34.5%) patients had a rebiopsy and TP53 status remained stable. In patients with TP53 mutation at the time of primary diagnosis, the same TP53 mutation persisted in the acquired resistance situation in 9/10 (90%) patients, in 1/10 (10%) cases the initial TP53 mutation was not detected most likely due to lack of material. 34/74 (46%) patients were tested for T790M and 20/34 (59%) developed a T790M after 1st line TKI. The risk of developing a T790M+ was independent of the presence of a TP53 mutation at diagnosis. Additional information is shown in the appendix (Supplementary Table 2).

Objective response rate

ORR was analyzed using the three TP53 classifications in 72 patients (1 died prior to start of TKI, 2 died prior of first assessment of response). The CR/PR rate in TP53 WT patients was 27/28 (96.4%) and 20/29 (69%) for non-disruptive/disruptive TP53 mutations. The odds to reach CR/PR was 12.15 for patients with WT vs. non-disruptive/disruptive TP53 mutation ($p < 0.02$). 15/22 (68.2%) of patients with a pathogenic and 5/7 (71.4%) of patients with a non-pathogenic TP53 mutation achieved a CR/PR. The odds to reach CR/PR was 4.98 for TP53 WT/ non-pathogenic mutations vs. pathogenic TP53 mutations ($p < 0.03$). 4/6 (66.7%) of patients with an exon 8 mutation and 16/23 (69.6%) of patients with a non-exon 8 mutation had a CR/PR. The odds to reach CR/PR was 13.5 for patients with TP53 WT vs. exon 8 mutations ($p < 0.05$). Thus TP53 co-mutations are a strong negative predictor of ORR. Additional information is shown in Table 2.

In the multivariate analysis TP53 non-disruptive/ disruptive and TP53 pathogenic mutations remained independent negative predictive factors for ORR: Other independent factors are EGFR uncommon mutations, ECOG status 2 and the variable no initial CNS metastases. The complete results are shown in the appendix (Supplementary Table 3).

Progression free survival

Statistically significant differences in median PFS were found in all TP53 mutation classifications (Table 3): mPFS in non-disruptive/disruptive TP53 mutations (n =
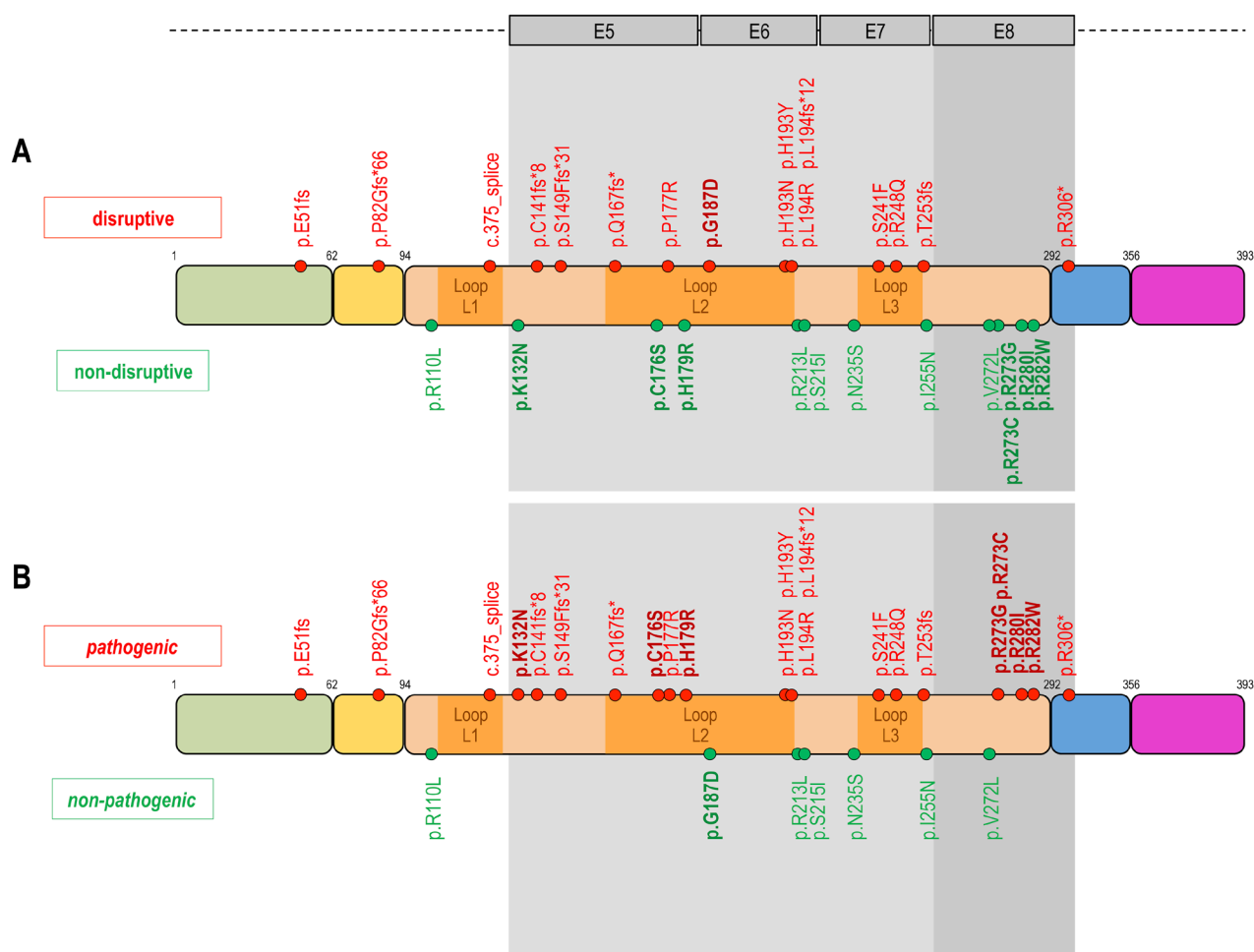
Figure 1: Classification of TP53 mutations. Proposed three different types of classifications of TP53 mutations. The upper DNA strand (**A**) shows the classification according to Poeta et al. 2007, which distinguishes between disruptive (n = 16) and non-disruptive mutations (n = 13). The disruptive mutations are highlighted in red to illustrate the negative impact on the clinical endpoints (PFS, OS, TKI response). The lower DNA strand (**B**) shows our structural/biophysical classification, which distinguishes between pathogenic and non-pathogenic mutations. The structural/biophysical classification shows more TP53 mutations with a pathogenic (n = 22) impact on clinical endpoints. The fields highlighted in grey show the classification according to Canale et al. 2017. Canale et al. grouped TP53 mutations according to the exon position (exon 8 vs. non-exon 8 mutations). TP53 mutations in exon 8 have a more negative impact on clinical endpoints than mutation in non-exon 8. Inspired by Poeta ML, Manola J, Goldwasser MA et al. 2007; Canale M, Petracci E, Delmonte A et al. 2017.

