Mutations in exon 8 of *TP53* are associated with shorter survival in patients with advanced lung cancer

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Abstract. Currently, in clinical settings, all *TP53* mutations have been considered equally. However, numerous studies have demonstrated that the position and type of mutation have differential effects on prognosis. Such discrepancy can be partially due to the lack of unifying classification system for *TP53* mutations. In the present study, two of the most frequently used systems were compared, according to the location of the mutation or its functional effects on p53 protein and the impact of *TP53* mutations on the overall survival (OS) time of 379 Chinese patients with advanced lung cancer was analyzed. Capture-based ultra-deep targeted sequencing on plasma samples of 379 patients with advanced lung cancer was performed. The present results suggested that mutations occurring in exon 8 may be associated with shorter OS in tyrosine kinase inhibitor-naïve patients (P=0.013) and in patients previously treated with one line of treatment (P=0.032). The results of the present study provided solid evidence that not all *TP53* mutations were associated with a similar prognosis. Mutations in exon 8 were found in a subgroup of patients with unfavorable prognosis across various treatment histories. To the best of our knowledge, the present study is the first to compare different *TP53* mutation classification systems in a large cohort of patients with advanced lung cancer.

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Introduction

TP53, the first tumor suppressor gene to be identified, acts as the guardian of the genome and is involved in the regulation of several essential cell processes, including, but not limited to, cell cycle regulation, apoptosis, cell differentiation, DNA repair and blood vessel formation (1,2). It is the most frequently mutated gene across a large spectrum of different types of cancer, including lung adenocarcinoma and lung squamous cell carcinoma, with a mutation rate of ~50% (2-4). Under normal conditions, *TP53* is rapidly degraded; however, upon cellular stress, it is activated and stabilized, resulting in protein accumulation in the nucleus (5,6). The activation of the *TP53* signaling pathway has been demonstrated to lead to DNA damage repair and cell cycle arrest (7,8). Mutations in *TP53* have been revealed to result in the loss of tumor-suppressor function, thus leading to an unstable genome and downregulating apoptosis (9). Accumulating evidence have suggested that, in addition to eliminating the tumor suppressor function, mutations in *TP53* can also induce new functions, including gain-of-function mutations, which can accelerate tumor progression and metastasis (2,9,10).

TP53 mutation is observed in ~50% of patients with non-small cell lung cancer (NSCLC), with a higher prevalence in squamous-cell carcinoma of the lung compared with lung adenocarcinoma (38 vs. 12%) (11,12). These alterations can include frameshift, nonsense, silent and missense mutations (11-13). Unlike other tumor suppressor genes, such as *APC*, *BRCA1* or RB transcriptional corepressor 1 *(RB1)* with truncating mutations being the major alteration type, the majority of *TP53* alterations are missense mutations, accounting for more than 75% of alterations (13,14). The majority of *TP53* mutations occur in the DNA-binding region, in exons 5-8, spanning 540 nucleotides with numerous recurring hotspot mutations, leading to a stable protein with a significant loss of activity (14-17). *In vitro* studies have shown that wild-type (WT) p53 promotes gefitinib-induced apoptosis (18). The prognostic and predictive values of *TP53* mutations have been investigated, however results are conflicting; a previous study demonstrated that non-disruptive *TP53* mutations are independently correlated with shorter OS in patients with advanced NSCLC, regardless of epidermal growth factor receptor (*EGFR)* and *KRAS* status (19). Another study revealed that a shorter OS was associated with adjuvant chemotherapy in patients presenting mutations in *TP53* with NSCLC and completely resected tumors (20). A previous study investigating clinical outcomes of patients with NSCLC with dual *EGFR* and *TP53* mutations revealed lower response rates and shorter progression-free survival (PFS) in such patients compared with patients with *EGFR* mutations (21). By contrast, other previous studies have revealed the lack of association between *TP53* mutations and OS or response to treatment (22-24). The lack of a unifying classification system may contribute to the controversy regarding the prognosis and predictive value of *TP53*. A variety of criteria have been used to categorize *TP53* mutations, including, but not limited to, functional effects on p53 (disruptive vs. non-disruptive) (15,17,19) and location ('hotspot' exons vs. 'non-hotspot' exons) (14,17).

