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TP53 mutations in circulating tumor DNA in advanced epidermal growth factor receptor-mutant lung adenocarcinoma patients treated with gefitinib

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ABSTRACT

Tumor protein p53 (TP53) is a tumor suppressor gene and TP53 mutations are associated with poor prognosis in non-small cell lung cancer. However, the in-depth classification of TP53 and its relationship with treatment response and prognosis in epidermal growth factor receptor (EGFR)-mutant tumors treated with EGFR tyrosine kinase inhibitors are unclear. Circulating tumor DNA was prospectively collected at baseline in advanced treatment-naïve EGFR-mutant lung adenocarcinoma patients treated with gefitinib in an open-label, single-arm, prospective, multicenter, phase 2 clinical trial (BENEFIT trial) and analyzed using next-generation sequencing. Survival was estimated using the Kaplan–Meier method. Of the 180 enrolled patients, 115 (63.9%) harbored TP53 mutations. The median progression-free survival (PFS) and overall survival (OS) of patients with TP53-wild type tumors were significantly longer than those of patients with TP53-mutant tumors. Mutations in exons 5–8 accounted for 80.9% of TP53 mutations. Mutations in TP53 exons 6 and 7 were significantly associated with inferior PFS and OS compared to wild-type TP53. TP53 mutation also influenced the prognosis of patients with different EGFR mutations. Patients with TP53 and EGFR exon 19 mutations had significantly longer PFS and OS than patients with TP53 and EGFR L858R mutations, and both groups had worse survival than patients with only EGFR mutations. Patients with TP53 mutations, especially in exons 6 and 7, had a lower response rate and shorter PFS and OS when treated with gefitinib. Moreover, TP53 exon 5 mutation divided TP53 mutations in disruptive and non-disruptive types.

Introduction

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for 80%–85% of all cases, and the primary cause of cancer-related deaths worldwide. NSCLC patients with activating epidermal growth factor receptor (EGFR) mutations usually show a high response rate to EGFR tyrosine kinase inhibitors (TKIs; e.g., gefitinib and erlotinib) and longer progression-free survival (PFS) than patients treated with standard chemotherapy [1–6]. The discovery of EGFR-TKIs was a significant advance in treating advanced lung cancer [7–9]. Three generations of EGFR-TKIs have been developed clinically: first-generation EGFR-TKIs, which include gefitinib, erlotinib, and ectinib; second-generation EGFR-TKIs, which include afatinib and dacinib; and third-generation TKIs, which target the EGFR T790M mutation, the most common mechanism of resistance to first- and second-generation EGFR-TKIs, and include osimertinib and amelatinib,

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Table 1
Basic information of the patients

No. of patients (%)		
Age (years)		
Median (Range)	59.6 (33.9 - 77.3)	
Gender		
Male	81 (45%)	
Female	99 (55%)	
Smoking status		
TP53 mutation	smoker	37 (32.2%)
	non-smoker	78 (67.8%)
TP53 wild type	smoker	12 (18.5%)
	non-smoker	53 (81.5%)
EGFR mutation (N=180)		
Exon 19 deletion	92 (51.1%)	
Exon 21 L858R	88 (48.9%)	
TP53 mutation (N=115)		
TP53 mutation	115 (63.9%)	
Non-TP53 mutation	65 (36.1%)	
TP53 hotexon mutation subtypes		
TP53 hotexon mutations	93 (80.9%) (4 of them shared repeated exons mutation)	
TP53 non-hotexon mutations	22 (19.1%)	
TP53 exon 4 mutation	14 (11.6%)	
TP53 exon 5 mutation	34 (28.1%)	
TP53 exon 6 mutation	19 (15.7%)	
TP53 exon 7 mutation	27 (22.3%)	
TP53 exon 8 mutation	17 (14.0%)	
TP53 exon 9-10 mutation	10 (8.3%)	
TP53 repeated exons mutation	6 (5.0%)	
TP53 disruptive mutation subtypes		
TP53 disruptive mutation	53 (46.1%)	
TP53 non-disruptive mutation	62 (53.9%)	

Our trial collected 180 patients with EGFR mutation, among them there were 115 patients combined with TP53 mutations. In our research, we also divided patients into various groups following their TP53 mutation types. TP53 hotexon mutations included exon 5, exon 6, exon 7 and exon 8 mutations. TP53 non-hotexon mutations mainly included the exon 4, exon 9 and exon 10 mutations.

among others [10,11]. Osimertinib is one of the standard options for first-line therapy based on the results of the FLAURA trial [12–15].

Despite the overall benefit of EGFR-TKIs, various studies have demonstrated heterogeneous treatment outcomes, with response rates of patients with EGFR-mutant tumors to first-line EGFR-TKIs ranging from 56% to 84% and PFS ranging from a few months to several years [16,17,19,20]. Additionally, Asian patients tend to have lower overall survival (OS), and most patients are still administered first-generation EGFR-TKIs [18]. These results suggests that EGFR-TKI monotherapy might not always be the optimal therapy for patients with EGFR-mutant NSCLC and highlight the need for in-depth research into the genomic landscape of such tumors toward the discovery and development of stratified therapeutic approaches. The molecular landscape of tumors can be explored using genomic technologies and high-throughput next-generation sequencing (NGS). Previous studies have confirmed that a fraction of EGFR-mutant tumors carry additional driver mutations that affect the activity of EGFR-TKIs in EGFR-mutant NSCLCs [21].

Tumor protein p53 (TP53) is a tumor suppressor involved in many biological processes, including DNA repair, cell senescence, apoptosis, and autophagy [22–24]. Genome-wide association studies and sequencing studies have identified TP53 mutations in approximately 50% of NSCLCs [25,26]. Moreover, previous reports indicate that 50%–60% of advanced EGFR-mutant lung cancers harbor TP53 mutations that are significantly associated with the resistance to therapy and confer a worse prognosis (shorter PFS and OS) after treatment with TKIs [27,28]. Although these findings indicate the significance of TP53, there is insufficient evidence on the prognostic value of TP53 and its correlation with EGFR mutations.