Table 1: Clinical characteristics of the TP53 patients

	TP53 Poeta et al. classification					
	non-disruptive *n* = 13	**disruptive** *n* = 16	**TP WT** *n* = 30	**TP53 unknown** *n* = 16	***p*-value**	**ALL** *n* = 75
Age (years)						
mean	63 (49–81)	65.5 (50–75)	65.2 (45–85)	67.6 (45–82)	0.72	65.4 (45–85)
median	63	67.5	69	67	0.66	66
Sex					0.56	
men	4 (30.8%)	7 (43.75%)	7 (23.3%)	5 (31.3%)		23 (30.7%)
women	9 (69.2%)	9 (56.25%)	23 (76.7%)	11 (68.7%)		52 (69.3%)
in total	13 (100%)	16 (100%)	30 (100%)	16 (100%)		75 (100%)
Smoking status					0.63	
never/light smoker	8 (61.5%)	12 (75%)	23 (76.7%)	8 (50%)		51 (68%)
ex/current smoker	5 (38.5%)	4 (25%)	7 (23.3%)	5 (31.25%)		21 (28%)
not known	0	0	0	3 (18.75%)		3 (4%)
in total	13 (100%)	16 (100%)	30 (100%)	16 (100%)		75 (100%)
ECOG					0.56	
0	6 (46.1%)	12 (75%)	17 (56.7%)	8 (50%)		43 (57.3%)
1	5 (38.5%)	4 (25%)	10 (33.3%)	4 (25%)		23 (30.7%)
2	2 (15.4%)	0	3 (10%)	3 (18.8%)		8 (10.7%)
3	0	0	0	1 (6.2%)		1 (1.3%)
in total	13 (100%)	16 (100%)	30 (100%)	16 (100%)		75 (100%)
CCI					0.48	
0–1	7 (53.8%)	6 (37.5%)	8 (26.7%)	3 (18.7%)		24 (32%)
2–3	3 (23.1%)	5 (31.25%)	16 (33.3%)	7 (43.8%)		31 (41.3%)
>3	3 (23.1%)	5 (31.25%)	6 (20%)	6 (37.5%)		20 (26.7%)
in total	13 (100%)	16 (100%)	30 (100%)	16 (100%)		75 (100%)
Exon status					0.94	
exon 19	9 (69.2%)	8 (50%)	16 (53.3%)	9 (56.25%)		42 (56%)
exon 21	4 (30.8%)	6 (37.5%)	12 (40%)	5 (31.25%)		27 (36%)
uncommon exons	0	2 (12.5%)	2 (6.7%)	2 (12.5%)		6 (8%)
in total	13 (100%)	16 (100%)	30 (100%)	16 (100%)		75 (100%)
Initial CNS **metastasis** CNS **primary**					0.57	
• yes	3 (23.1%)	3 (18.7%)	9 (30%)	2 (12.5%)		17 (22.7%)
• no	10 (76.9%)	13 (81.3%)	21 (70%)	14 (87.5%)		58 (77.3%)
in total	13 (100%)	16 (100%)	30 (100%)	16 (100%)		75 (100%)
CNS **metastasis PD**					0.02	
• yes	6 (46.2%)	4 (25%)	2 (6.7%)	5 (31.3%)		17 (22.7%)
• no	7 (53.8%)	12 (75%)	28 (93.3%)	11 (68.7%)		58 (77.3%)
in total	13 (100%)	16 (100%)	30 (100%)	16 (100%)		75 (100%)
	TP53 structural/biophysical classification					
	non-pathogenic *n* = 7	**pathogenic** *n* = 22	**TP WT** *n* = 30	**TP53 unknown** *n* = 16	***p*-value**	**All** *n* = 75
Age (years)						
mean	67.3 (54–81)	63.5 (49–75)	65.2 (45–85)	67.6 (45–82)	0.65	65.4 (45–85)
median	67	63.5	69	67	0.55	66
Sex					0.60	
men	2 (28.6%)	9 (40.9%)	7 (23.3%)	5 (31.3%)		23 (30.7%)
women	5 (71.4%)	13 (59.1%)	23 (76.7%)	11 (68.7%)		52 (69.3%)
in total	7 (100%)	22 (100%)	30 (100%)	16 (100%)		75 (100%)