Currently, in clinical settings, all *TP53* mutations have been considered equally, without major differences among the various types of mutations. However, an increasing number of studies suggested that the type and position of the mutation may be important, and the present study aimed to investigate this possibility in lung cancer. In fact, numerous studies have revealed that the position and the type of mutation have differential effects on prognosis (2,11,17). Important functional differences among various mutant forms of p53 have been elucidated, including mutations in the amino-terminal (AT) domain, the oligomerization domain (OD) and the DNA-binding domain (DBD) (25-28). AT-domain mutations often result in the disruption of the expression of full-length p53 (26). Alternatively, translation from the start codon in exon 4 results in the expression of p47, which retains the apoptotic function of p53 (26). A previous study has suggested that sporadic human cancers with AT-domain mutations are often more responsive to treatment (26). Mutations occurring in the OD, which is important for the tetramerization of p53, often behave as loss-of-function mutations (25). Patients harboring such mutations are less responsive to therapies that rely on p53-mediated cytotoxic effects (25). In total, ~80% of *TP53* mutations affect the DBD, encoded by exons 5-8. In addition to the loss of functional effects, mutations in DBD can also acquire additional oncogenic properties after the loss of the WT allele (2,28).

In the present study, capture-based ultra-deep targeted sequencing was performed on the plasma samples of 379 Chinese patients with advanced lung cancer to investigate clinical outcomes associated with *TP53* mutations. The *TP53* mutation classification systems, based on the functional effect and location of the mutation, were also compared.

Materials and methods

Patient selection. *TP53* status was retrospectively analyzed and its predictive and prognostic values were examined in 379 patients with advanced lung cancer (Stage IIIB-IV) harboring at least one classic NSCLC driver mutation. Staging of the primary lung tumor, lymph node status and metastasis were assessed based on the American Joint Committee on Cancer 7th edition Tumor, Node and Metastasis (TNM) staging system of NSCLC (29). Patients (female to male ratio, 1:1.3; median age, 56.5 years; range, 26-82 years) were treated at any of the nine participating centers between September 2015 and October 2016. The inclusion criteria were: i) Patients diagnosed with advanced-stage lung cancer (stage IIIB-stage IV) of any histology harboring at least one classic NSCLC driver mutation; and ii) the patient was treated at any of the nine participating centers between September 2015 and October 2016. The exclusion criteria was patients with early-stage lung cancer (stage IA-IIIA) of any histology. Written informed consent was obtained from each patient for participation in the present study. Capture-based targeted sequencing was performed on the plasma samples using a panel consisting of 168 lung cancer-associated genes, spanning 160 kb of the human genome. The present study was approved by the Ethics Committee of Jiangsu Province Hospital (Nanjing, China).

Next generation sequencing library preparation and capture-based targeted DNA sequencing. Next generation sequencing was performed using a commercial panel comprising 168 lung cancer-associated genes (Lung Plasma; Burning Rock Biotech) in a Clinical Laboratory Improvement Amendments-certified laboratory as previously described (30). Briefly, circulating cell-free DNA was acquired from 4-5 ml of plasma using the QIAamp Circulating Nucleic Acid kit (Qiagen China Co., Ltd.) according to the manufacturer's protocol. A minimum of 50 ng of DNA is required for next-generation sequencing library construction. A DNA library for the next-generation sequencing experiments were constructed. Fragments between 200 to 400 base pairs (bp) from the DNA were end-repaired, phosphorylated and ligated with adaptors (Agencourt AMPure XP kit; Beckman Coulter, CA, USA). Purified DNA with adaptors were then hybridized with capture probes baits, underwent hybrid selection with magnetic beads, and PCR amplified. The quality and the size of the fragments were assessed using a Qubit 2.0 fluorimeter (Thermo Fisher Scientific, Inc., Waltham, MA, USA) with a dsDNA high-sensitivity assay kit (Thermo Fisher Scientific, Inc.). Indexed samples were sequenced on a Nextseq500 sequencer (Illumina, Inc.) with pair-end reads. An average coverage of 11,816x was reached with a limit of detection of 0.2%.

Sequence data analysis. Data were analyzed using optimized pipeline for somatic mutation calling as previously

described (30). Briefly, the sequence data were mapped to the reference human genome (hg19) using Burrows-Wheeler Aligner (version 0.7.10) (31). Local alignment optimization and variant calling were performed using Genome Analysis Tool kit (version 3.2) (32,33) and VarScan (version 2.4.3) (34). Variants were filtered using the VarScan fpfilter pipeline; loci with depth <100 were filtered out. Base calling in plasma samples required ≥8 supporting reads for single nucleotide variations and 5 supporting reads for insertion-deletion variations. Variants with population frequency >0.1% in the ExAC (http://exac.broadinstitute.org/), 1,000 Genomes (35), dbSNP (https://www.ncbi.nlm.nih.gov/snp/) (36) or ESP6500SI-V2 (https://evs.gs.washington.edu/EVS/) databases were grouped as single nucleotide polymorphisms and excluded from further analysis. Remaining variants were annotated with ANNOVAR (2016-02-01 release) (37) and SnpEff (version 3.6) (38). Analysis of DNA translocation was performed using Factera (version 1.4.3) (39). Copy number variations (CNV) were analyzed based on the depth of coverage data of capture intervals using an in-house developed algorithm. The limit of detection for CNVs was 1.5 and 2.64 for deletions and amplifications, respectively.