Our research is based on the results of the Blood Detection of EGFR Mutation for Iressa Treatment (BENEFIT) trial [23], which was performed at the Cancer Hospital of the Chinese Academy of Medical Sciences. BENEFIT is a prospective clinical trial and the first to use circulating tumor DNA (ctDNA) to explore the relationship between EGFR mutation status and the efficacy of first-generation EGFR-TKIs. The detailed results have been previously published [22]. The trial showed that a considerable number of patients with EGFR mutations have co-mutations at diagnosis, including mutations in TP53, ERBB2, and PTEN, and the drug efficacy and survival data of these patients were significantly different from those with only EGFR mutations. Therefore, we conducted a further study examining concomitant TP53 mutations in patients with EGFR-mutated NSCLC to explore the difference in response to EGFR-TKI treatment under different TP53 co-mutation states.

Materials and methods

Study design and participants

The BENEFIT trial was an open-label, multicenter, phase 2 clinical trial in China (ClinicalTrials.gov, number NCT02282267) [23]. The inclusion criteria were as follows: age 18 to 75 years old; no previous chemotherapy or immunotherapy; histologically confirmed, stage IV lung adenocarcinoma; and EGFR mutation (exon 19 del or L858R) as assessed by the highly sensitive and specific droplet digital PCR (ddPCR) method. Written informed consent was obtained from all participants, and we obtained approval for independent ethics experiments from each research committee. Moreover, the research was conducted in accordance with the requirements of local laws and the biomedical research general principles of the International Ethical Guidelines.

Procedures

Qualified patients were administered oral gefitinib 250 mg/d (AstraZeneca, Macclesfield, UK) as first-line treatment until disease progression, as defined by the RECIST Version 1.1 guidelines [40]. After disease progression, patients were followed up every 12 weeks until the time of death or loss to follow-up. Blood samples were collected within 7 days before the first treatment and every 8 weeks thereafter until disease progression. We analyzed biomarkers in blood and tumor tissue samples, and samples were sent to a designated central laboratory (Amoy Diagnostics, China) for EGFR mutation testing. We also obtained tumor tissue during initial diagnosis or biopsy 14 days before the first treatment and stored specimens as formalin-fixed, paraffin-embedded (FFPE) samples.

We defined EGFR mutation types, including EGFR exon 19 deletions, L858R mutations by testing ctDNA by ddPCR. The EGFR mutation sequencing in FFPE tissue specimens was tested using the ADX-Amplification Refractory Mutation System. We also used a next-generation sequencing (NCG) platform [41] and a lung plasma panel (Lung Plasma, Burning Rock Biotech, Guangzhou, China) to detect additional mutations in 168 oncogenes and tumor suppressors.

Statistical analysis

We intended to gather 180 patients in this study. χ^2-test and Fisher's exact test were used to compare the proportions of patients who achieved an objective response in different subgroups (EGFR and TP53 co-mutations, TP53 hotexon mutations and TP53 disruptive mutations). The progression-free survival (PFS) and overall survival (OS) were estimated by using the Kaplan–Meier method. Moreover, the comparison of subgroups was performed using a Cox proportional hazards model. In the Cox model, Breslow's approximate likelihood method was used to identify relationships between variables. All analyses were performed using SAS software (version 9.4).

Results

Patients

From December 25, 2014 to January 16, 2016, 426 patients were screened, there were 188 patients with EGFR mutations and treated with