					p-value	
Smoking status					0.77	
never/light smoker	5 (71.4%)	15 (68.2%)	23 (76.7%)	8 (50%)		51 (68%)
ex/current smoker	2 (28.6%)	7 (31.8%)	7 (23.3%)	5 (31.25%)		21 (28%)
not known	0	0	0	3 (18.75%)		3 (4%)
in total	7 (100%)	22 (100%)	30 (100%)	16 (100%)		75 (100%)
ECOG					0.83	
0	4 (57.1%)	14 (63.6%)	17 (56.7%)	8 (50%)		43 (57.3%)
1	3 (42.9%)	6 (27.3%)	10 (33.3%)	4 (25%)		23 (30.7%)
2	0	2 (9.1%)	3 (10%)	3 (18.8%)		8 (10.7%)
3	0	0	0	1 (6.2%)		1 (1.3%)
in total	7 (100%)	22 (100%)	30 (100%)	16 (100%)		75 (100%)
CCI					0.47	
0–1	1 (14.2%)	5 (22.7%)	8 (26.7%)	3 (18.7%)		17 (22.6%)
2–3	3 (42.9%)	12 (54.6%)	16 (33.3%)	7 (43.8%)		38 (50.6%)
>3	3 (42.9%)	5 (22.7%)	6 (20%)	6 (37.5%)		20 26.6%)
in total	7 (100%)	22 (100%)	30 (100%)	16 (100%)		75 (100%)
Exon status					0.95	
exon 19	4 (57.1%)	13 (59.1%)	16 (53.3%)	9 (56.25%)		42 (56%)
exon 21	2 (28.6%)	8 (36.4%)	12 (40%)	5 (31.25%)		27 (36%)
uncommon Exon	1 (14.3%)	1 (4.5%)	2 (6.7%)	2 (12.5%)		6 (8%)
in total	7 (100%)	22 (100%)	30 (100%)	16 (100%)		75 (100%)
Initital CNS metastasis					0.54	
• yes	1 (14.3%)	5 (22.7%)	9 (30%)	2 (12.5%)		17 (22.7%)
• no	6 (85.7%)	17 (77.3%)	21 (70%)	14 (87.5%)		58 (77.3%)
in total	7 (100%)	22 (100%)	30 (100%)	16 (100%)		75 (100%)
CNS metastasis PD					0.01	
• yes	4 (57.1%)	6 (27.3%)	2 (6.7%)	5 (31.3%)		17 (22.7%)
• no	3 (42.9%)	16 (72.7%)	28 (93.3%)	11 (68.7%)		58 (77.3%)
in total	7 (100%)	22 (100%)	30 (100%)	16 (100%)		75 (100%)

TP53 exon 8 vs. non-exon 8

	TP53 exon 8 $n = 6$	TP53 non-exon 8 $n = 26$	TP WT $n = 30$	TP53 unknown $n = 16$	*p*-value	ALL $n = 75$
Age (years)						
mean	59.3 (49–67)	65.7 (50–81)	65.2 (45–85)	67.6 (45–82)	0.08	65.4 (45–85)
median	60	66	69	67	0.35	66
Sex					0.08	
men	0	11 (47.8%)	7 (23.3%)	5 (31.3%)		23 (30.7%)
women	6 (100%)	12 (52.2%)	23 (76.7%)	11 (68.7%)		52 (69.3%)
in total	6 (100%)	23 (100%)	30 (100%)	16 (100%)		75 (100%)
Smoking status					0.60	
never/light smoker	5 (83.3%)	15 (65.2%)	23 (76.7%)	8 (50%)		51 (68%)
ex/current smoker	1 (16.7%)	8 (34.8%)	7 (23.3%)	5 (31.25%)		21 (28%)
not known	0	0	0	3 (18.75%)		3 (4%)
in total	6 (100%)	23 (100%)	30 (100%)	16 (100%)		75 (100%)
ECOG					0.83	
0	3 (50%)	15 (65.2%)	17 (56.7%)	8 (50%)		43 (57.3%)
1	2 (33.3%)	7 (30.4%)	10 (33.3%)	4 (25%)		23 (30.7%)
2	1 (16.7%)	1 (4.4%)	3 (10%)	3 (18.8%)		8 (10.7%)
3	0	0	0	1 (6.2%)		1 (1.3%)
in total	6 (100%)	23 (100%)	30 (100%)	16 (100%)		75 (100%)

						p-value
CCI						0.27
0–1	3 (50%)	3 (13%)	8 (26.7%)	3 (18.7%)	17 (22.7%)	
2–3	2 (33.3%)	13 (56.5%)	16 (33.3%)	7 (43.8%)	38 (50.6%)	
>3	1 (16.7%)	7 (30.5%)	6 (20%)	6 (37.5%)	20 (26.7%)	
in total	6 (100%)	23 (100%)	30 (100%)	16 (100%)	75 (100%)	
Exon status						
exon 19	5 (83.3%)	12 (52.2%)	16 (53.3%)	9 (56.25%)	42 (56%)	
exon 21	1 (16.7%)	9 (39.1%)	12 (40%)	5 (31.25%)	27 (36%)	
uncommon Exon	0	2 (8.7%)	2 (6.7%)	2 (12.25%)	6 (8%)	
in total	6 (100%)	23 (100%)	30 (100%)	16 (100%)	75 (100%)	
Initial CNS metastasis						0.45
• yes	2 (33.3%)	4 (17.4%)	9 (30%)	2 (12.5%)	17 (22.7%)	
• no	4 (66.7%)	19 (82.6%)	21 (70%)	14 (87.5%)	58 (77.3%)	
in total	6 (100%)	23 (100%)	30 (100%)	16 (100%)	75 (100%)	
CNS metastasis PD						0.06
• yes	2 (33.3%)	8 (34.8%)	2 (6.7%)	5 (31.3%)	17 (22.7%)	
• no	4 (66.7%)	15 (65.2%)	28 (93.3%)	11 (68.7%)	58 (77.3%)	
in total	6 (100%)	23 (100%)	30 (100%)	16 (100%)	75 (100%)	