Classification of TP53 mutations. Disruptive mutations, as described previously (15), were defined as any mutation leading to a stop codon or missense mutations occurring within the L2-L3 loop of the DNA-binding domain, leading to a substitution with an amino acid of a different polarity or charge group. All other mutations were defined as non-disruptive. Hotspot exons were defined as exons 5-8, as previously described (17).

Statistical analysis. Since the data were not equally distributed, data are presented as the median. All statistical tests were conducted in R version 3.3.3 (The R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org) and R Studio version 1.1.383 software (40), and all tests were two-sided unless otherwise specified. Pearson's correlation test was used to assess correlation between two continuous variables. Fisher's exact test was used to assess the association between two categorical variables. Survival times were illustrated by Kaplan-Meier curves with the P-value determined by log-rank tests or Cox regression models when a co-variant was included. All survival analyses were adjusted for age, sex, smoking history, stage and histology. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. The *TP53* status of 379 patients with advanced lung cancer (Stage IIIB to IV) harboring at least one classic NSCLC driver mutation with various histological types was assessed in the present study. Among them, 294 patients had *EGFR* mutations, 24 had anaplastic lymphoma kinase (*ALK*) rearrangements, 14 had erb-b2 receptor tyrosine kinase 2 mutations, five had MET Proto-Oncogene (*MET*) mutations, two had B-raf proto-oncogene mutations, four had ROS proto-oncogene 1, receptor tyrosine kinase fusions, 11 had *KRAS* fusions and five had ret proto-oncogene fusions; the remaining 20 patients had dual driver mutations (Table I). In the examined cohort, 213 (56.2%) were female and 166 (43.8%) were male patients, and 156 had a history of smoking. The median age of this cohort was 56.5 years, ranging between 26 and 82 years. The cohort primarily consisted of adenocarcinoma (332/379), followed by squamous cell carcinoma (SqCC) (11/379) and small cell lung cancer (10/379). A total of 165 patients (43.5%) had not received tyrosine kinase inhibitors (TKI) as a treatment regimen and were considered TKI-naïve; among them, 84 were treatment-naïve and the remaining were previously treated with chemotherapy. A total of 214 patients (56.5%) were previously treated with TKI; among them, 184 patients were previously treated with one line of treatment. A total of 173 patients were treated with EGFR-TKI and the remaining 11 were treated with crizotinib, an ALK inhibitor (41). A total of 30 patients received two lines of treatment. Among them, 24 were treated with first and third generation EGFR-TKIs, two patients were treated with first generation EGFR-TKIs followed by a tyrosine-protein kinase MET inhibitor and the remaining three patients were treated with ALK inhibitors, Crizotinib followed by ceritinib (41). Table I summarized the detailed clinical characteristics of the cohort investigated.

TP53 mutation prevalence and associations with clinical parameters. A capture-based ultra-deep targeted sequencing analysis was performed as described in Materials and methods and (30) on the plasma samples obtained from 379 patients with advanced lung cancer to investigate their *TP53* status and the prognostic value of the *TP53* mutations. The prevalence of *TP53* mutations in the cohort was 49.9% (189/379), which is comparable to its prevalence in the western population (22). Among them, 163 patients harbored mutations in hotspot exons: 48 Mutations were on exon 5, 31 on exon 6, 40 on exon 7 and 46 on exon 8 (Table I). Two patients had two *TP53* mutations (data not shown). A total of 84 patients had disruptive mutations. The distribution of *TP53* mutations is also presented in Fig. S1. No association between *TP53* mutations and smoking history was observed when all *TP53* mutations were taken into consideration (data not shown). The examined cohort primarily consisted of patients with adenocarcinoma, squamous cell carcinoma or small cell lung cancer. The percentages of *TP53* mutations were comparable in patients with adenocarcinoma (48.3%) and small cell lung cancer (48.4%) (data not shown). All patients investigated in the present study with small cell lung cancer carried *TP53* mutations, in line with previous studies (42), suggesting that a significant percentage of patients with small cell lung cancer have *TP53* mutations. Next, the mutation spectra of patients with *TP53* mutations were compared with patients without *TP53* mutation (Fig. S2). The present results revealed that the most frequent mutation was *EGFR* in both groups. In addition, a larger number of *RB1* mutations and *MET* amplifications were present in patients with *TP53* mutation (Fig. S2).