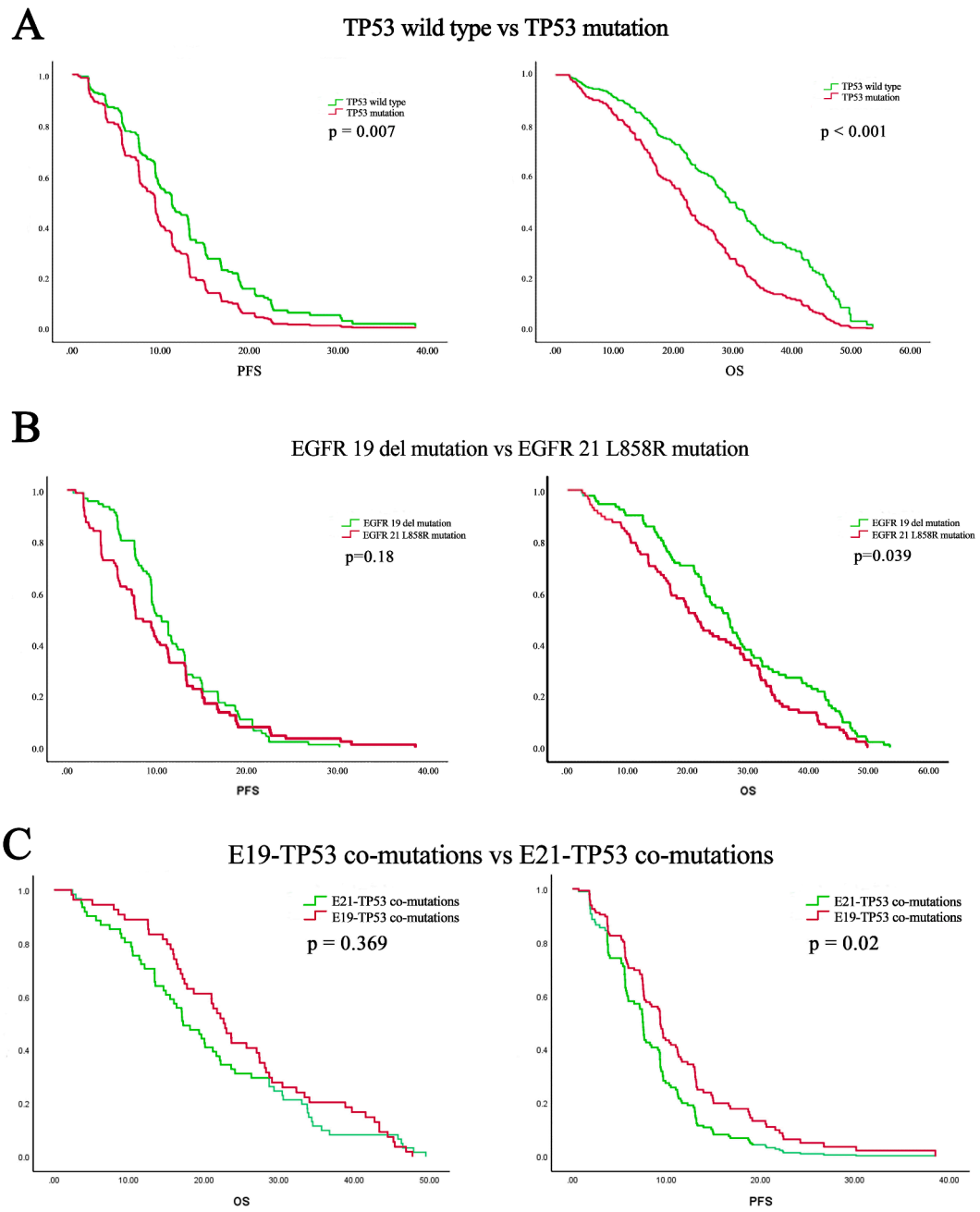

Fig. 1. A: The survival trend of the patients with TP53 mutations; B: The survival trend of the patients with EGFR mutations; C: The survival trend of the patients with TP53 and EGFR co-mutations;.

gefitinib therapy, and 180 patients with EGFR mutations (179 included in the BENEFIT analysis and 1 was added after the BENEFIT analysis) and available ctDNA samples at baseline for NGS analysis were enrolled for gefitinib therapy. By the cut-off time of June 1st, 2019, 31 patients were still alive.

The proportions of EGFR exon 19 deletions (51.1%) and L858R mutations (48.9%) were similar. The average age of the patients was 59.6 years (range 33.9 to 77.3 years). Ninety-nine patients (55%) were women, and 131 (72.8%) were non-smokers. At the data cutoff date (April 1, 2020), 173 patients (96.1%) had developed disease progression and 31(17.2%) patients were still alive before the cutoff data (June 1st, 2019). The average PFS and OS were 10.78 months (range 9.81–11.75 months) and 25.1 months (22.2–28.2 months), respectively (Table 1).

In our study, we found that there were 37 patients (32.2%) combined with smoking history among those with TP53 mutation. However, only 12 patients (18.5%) shared smoking habits in patients with TP53 wild type. Therefore, we believed that TP53 mutations in lung cancer are strongly associated with smoking status of patients.

TP53 and EGFR co-mutations

A total of 115 (63.9%) patients harbored TP53 mutations. We examined the location of TP53 mutations, as well as whether they were disruptive or non-disruptive. Exons 5–8 (“hot exons”) were the most common mutation sites, accounting for 80.9% of TP53 mutations. There were also mutations in exons 9 and 10 (8.7%). The sequence-specific transcriptional activity mediated by the DNA-binding domain (DBD) of p53 is the primary mechanism behind its tumor suppressor activity. The DBD is encoded by exons 5 to 8 and comprises residues 102–292. Within the DBD, the L2 (codons 163–195) and L3 (codons 236–251)

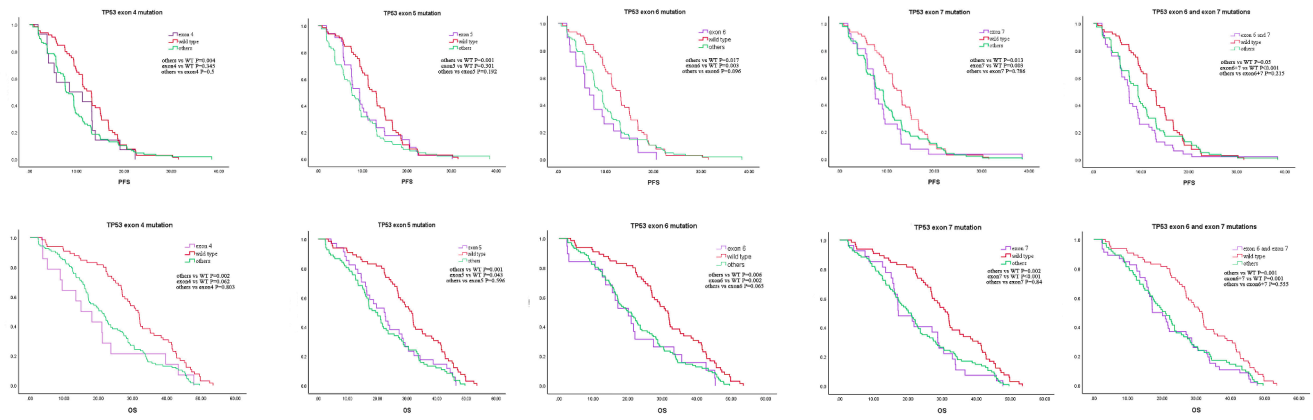

Fig. 2. The survival trend of the patients with TP53 various exon mutations.