29) vs. WT TP53 mutations ($n = 29$) was 12 vs. 18 months, respectively ($p < 0.004$) (Figure 2A) (HR 0.41; $p < 0.005$). The median PFS of TP53 WT/non-pathogenic mutations ($n = 36$) was significantly different to pathogenic TP53 mutated patients ($n = 22$) with 17 months vs. 11 months respectively ($p < 0.001$) (Figure 2B) (HR 0.28; $p < 0.001$). Patients with a TP53 mutation in exon 8 ($n = 6$) had a median PFS of 7 months vs. 12 months for patients with non-exon 8 TP53 mutations ($n = 23$) ($p < 0.006$) (Figure 2C) (HR 0.15; $p < 0.001$). The negative impact of TP53 co-mutations for mPFS was seen in both del19 and exon21 mutated patients (Table 3).

In the multivariate analysis, TP53 non-disruptive/ disruptive mutations, TP53 pathogenic mutation, ECOG status 2 and the EGFR mutation status were independent predictive factors for PFS. A shorter median PFS is associated with TP53 non-disruptive/disruptive mutations vs. TP53 WT (HR 3.07; $p < 0.003$) and with TP53 pathogenic mutations vs. TP53 WT/non-pathogenic mutations (HR 6.19; $p < 0.001$). The full results are shown in the appendix (Supplementary Table 3).

Overall survival

mOS was different in EGFR mt+ patients based on the TP53 mutation status and the different classifications (Table 4). The median OS of patients with non-disruptive/ disruptive TP53 mutation ($n = 29$) was 24 months compared to 42 months in patients with TP53 WT ($n = 30$, $p < 0.009$, Figure 2D) (HR 0.40; $p < 0.012$) and 42 months in TP53 WT/non-pathogenic mutations ($n = 37$) vs. 23 in patients with pathogenic TP53 mutations ($n = 22$, $p < 0.001$, Figure 2E) (HR 0.33; $p < 0.002$). The median OS in patients with TP53 exon 8 mutations ($n = 6$) was 12 months vs. 28 months in patients with a non-exon 8 TP53

mutation ($n = 23$, $p < 0.024$, Figure 2F) vs. TP53 WT (42 months) (HR 0.21; $p < 0.004$). TP53 mutations were an independent predictive factor in both del19 and exon 21 mutated patients for median OS. Detailed information is shown in Table 4.

In the multivariate analysis for OS including the covariates TP53 non-disruptive/disruptive mutation, ECOG status 2, type of EGFR mutation, TP53 pathogenic mutation and no initial CNS metastasis, TP53 mutations, only non-disruptive/disruptive and pathogenic TP53 mutations remained as independent factors for survival: HR 4.08, $p < 0.001$ and HR 4.88, $p < 0.001$, respectively. Additional information is shown in the appendix (Supplementary Table 3).

DISCUSSION

This paper extensively studies the impact of TP53 co-mutations in EGFR mutated NSCLC homogeneously treated with EGFR TKI in first line on clinically relevant endpoints ORR, PFS and OS. In our study of 75 patients with EGFR mt+ tumors, the incidence of TP53 mutations was 29/59 patients (49%) and thus more frequent than previously reported using Sanger sequencing yielding 26% [4] and 30% [18], however consistent with more sensitive methods used by VanderLaan et al. (TP53 incidence 50%) [20] and Labbé et al. (41%) [5], the Cologne group (48%) [21] and up to 62% as observed by Yu et al. [22] using hybrid capture methodology.

Although TP53 mutations have been associated with exogenous toxins such as smoking, no dependence with to the smoking status categorizing patients in never/light and heavy smokers in our cohort was observed. Also, TP53 mutations were not associated with any other prognostically significant factors such as age, ECOG, CNS metastases at