The correlations between *TP53* mutations, classified according to different systems, and clinical parameters, including but not limited to the TNM stage, smoking history and metastatic sites were further investigated. A positive correlation was identified between *TP53* mutations and the TNM stage when all mutations were considered collectively. Patients with *TP53* mutations were more likely to have advanced N (P=0.004, r=0.161) and M (P=0.004, r=0.151) stages of the

Table I. Patient demographics and clinical characteristics.

Characteristics	Value
Total, n	379
Sex	
Male, n (%)	166 (43.8)
Female, n (%)	213 (56.2)
Age, median (range)	56.5 years (26-82 years)
Smoking history	
Smokers, n (%)	156 (41.2)
Non-smokers, n (%)	200 (52.8)
No data, n (%)	39 (10.3)
Histology	
Adenocarcinoma, n (%)	332 (87.5)
Squamous cell carcinoma, n (%)	11 (2.9)
Small cell lung cancer, n (%)	10 (2.64)
Others, n (%)	26 (6.9)
Treatment History	
TKI-naïve, n (%)	165 (43.5)
One line of TKI treatment, n (%)	184 (48.5)
EGFR-TKIs, n (%)	173 (94)
ALK-TKIs, n (%)	11 (6)
Two lines of TKI treatment, n (%)	30 (8)
1st and 3rd EGFR-TKI, n (%)	24 (80)
EGFR-TKI and c-MET-TKI, n (%)	3 (10)
ALK-TKIs, n (%)	3 (10)
TP53 status, n (%)	
WT, n (%)	190 (50.1)
Mutated, n (%)	189 (49.9)
Disruptive mutation, n (%)	84 (44.4)
Non-disruptive mutation, n (%)	105 (55.6)
Exon 5, n (%)	48 (25.4)
Exon 6, n (%)	31 (16.4)
Exon 7, n (%)	40 (21.2)
Exon 8, n (%)	46 (24.3)
Driver mutation	
EGFR, n (%)	294 (77.6)
ALK, n (%)	24 (6.3)
ERBB2, n (%)	14 (3.7)
MET, n (%)	5 (1.3)
BRAF, n (%)	2 (0.5)
ROS1, n (%)	4 (0.1)
KRAS, n (%)	11 (2.9)
RET, n (%)	5 (1.3)
Dual drivers, n (%)	20 (5.3)

TKI, tyrosine kinase inhibitor; WT, wild-type; ALK, anaplastic lymphoma kinase; EGFR, epidermal growth factor receptor; ERBB2, erb-b2 receptor tyrosine kinase 2; MET, MET proto-oncogene, receptor tyrosine kinase; ROS1, ROS proto-oncogene 1, receptor tyrosine kinase; RET, ret proto-oncogene.

disease (Fig. 1A and B). A positive correlation was also identified between liver metastasis and *TP53* mutations when all mutations were considered collectively (P=0.001, r=0.187). In the cohort, 67.9% patients (55/81) with *TP53* mutations had liver metastasis; in contrast, 45.5% (127/279) of patients without a *TP53* mutation had liver metastasis (P=0.001) (Fig. 1C). These trends also existed when *TP53* mutations were classified according to the location of the mutation or functional effects on the p53 protein. Hotspot exon and non-hotspot exon mutations demonstrated a significant correlation with N (P=0.036 and 0.012, respectively) and M (P=0.012 and 0.001, respectively) stage (data not shown). When *TP53* mutations were classified as disruptive or non-disruptive mutations, *TP53* disruptive mutations exhibited a non-significant correlation with N (P=0.08) and M (P=0.1) stages. A strong correlation was observed between liver metastasis and *TP53* mutation, regardless of the classification system (P<0.001). Only *TP53* hotspot exon mutations were significantly correlated with bone metastasis (P=0.032) (data not shown). Collectively, the present results suggested that only certain types of *TP53* mutations were correlated with the clinical parameters analyzed, providing evidence for the hypothesis that not all *TP53* mutations are equal.