Table 2
Result of the comparison in different TP53 mutation types

Types	OS (months)	PFS (months)
TP53 $^{exon\ 4}$	14.9 [95% CI 6.3–23.5] (vs TP53 ^{wild}:P=0.062, HR=2.03 [95% CI 1.13–3.66])	8.4 [95% CI 0.91–15.9] (vs TP53 ^{wild}:P=0.345, HR=1.38 [95% CI 0.76-2.52])
TP53 $^{exon\ 5}$	22.2 [95% CI 16.3–28.1] (vs TP53 ^{wild}: P=0.043, HR=1.79 [95% CI 1.16–2.74])	9.3 [95% CI 6. 9-11.8] (vs TP53 ^{wild}:P=0.195, HR=1.33 [95% CI 0.86-2.04])
TP53 $^{exon\ 6}$	20.1 [95% CI 15.5–35.6] (vs TP53 ^{wild}: P=0.002, HR=2.27 [95% CI 1.34–4.04])	6.6 [95% CI 5.4–13.2] (vs TP53 ^{wild}:P=0.003, HR=2.17 [95% CI 1.27–3.58])
TP53 $^{exon\ 7}$	21.2 [95% CI 16.0–30.4] (vs TP53 ^{wild}:P <0.001, HR=2.27 [95% CI 1.36–3.63])	7.5 [95% CI 7.13–9.63] (vs TP53 ^{wild}:P=0.003, HR=2.00 [95% CI 1.16–2.94])
TP53 $^{exon\ 6+7}$	22.0 (vs TP53 ^{wild}:P<0.001, HR=2.207 [95% CI 1.45–3.37])	8.7 (vs TP53 ^{wild}:P=0.001, HR=1.92 [95% CI 1.30–2.85])
TP53 ^{dis}	17.13 [95% CI 14.27–20.00] (vs TP53 ^{wild}:P<0.001, HR = 2.287 [95% CI 1.513–3.458])	9.33 [95% CI 7.92–10.45] (vs TP53 ^{wild}:P=0.03, HR = 1.523 [95% CI 1.05–2.22])
TP53 $^{non\text{-}dis}$	21.73 [95% CI 18.69–24.77] (vs TP53 ^{wild}:P<0.001, HR = 1.941 [95% CI 1.31–2.87])	7.57 [95% CI 7.35–7.79] (vs TP53 ^{wild}:P = 0.01, HR = 1.628 [95% CI 1.14–2.33])
TP53 ^{wild}	31.6 [95% CI 28.4–37.8]	13.0 [95% CI 11.1–15.0]

loops bind to a zinc atom and play an important role in the interaction with DNA [28,29]. Therefore, mutations in the DBD lead to accumulation of p53 in the nucleus but loss of activity. TP53 disruptive mutations included the following: (1) alterations that introduce a stop codon resulting in disruption of p53 protein production (nonsense, frameshift, and intronic), (2) amino acid substitutions from one polarity/charge category to another, and (3) in-frame deletions within the L2 or L3 loops [30,31]. Non-disruptive TP53 mutations included all those not classified as disruptive mutations, including mutations outside the L2 or L3 loops, such as missense mutations and in-frame deletions, and mutations within the L2 or L3 loops that substitute one amino acid residue with another of the same polarity [30,31]. Fifty-three patients (46.1%) had TP53 disruptive mutations, and 62 (53.9%) had TP53 non-disruptive mutations. Additionally, 13 patients (7.2%) had TP53 disruptive mutations in exon 5, and 21 patients (11.7%) had TP53 non-disruptive mutations in non-hot exons (Table 1). Forty-three patients (37.4%) had EGFR exon 19 and TP53 hot exon mutations, and 50 patients (43.5%) had EGFR L858R and TP53 hot exon mutations.

Relationship between TP53 mutation and clinical efficacy of TKIs

Patients with wild-type TP53 showed a trend toward a better disease control rate (DCR) than patients with mutated TP53 but a trend toward a worse objective response rate (ORR) (DCR: 95.38% vs 67.83% and ORR: 80.00% vs 89.57%). The ORR and DCR of patients with TP53 exon 6 and 7 mutations were 90.70% and 67.00%, respectively.

We further explored the DCR and ORR of patients harboring EGFR mutations and TP53 mutations. Patients with EGFR exon 19 and TP53 mutations had a higher DCR and ORR than those with EGFR L858R mutations and TP53 mutations (DCR 80.00% vs 58.33% and ORR: 94.55% vs 86.67%). We next examined the DCR and the ORR in patients with different TP53 mutations. Those with EGFR exon 19 and TP53 hot exon mutations had the highest ORR (94.5%) and DCR (80.0%) among all the patients with TP53 mutations. The lowest ORR (87.1%) and DCR (67.0%) were observed in patients with EGFR exon 19 and TP53 non-disruptive mutations. (Table 4)

Moreover, we also found that patients with E19-TP53^{mut} shared a significantly different DCR than those with E19-TP53^{wild} (DCR: 80.0% vs 95.4%, $p = 0.005$), which means E19-TP53^{mut} patients shared poor curative effect. We also found patients with TP53 disruptive mutations also shared better curative effect than those with TP53 non-disruptive mutations. (ORR: 94.0% vs 87.1%, $p = 0.001$).

The prognostic value of TP53 mutations and subtypes

TP53 mutation is predictive of poor survival

We analyzed the OS and PFS in patients with different types of TP53 mutations. Both the OS and PFS of patients with mutated TP53 were significantly lower than those of patients with wild-type TP53 (OS: 21.2 months [95% CI 17.4–23.6] vs 32.0 months, [95% CI 27.60–33.96], $p < 0.001$, HR = 0.54 [95% CI 0.40–0.74]; PFS: 8.4 months [95% CI 7.5–9.63] vs 12.81 months [95% CI 11.27–14.35], $p = 0.007$, HR = 0.66 [95% CI 0.48–0.89]). (Fig. 1A)

Prognostic value of EGFR and TP53 co-mutations

Of the 180 patients with EGFR mutations, 92 harbored EGFR exon 19 deletions and 88 harbored EGFR L858R mutations. There was no significant difference in PFS between EGFR exon 19 deletions and EGFR L858R mutations (11.0 months [95% CI 9.20–12.68] vs 9.2 months [95% CI 7.06–11.14], $p = 0.18$, HR = 0.8 [95% CI 0.57–1.11]). However, there was a significant difference in OS between the two groups (26.8 months [95% CI 24.85–30.28] vs 21.5 months [95% CI 20.43–25.92], $p = 0.039$, HR = 1.37 [95% CI 1.02–1.84]) (Fig. 1B). Conversely, patients harboring EGFR exon 19 mutations and TP53 mutations had a longer PFS and OS than those with EGFR L585R mutations and TP53 mutations (PFS 9.47 months [95% CI 9.07–13.0] vs 7.17 months [95% CI 5.57–9.3], HR = 1.532 [95% CI 1.06–2.22]; OS 22.6 months [95% CI 19.9–25.3] vs 17.2 months [95% CI 13.59–20.81], $p = 0.369$, HR = 0.769) (Fig. 1C).