Table 2: Objective response rate

		ORR in percent % (n)			
		EGFR exon status			
		exon 19	**exon 21**	**uncommon EGFR mt+**	
ORR	CR/PR	92.9% (39)	76% [19]	40% [2]	
	SD/PD	7.1% [3]	24% [6]	60% [3]	
	total	100% (42)	100% [25]	100% [5]	
		Odds Ratio CR/PR		**p-value**	
exon 19 vs. Exon 21		4.11 [0.92–18.22]		0.06	
exon 19 vs. uncommon EGFR mt+		19.50 [2.29–165.76]		0.007	
exon 21 vs. uncommon EGFR mt+		4.75 [0.64–35.48]		0.13	
		EGFR exon 19/21 and TP53 mutation status			
		exon 19/TP53 mt+	**exon 19/TP53 WT**	**exon 21/TP53 mt+**	**exon 21/TP53 WT**
ORR	CR/PR	77.8% [14]	100% [16]	60% [6]	90.9% [10]
	SD/PD	22.2% [4]	0	40% [4]	9.1% [1]
	total	100% [18]	100% [16]	100% [10]	100% [11]
		Odds Ratio CR/PR		**p-value**	
exon 19/TP53 WT vs. exon 19/TP53 mt+		10.24 [0.51–206.88]		0.13	
exon 21/TP53 WT vs. exon 21/TP53 mt+		6.67 [0.60–74.51]		0.12	
exon 19/TP53 mt+ vs. exon 21 TP53 mt+		2.33 [0.42–12.57]		0.32	
		TP53 mutation and T790M+/-			
		TP53 mt/T790M+		**TP53 WT/T790M+**	
ORR	CR/PR	100% [10]		100% [9]	
	SD/PD	0		0	
	total	100% [10]		100% [9]	
		Odds Ratio CR/PR		**p-value**	
TP53 mt/T790M+ vs. TP53WT/T790M+		1		1	
		TP53 Poeta et al. classification			
		non-disruptive	**disruptive**	**TP53 WT**	**TP53 unknown**
ORR	CR/PR	61.5% [8]	75% [12]	96.4% [27]	86.7% [13]
	SD/PD	38.5% [5]	25% [4]	3.6% [1]	13.3% [2]
	total	100% [13]	100% [16]	100% [28]	100% [15]
		Odds Ratio CR/PR		**p-value**	
disruptive vs. non-disruptive		1.88 [0.38–9.20]		0.44	
TP53 WT vs. non-disruptive		16.88 [1.71–166.21]		0.02	
TP53 WT vs. disruptive		9.00 [0.91–89.27]		0.06	
TP53 WT vs. non-disruptive/disruptive		12.15 [1.42–103.82]		0.02	
		TP53 Structural/biophysical classification			
		non-pathogenic	**pathogenic**	**TP53 WT**	**TP53 unknown**
ORR	CR/PR	71.4% [5]	68.2% [15]	96.4% [27]	86.7% [13]
	SD/PD	28.6% [2]	31.8% [7]	3.6% [1]	13.3% [2]
	total	100% [7]	100% [22]	100% [28]	100% [15]
		Odds Ratio CR/PR		**p-value**	
non-pathogenic vs. pathogenic		1.17 [0.18–75.6]		0.87	
TP53 WT vs. non-pathogenic		10.80 [0.82–142.98]		0.07	
TP53 WT vs. pathogenic		12.60 [1.41–112.39]		0.02	
TP53 WT/non-pathogenic vs. pathogenic		4.98 [1.13–21.98]		0.03	
		TP53 exon 8 vs. non-exon 8 classification			
		Exon 8	**other Exons**	**TP53 WT**	**TP53 unknown**
ORR	CR/PR	66.7% [4]	69.6% [16]	96.4% [27]	86.7% [13]
	SD/PD	33.3% [2]	30.4% [7]	3.6% [1]	13.3% [2]
	total	100% [6]	100% [23]	100% [28]	100% [15]
		OR CR/PR		**p-value**	
non-Exon 8 vs. Exon 8		1.14 [1.33–104.98]		0.89	
TP53 WT vs. Exon 8		13.50 [0.98–185.45]		0.05	
TP53 WT vs. non-exon 8		11.81 [1.33–104.48]		0.03	

Table 3: Median PFS in months

	Median PFS	95% CI	n	p-value
Median PFS in months				
EGFR exon status				
	Median PFS	**95% CI**	**n**	**p-value**
EGFR exon 19	13	10.228–15.772	42	
EGFR exon 21	16	11.983–20.017	27	
EGFR uncommon mutation	9	4.022–13.978	5	
				0.792
	Median PFS	**95% CI**	**n**	**p-value**
EGFR exon 19/TP53 mt+	11	8.359–13.641	18	
EGFR exon 21/TP53 WT	20	12.351–27.649	16	
				<0.001
	Median PFS	**95% CI**	**n**	**p-value**
EGFR exon 21/TP53 mt+	10	4.300–15.700	10	
EGFR exon 21/TP53 WT	17	8.161–25.835	12	
				0.453
TP53 mutation and T790M+/-				
	Median PFS	**95% CI**	**n**	**p-value**
TP53/T790M+	7	5.379–8.621	10	
TP53 WT/T790M+	10	2.679–17.321	9	
				0.23
TP53 Poeta et al. classification				
	Median PFS	**95% CI**	**n**	**p-value**
non-disruptive	10	6.721–13.279	13	
disruptive	12	7.633–16.367	16	
TP53 WT	18	13.5–22.5	29	
TP53 unknown	15	6.909–23.091	16	
				0.18
	Median PFS	**95% CI**	**n**	**p-value**
non-disruptive	10	6.721–13.279	13	
disruptive	12	7.633–16.367	16	
TP53 WT	18	13.5–22.5	29	
				0.007
	Median PFS	**95% CI**	**n**	**p-value**
non-disruptive	10	6.721–13.279	13	
disruptive	12	7.633–16.367	16	
				0.224
	Median PFS	**95% CI**	**n**	**p-value**
non-disruptive	10	6.721–13.279	13	
TP53 WT	18	13.5–22.5	29	
				0.004
	Median PFS	**95% CI**	**n**	**p-value**
disruptive	12	7.633–16.367	16	
TP53 WT	18	13.5–22.5	29	
				0.033
	Median PFS	**95% CI**	**n**	**p-value**
non-disruptive/disruptive	12	9.271–14.729	29	
TP53 WT	18	13.5–22.5	29	
				0.004