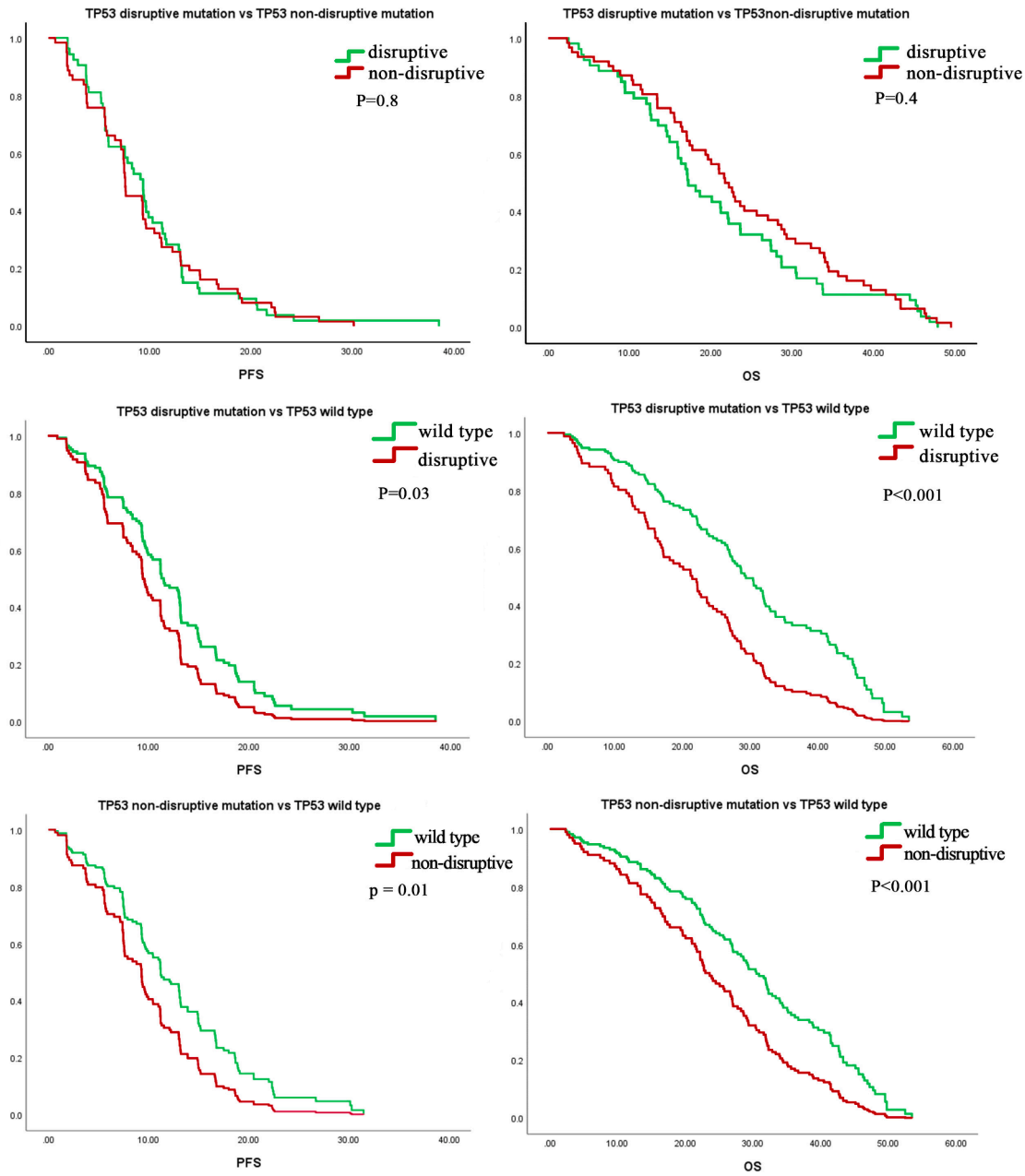

Fig. 3. The survival trend of TP53 disruptive mutation and TP53 non-disruptive mutation compared with TP53 wild type.

Table 3
TP53 disruptive mutations redivided by TP53 disruptive and non-disruptive mutations.

Types	No. of patients (%)	PFS (months)	OS (months)	
TP53 disruptive				
(N=53,3 of them shared exon co-mutations)	exon4	8 (15.1%)	5.2 (95%CI 0.0-11.3)	15.0 (95%CI 2.8-27.0)
	exon5	13 (24.5%)	9.3 (95%CI 5.2-13.4)	14.5 (95%CI 8.4-20.5)
	exon6	11 (20.8%)	7.5 (95%CI 5.2-9.7)	17.1 (95%CI 9.9-24.2)
	exon7	14 (26.4%)	9.1 (95%CI 7.1-11.1)	17.1 (95%CI 9.2-25.1)
	exon8	2 (3.8%)	1.9 (95%CI ——)	17.2 (95%CI ——)
	exon 9&10	8 (15.1%)	11.3 (95%CI 10.9-11.7)	23.6 (95%CI 17.9-29.4)
TP53				
non-disruptive				
(N=62, 1 of them shared exon co-mutations)	exon4	6 (9.7%)	11.1 (95%CI 1.2-21.0)	13.4 (95%CI 0.0-28.0)
	exon5	21 (33.9%)	9.3 (95%CI 6.7-11.9)	24.1 (95%CI 15.9-32.3)
	exon6	8 (12.9%)	5.6 (95%CI 0.0-11.4)	21.0 (95%CI 12.4-29.5)
	exon7	12 (19.4%)	7.4 (95%CI 7.0-7.8)	17.0 (95%CI 9.0-25.1)
	exon8	14 (22.6%)	3.8 (95%CI 0.0-11.0)	19.3 (95%CI 3.8-34.8)
	exon 9&10	2 (3.2%)	0.7 (95%CI ——)	2.6 (95%CI ——)

Table 4
The comparison of the ORR in various TP53 mutations.

Types	ORR	DCR
TP53 wild-type	80.00%	95.38%
TP53 mutations	89.57%	67.83%
TP53 exon 6 and 7 mutations	90.70%	67.00%
E19-TP53 hotexon mutations	94.5%	80.0%
E19-TP53 mutations	94.55%	80.00%
E21-TP53 mutations	86.67%	58.33%
E19-TP53 non-disruptive	87.1%	67.0%

The influence of TP53 exon mutation on prognosis

Because most of the TP53 mutations were found in exons 5–8, we examined differences in survival in patients with TP53 exon 5–8 mutations. Ninety-three patients had mutations in TP53 exons 5–8, including 34 patients with mutations in exon 5, 19 with mutations in exon 6, 27 with mutations in exon 7, and 17 with mutations in exon 8 (4 patients had two TP53 mutations) (Table 1,Fig. 6). Patients with mutations in exons 6 or 7 had a significantly shorter OS and PFS than patients with wild-type TP53, the result had been shown. (Fig. 2,Table 2)

TP53 disruptive mutations decrease OS

We next examined the association between prognosis and disruptive/non-disruptive mutations in TP53. Patients were divided into three groups: those with disruptive TP53 mutations, those with non-disruptive TP53 mutations, and those with wild-type TP53. When compared with wild-type patients, patients with both disruptive and non-disruptive mutations had a significantly shorter OS and a shorter PFS (Table 2, Fig. 3). However, there was no difference in survival between patients with disruptive and non-disruptive mutations (OS 17.13 months [95% CI 14.27–20.00] vs 21.73 months [95% CI 18.69–24.77], $p = 0.466$ and PFS 9.33 months [95% CI 7.92–10.45] vs 7.57 months [95% CI 7.35–7.79], $p = 0.469$) (Fig. 3).

TP53 exon 5 mutations influence the prognostic utility of disruptive and non-disruptive TP53 mutations

Because there was no obvious difference in OS between patients with disruptive and non-disruptive mutations, we divided patients based on mutation type and exon mutations (Table 3,Fig. 4). We found that patients with non-disruptive mutations in exon 5 had the highest OS, whereas patients with disruptive mutations in exon 5 had lower OS (24.10 months [95% CI 15.94–32.26) vs 14.47 months [95% CI 8.40–20.54], $p < 0.05$, HR = 2.04 [95% CI 0.99–4.19]). Therefore, disruptive mutations in TP53 $^{\text{exon 5}}$ could be considered as a factor in influencing the prognosis of patients (Fig. 5A).

We speculate that the related mechanism of this phenomenon might be the exon 5 was included in the DNA-binding domain [28,29], which was related to the core function of the protein transcribed by TP53. With the mutation of exon 5, the function of TP53 tumor suppressor gene was inhibited accordingly.

EGFR and TP53 co-mutations when combined with other mutations shared worse prognosis

As described above, TP53 mutations are associated with worsened OS and PFS. Therefore, we further analyzed patients with EGFR-TP53 co-mutations and other mutations (RB1 and PTEN mutations) and found that patients with EGFR-TP53-other mutations had a shorter OS and PFS than those with EGFR-TP53 co-mutations alone (EGFR-TP53 co-mutations vs EGFR-TP53-other mutations: OS 20.97 months [95% CI 17.44–24.50] vs 12.50 [95% CI 5.58–19.419], $p = 0.029$, HR=0.527 [95% CI 0.309–0.897]; PFS 8.23 months [95% CI 6.78–9.69] vs 3.70 months [95% CI 6.24–8.90], $p<0.001$, HR=0.332 [95% CI 0.192–0.571]) (Fig. 5B).