TP53 structural/biophysical classification				
	Median PFS	**95% CI**	***n***	***p*-value**
non-pathogenic	13	3.398–22.602	7	
pathogenic	11	8.63–13–37	22	
TP53 WT	18	13.5–22.5	29	
TP53 unknown	15	6.909–23.091	16	
				0.001
	Median PFS	**95% CI**	***n***	***p*-value**
non-pathogenic	13	3.398–22.602	7	
pathogenic	11	8.63–13–37	22	
TP53 WT	18	13.5–22.5	29	
				0.001
	Median PFS	**95% CI**	***n***	***p*-value**
non-pathogenic	13	3.398–22.602	7	
TP53 WT	18	13.5–22.5	29	
				0.542
	Median PFS	**95% CI**	***n***	***p*-value**
pathogenic	11	8.63–13–37	22	
TP53 WT	18	13.5–22.5	29	
				<0.001
	Median PFS	**95% CI**	***n***	***p*-value**
non-pathogenic	13	3.398–22.602	7	
pathogenic	11	8.63–13–37	22	
				0.059
	Median PFS	**95% CI**	***n***	***p*-value**
TP53 WT/non-pathogenic	17	14.458–19.541	36	
pathogenic	11	8.63–13–37	22	
				<0.001
TP53 exon 8 vs. non-exon 8 classification				
	Median PFS	**95% CI**	***n***	***p*-value**
exon 8	7	0.000–15.402	6	
non-exon 8	12	8,757–15.243	23	
TP53 WT	18	13.5–22.5	29	
TP53 unknown	15	6.909–23.091	16	
				<0.001
	Median PFS	**95% CI**	***n***	***p*-value**
exon 8	7	0.0–15.402	6	
non-exon 8	12	8.757–15.243	23	
TP53 WT	18	13.5–22.5	29	
				<0.001
	Median PFS	**95% CI**	***n***	***p*-value**
exon 8	7	0.0–15.402	6	
non-exon 8	12	8.757–15.243	23	
				0.006
	Median PFS	**95% CI**	***n***	***p*-value**
exon 8	7	0.0–15.402	6	
TP53 WT	18	13.5–22.5	29	
				<0.001
	Median PFS	**95% CI**	***n***	***p*-value**
non-exon 8	12	8.757–15.243	23	
TP53 WT	18	13.5–22.5	29	
				0.024

Table 4: Median OS in months

	Median OS	95% CI	n	p-value
Median OS in months				
EGFR exon status				
EGFR exon 19	31	24.904–37.096	42	
EGFR exon 21	20	12.943–27.057	27	
EGFR uncommon mutation	8	0.000–46.408	6	
				0.166
EGFR exon 19/TP53 mt+	27	20.266–33.734	18	
EGFR exon 19/TP53 WT	55	-	16	
				0.005
EGFR exon 21/TP53 mt+	11	0.000–22.833	10	
EGFR exon 21/TP53 WT	22	0.000–47.183	12	
				0.09
TP53 mutation and T790M+/-				
TP53/T790M+	35	17.915–52.081	10	
TP53 WT/T790M+	55	34.295–75.705	9	
				0.094
TP53 Poeta et al. classification				
non-disruptive	27	11.065–42.935	13	
disruptive	24	17.040–30.960	16	
TP53 WT	42	28.754–55–246	30	
TP53 unknown	15	7.160–22.840	16	
				0.010
non-disruptive	27	11.065–42.935	13	
disruptive	24	17.040–30.960	16	
TP53 WT	42	28.754–55–246	30	
				0.032
non-disruptive	27	11.065–42.935	13	
disruptive	24	17.040–30.960	16	
				0.862
non-disruptive	27	11.065–42.935	13	
TP53 WT	42	28.754–55–246	30	
				0.077
disruptive	24	17.040–30.960	16	
TP53 WT	42	28.754–55–246	30	
				0.018
non-disruptive/disruptive	24	17.019–30.981	29	
TP53 WT	42	28.754–55–246	30	
				0.009

TP53 structural/biophysical classification				
	Median OS	**95% CI**	***n***	***p*-value**
non-pathogenic	42	40.360–43.640	7	
pathogenic	23	17.782–28.218	22	
TP53 WT	42	28.754–55–246	30	
TP53 unknown	15	7.160–22.840	16	
				0.002
	Median OS	**95% CI**	***n***	***p*-value**
non-pathogenic	42	40.360–43.640	7	
pathogenic	23	17.782–28.218	22	
TP53 WT	42	28.754–55–246	30	
				0.005
	Median OS	**95% CI**	***n***	***p*-value**
non-pathogenic	42	40.360–43.640	7	
TP53 WT	42	28.754–55–246	30	
				0.876
	Median OS	**95% CI**	***n***	***p*-value**
pathogenic	23	17.782–28.218	22	
TP53 WT	42	28.754–55–246	30	
				0.003
	Median OS	**95% CI**	***n***	***p*-value**
non-pathogenic	42	40.360–43.640	7	
pathogenic	23	17.782–28.218	22	
				0.127
	Median OS	**95% CI**	***n***	***p*-value**
WT/non-pathogenic TP53	42	34.711–49.289	37	
pathogenic	23	17.782–28.218	22	
				0.001
TP53 exon 8 vs. non-exon 8 classification				
	Median OS	**95% CI**	***n***	***p*-value**
exon 8	12	8.399–15.601	6	
non-exon 8	28	15.168–40.184	23	
TP53 WT	42	28.754–55–246	30	
TP53 unknown	15	7.160–22.840	16	
				0.001
	Median OS	**95% CI**	***n***	***p*-value**
exon 8	12	8.399–15.601	6	
non-exon 8	28	15.168–40.184	23	
TP53 WT	42	28.754–55–246	30	
				0.002
	Median OS	**95% CI**	***n***	***p*-value**
exon 8	12	8.399–15.601	6	
non-exon 8	28	15.168–40.184	23	
				0.024
	Median OS	**95% CI**	***n***	***p*-value**
exon 8	12	8.399–15.601	6	
TP53 WT	42	28.754–55–246	30	
				0.001
	Median OS	**95% CI**	***n***	***p*-value**
non-exon 8	28	15.168–40.184	23	
TP53 WT	42	28.754–55.246	30	
				0.051

diagnosis or type of EGFR mutation. The high frequency of TP53 mutations seems to be a hallmark of EGFR mutated NSCLC, as they are much less frequent in ALK+ tumors, another tumor predominantly observed in never or light smokers. Furthermore, TP53 mutations seem to be a stable genetic marker in EGFR mt+ NSCLC as no conversions of the TP53 status were observed at the time point of acquired TKI resistance. This is also in contrast to ALK+ tumors, where TP53 mutations respresent on of multiple resistance mechanisms of ALK inhibitors [23].

In this retrospective analysis of EGFR mutated patients, a significant impact of TP53 mutations on ORR, PFS and OS was found. This unfavorable impact was independent of the type of EGFR mutation (del19 vs. exon 21), and in a multivariate analysis TP53 mutations represented an independent unfavorable predictor for ORR, PFS and OS. The hypothesis that TP53 mutations are associated with a lower incidence of T790M mutations could be discarded in the cohort described. 10/29 (34.5%) patients with a TP53 mutation and 9/29 (31%) patients with a TP53 WT developed a T790M resistance mutation after 1st line TKI. When patients with acquired resistance were rebiopsied, the incidence of T790M, which is associated with a more favorable outcome than non T790M resistance mutations [24], there was no significant difference in the incidence of TP53 mutations. Also when patients with and without T790M were analyzed, TP53 mutations still had a significant impact on PFS and OS.

One potential explanation for the unfavorable outcome of the TP53 mutated patients might be the risk of developing CNS metastases during the course of the disease: while the incidence of CNS metastases was not different in the TP53 WT vs. TP53 mt+ cohorts at diagnosis, the risk of developing CNS metastases during the course of the disease was higher in the TP53 mutation subgroup than in the TP53 WT cohort (10/29; (34.4%) of TP53 mt+ patients vs. 2/29; (6.9%) of TP53 WT patients; $p < 0.02$; $p < 0.01$). Thus it might be speculated that TP53 mutations are associated with a higher risk of seeding in the brain. In ALK+ NSCLC MRI of the brain are performed every 3 to 6 months due the high incidence of brain metastastases. In contrast there is no widely accepted follow up regimen in EGFR mt+ NSCLC regarding brain imaging. The data presented for EGFR mutated and TP53 co-mutated NSCLC patients might stipulate for a systematic and regular MRI imaging of the brain as part of the follow up of these patients at high risk for developing brain metastases.

In the current paper, we compared different classifications of TP53 mutations that were used in the literature. Overall, the unfavorable impact on clinical outcome parameters was seen for all TP53 mutations, however the "structural/biophysical classification" and the classification using TP53 exon 8 mutations [17] correlated more precisely with outcome parameters than the classification based on Poeta et al. [16] and used by the Molina-Vila et al. [4]. However, we cannot formally distinguish between p53 being a predictive or a prognostic marker because in our study we do not have data on EGFR mt+ patients that were untreated or that received chemotherapy instead of TKI. Prognostic factors are defined as factors independent of treatment and predictive factors as those dependent of treatment and predictive of
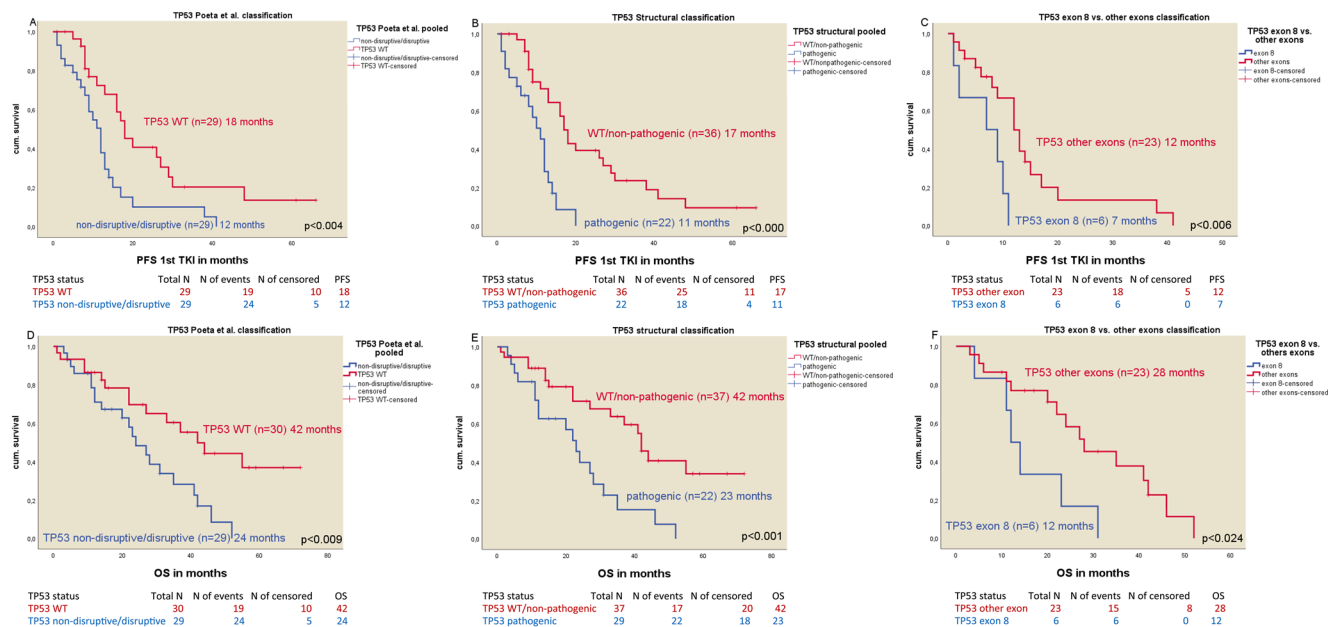
Figure 2: Kaplan-Meier plots of the different classification of TP53 mutations. (**A**) Median PFS of TP53 Poeta et al. non-disruptive/disruptive mt+ vs. TP53 WT. (**B**) Median PFS of TP53 structural/biopysical classification TP53 non-pathogenic/TP53 WT vs. TP53 pathigenic mt+. (**C**) Median PFS of TP53 exon 8 vs. TP53 non-exon 8 mt+. (**D**) Median OS of TP53 Poeta et al. non-disruptive/disruptive mt+ vs. TP53 WT. (**E**) Median OS of TP53 structural/biophysical classification TP53 non-pathogenic/TP53 WT vs. TP53 pathogenic mt+. (**F**) Median OS of TP53 exon 8 vs. TP53 non-exon 8 mt+.