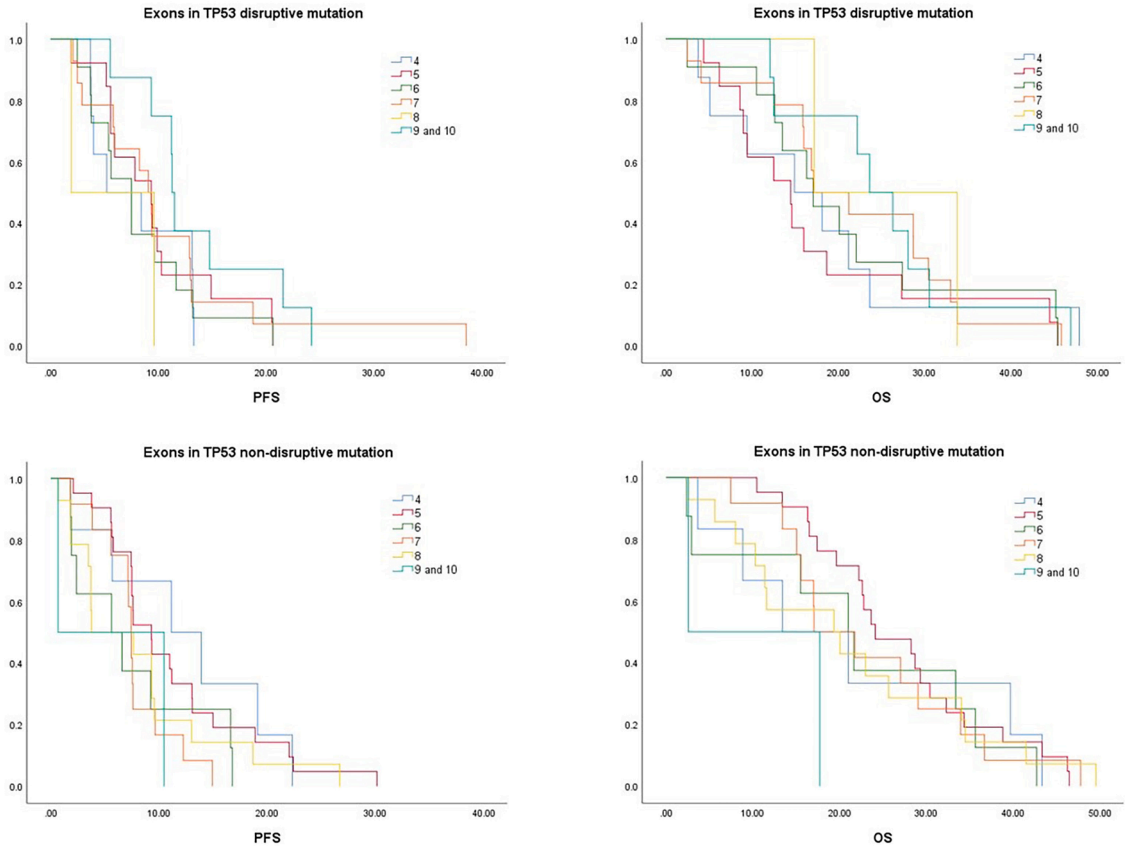

Fig. 4. The difference of patients with TP53 disruptive/non-disruptive and TP53 various exon mutations.

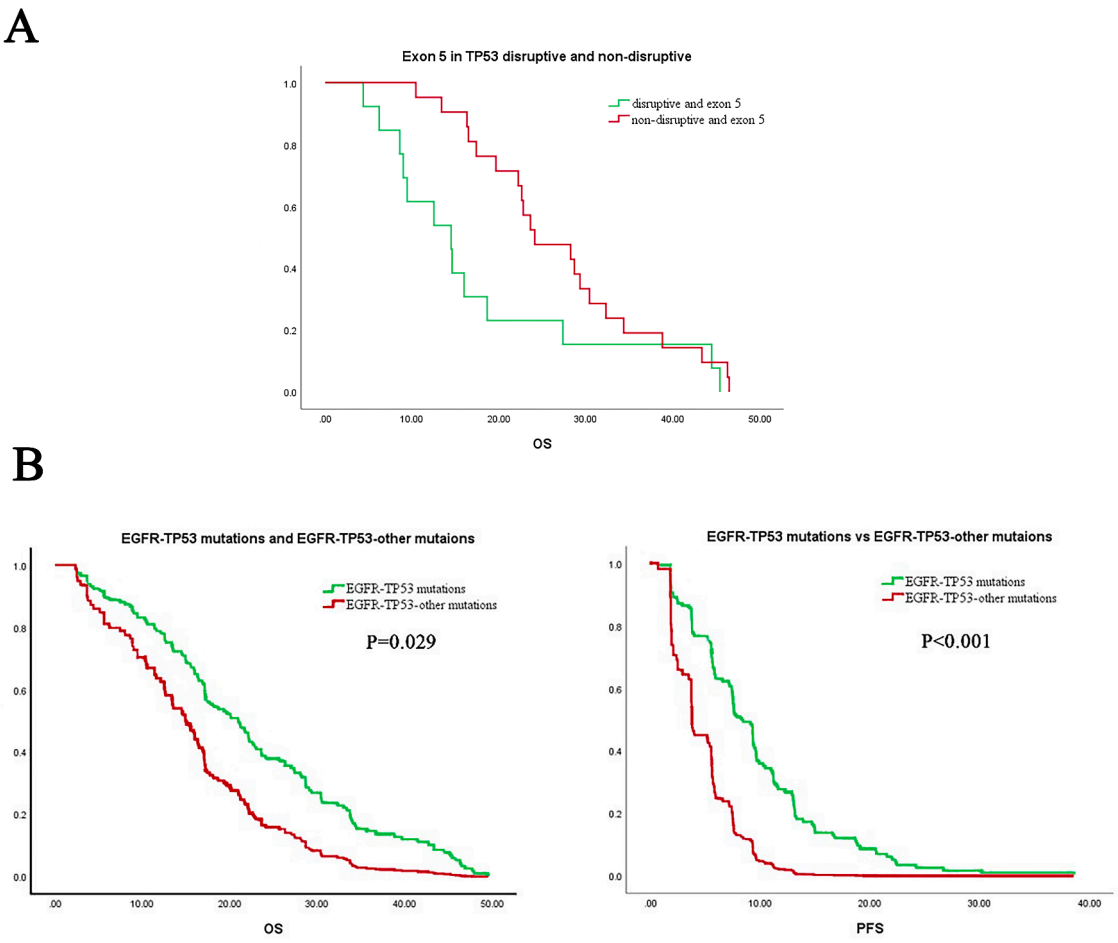

Fig. 5. A: The influence of exon 5 in divided patients with TP53 disruptive and non-disruptive mutations. B: The survival trend of patients with EGFR-TP53 co-mutations and EGFR-TP53-other co-mutations.