the the effectiveness of treatments [25]. Therefore, as we included only patients treated with TKI in our study, we opted for defining TP53 mutations as being predictive for the effectiveness of TKI treatment. To definitively answer the question of TP53 being a predictive or prognostic marker, a multicenter retrospective study that compares the outcome with and without TKI therapy of TP53 comutations would be necessary.

Recently the combination of chemotherapy and EGFR TKI was studied in a prospective phase III trial that yielded a significant OS benefit for the combination therapy [26]. It might be speculated that TP53 mutated tumors might preferentially benefit from a broad cytotoxic chemotherapeutic approach in combination with EGFR TKI. A stratification for TP53 mutations should at least be performed in the upcoming EGFR TKI and chemotherapy trials.

An even more attractive approach to tackle TP53 mutated EGFR mt+ tumors might be the specific targeting of TP53 mutations by TP53 inhibitors. This approach is being discussed extensively in a variety of cancers, such as breast cancer [27] and hematologic malignancies [28]. Recently a trial combining a TP53 inhibitor (APREA 246) in combination with azacitidine in TP53 mutant MDS showed a 100% response rate with 8/9 patients even achieving complete remission with median PFS and median OS not reached. As to the knowledge of the authors trials combining TP53 targeted therapies and EGFR TKI have not been initiated in EGFR mt+ NSCLC but this patient population could potentially represent a good target for such a specific approach.

MATERIALS AND METHODS

Between 2009 and 2016 465 patients from a single center (Pius-Hospital Oldenburg, Germany) diagnosed with NSCLC IV were studied for the presence of EGFR mutations. In routine diagnostic patients were prospectively tested for EGFR and other biomarker.

Patients

75/465 (16%) patients with TTF1 positve adenocarcinoma of the lung ICD-O M8140/3 harboring sensitizing EGFR mutations and intended for 1st line EGFR TKI treatment were included in this retrospective analysis and were further analyzed for the presence of TP53 mutations. Two cycles of 1st line chemotherapy were allowed because of turn around time of the EGFR results. Patients' clinical and molecular data including sex, age, histology, smoking status, ECOG, presence of CNS metastases at baseline and during course of disease and comorbidities, as well as types of EGFR and TP53 mutations were captured. ORR, PFS and OS were assessed. Patients gave informed and written consent and the project was approved by the University of Oldenburg's ethics committee (FP-Projekt 2014-I).

TP53 co-mutation

Mutational analyses were performed by standard methods, mostly by hybrid capture assays. DNA was extracted from FFPE tissue and subjected to molecular analysis for EGFR, TP53 and other therapeutically relevant genes. TP53 mutations were classified according to different algorithms 1: as previously described by Poeta et al. [16], 2: by an extended algorithm based on Poeta et al. [16] with additional parameters like structural prediction and GVGD biophysical analysis [17] and 3: based on exon 8 vs. non-exon 8 mutations [18]. More details are given in the appendix (Supplementary Materials - Patients and Methods).

Statistics

For this retrospective analysis, bivariate dependencies with OS and PFS were using Kaplan Meier curves and the log rank test for the calculation of p-values. For ORR a logistic regression considering the covariates (sex, age, ECOG, smoking status, EGFR exon status, CNS metastasis and the CCI) was used. The logistic regression results are reported as odds ratios with 95% confidence intervals. Furthermore we constructed Cox regression models to predict the PFS and the OS separately in multivariate analyses. The results are presented as hazard ratios with 95% confidence intervals and p-values.

Abbreviations

APREA: APREA therapeutics; CCI: Charlson Comorbidity Index; CI: Confidence Interval; CNS: Central Nervous System; CR/PR: Complete Response/ Partial response; DCR: Disease Control Rate; del19: Deletion 19; DFG: Deutsche Forschungsgemeinschaft; DNA: Deoxyribonucleic Acid; ECOG: Eastern Cooperative Oncology Group; EGFR: Epidermal Growth Factor-Receptor; FFPE: Formalin-Fixed, Paraffin-Embedded; FP: Forschungspool; GVGD: Grantham Variation Grantham Deviation; HR: Hazard Ratio; i.e.: id est; MDS: Myelodysplastic Syndromes; MRI: Magnetic Resonance Imaging; mt+: mutated; NSCLC: Non-Small-Cell Lung Cancer; OR: Odds Ratio; ORR: Objective Response Rate; OS: Overall Survival; PFS: Progression Free Survival; pts: patients; Qol: Quality of Life; TKI: Tyrosinkinase-Inhibitor; TP53: Tumor suppressor gene (p53); UICC: Union for International Cancer Control; vs.: versus; WT: Wild Type.

Author contributions

Julia Roeper – study design, data collection, data analysis, writing and editing the manuscript, Markus Falk – molecular genetic analysis, writing the manuscript (partly), Athena Chalaris-Rißmann – molecular genetic analysis, Anne Ch. Lueers – helped with data collection, Hayat

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