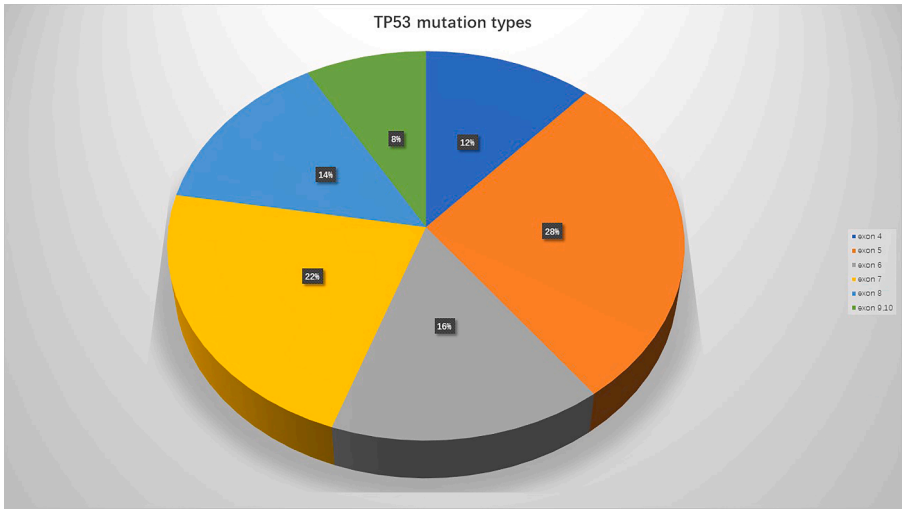

Fig. 6. The proportion of patients with TP53 exon mutations.

Discussion

The BENEFIT trial was the first clinical trial to demonstrate the value of using ctDNA circulating in plasma to detect EGFR mutations and identify patients that might benefit from EGFR-TKIs therapy [23]. Compared with the detection of solid tumor tissue, the detection of ctDNA in blood has the following advantages: firstly, it overcomes the heterogeneity of tumor and can evaluate the tumor mutation completely. Secondly, it is easy to dynamically follow up the mutations of tumor gene. Finally, the sample is convenient for biopsy, which can reduce the damage caused by tissue puncture biopsy. This was a novel approach compared to the most of previous studies which detected biomarkers by sequencing solid tumor tissue [30,32,34], and this may partly explain the conflicting results regarding the value/efficacy of

treatment with EGFR-TKIs. For example, previous studies have suggested that TP53 mutation adversely affects patient survival, but this was not consistently demonstrated in our study population. VanderLaan et al. [29] suggested that TP53 mutation decreases the response rate to EGFR-TKIs, and the ORR of patients with wild-type TP53 is longer than that of patients with mutant TP53. However, other groups have found no prognostic benefit of TP53 mutation in patients who received TKIs [33, 34]. Furthermore, various types of TP53 mutations have different prognostic values. Jiao et al. [32] suggested that patients with mutations in the TP53 coding region have a lower OS than patients with TP53 ^{wild} type (with rare mutations at exons 2, 3, and 10). They also suggested that TP53 $^{exon\ 7}$ mutations were correlated with a moderate prognosis and TP53 $^{exon\ 6}$ mutations with a poor prognosis.

It is worth to mention that our study had some controversial results which were inconsistent with the results of previous studies based on sequencing solid tumor tissue. First, we found that TP53 mutation had independent prognostic value for the PFS of patients harboring EGFR exon 19 del and L858R mutations. Further, our study also demonstrated that TP53 mutations (hot exon mutations, disruptive and non-disruptive mutations, and co-mutations) all have prognostic value in patients with stage IIIA, IIIB and IV cancer treated with gefitinib, and the clinical efficiency was significant. We believed that TP53 mutations are important determinants of poor survival, which is different from the results of previous studies [33,34]. Regarding mutations in different TP53 exons, we found that patients with mutations in TP53 $^{exons\ 6\ \&\ 7}$ had a significantly poorer survival than patients with TP53 ^{wild}. We also found that exon 5 mutations were an independent factor influencing the prognosis of patients with disruptive and non-disruptive TP53 mutations.

Targeted therapy and immunotherapy are the main treatments with proven efficacy for NSCLC. Wu et al. [35] indicated that stage-differential gene signatures can identify TP53 ^{mut} and TP53 ^{wild} patients to determine those best suited for immunotherapy. Other groups have also demonstrated that patients with TP53 and Kras co-mutation could benefit from PD-1 therapy [36,37]. In our study, we assessed the long-term efficacy of immunotherapy. However, drugs targeting TP53 currently only target specific sites, and therefore therapeutic options are relatively limited. Based on our results, we propose that the efficiency of single-targeted therapy is poor, and patients with different co-mutation types should be administered combined therapy. Many studies indicated that combined therapy might lead to a better outcome [38,39]. Therefore, a prospective clinical trial is conducting in our research group. It is a phase III, multicenter, randomized controlled clinical trial that subdivided patients with co-mutations into 2 groups (one of the groups continued targeted inertial chemotherapy and the other one received combined chemotherapy), mainly focused on PFS and less emphasized on ORR, AE side reaction, which has been planned to investigate targeted therapy for patients with different TP53 co-mutation states.

This study has some limitations. Firstly, the study population was small, and some of the subgroups shared several patients. Future studies with a larger sample population are warranted to validate the results described in this study. Secondly, we were unable to fully elucidate the mechanisms of drug resistance, which may be due to the NCG test with limited target locations. Thirdly, our results may have been influenced using different types of samples and cancer stages. Finally, these results should be validated in clinical studies.

In conclusion, by sequencing ctDNA in plasma, we demonstrate that EGFR mutation and EGFR mutations combined with various TP53 mutation types affect patient prognosis. Confirming the patient's mutation status prior to treatment may optimize treatment response and reduce the emotional and financial strain of treatment failure.

Declaration of Competing Interest

None.

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Statement of translational relevance

We conducted a study examining concomitant TP53 mutations in patients with EGFR-mutated NSCLC to explore the difference in response to EGFR-TKI treatment under different TP53 co-mutation states. We assessed the long-term efficacy of immunotherapy. However, drugs targeting TP53 currently only target specific sites, and therefore therapeutic options are relatively limited. Based on our results, we propose that the efficiency of single-targeted therapy is poor, and patients with different co-mutation types should be administered combined therapy. This study demonstrates that TP53 and EGFR mutation status affects the survival of patients with lung cancer treated with gefitinib. Confirming the patient's TP53 mutation status prior to treatment may optimize treatment response and reduce the emotional and financial strain of treatment failure.

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