Prognostic values of TP53 mutations. Conflicting findings regarding the prognostic values of the *TP53* status were reported, which can be partially attributed to the lack of a unifying classification system (21-24). The prognostic value of *TP53* mutations was evaluated using the two aforementioned classification systems. Patients were grouped into three groups based on their treatment history: i) TKI-naïve (n=165); ii) previously treated with one line of TKI (n=184); and iii) treated with ≥2 TKI (n=30). In the TKI-naïve patients, 74 patients had *TP53* mutations. Among them, 64 had mutations in hotspot exons (exons 5-8) (2,28) and the remaining 10 had mutations in other exons. A total of 33 patients had disruptive mutations and 45 had non-disruptive mutations. No association was observed between *TP53* mutations and OS when all *TP53* mutations were considered collectively or classified according to their location (hotspot exon vs. non-hotspot exon mutations) and functional effects on p53 protein (disruptive vs. non-disruptive; Fig. 2A-C). Notably, mutations occurring on exon 8 were found to be associated with OS (P=0.013) when controlling for age, sex, stage and histology. A total of 14 patients with mutations in exon 8 had a shorter median OS compared with the remaining 91 patients who had no mutations in exon 8 (Fig. 2D).

In patients previously treated with one line of TKI treatment (n=184), an analysis revealed that *TP53* status, when all mutations were considered collectively, was found to be marginally associated with OS (P=0.05). Patients with *TP53* mutations had a shorter OS compared with patients with WT *TP53* (Fig. 3A). Such associations were significantly enhanced when only mutations occurring on exon 8 were considered (P=0.032; Fig. 3B). A total of 26 patients had mutations in exon 8, including 22 missense, three frameshift and one nonsense mutation (Fig. 3B). However, when all hot exon mutations were considered collectively, no association was observed (P=0.083; Fig. 3C). The same trend was observed in TKI-naïve patients, disruptive (P=0.081) and non-disruptive (P=0.106) mutations were not significantly associated with OS (Fig. 3D).

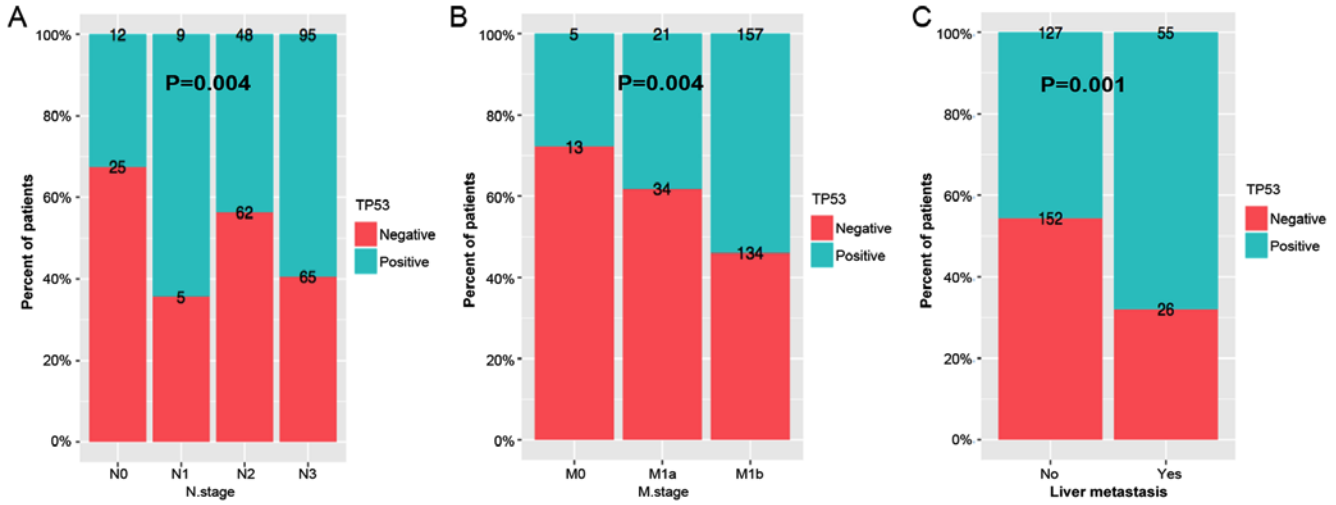

Figure 1. Correlations between *TP53* status and clinical parameters. Patients with *TP53* mutations are more likely to have more advanced (A) N and (B) M stage as well as (C) liver metastasis. Blue bars denote patients with mutations in *TP53* and red bars denote patients with wild-type *TP53*. Pearson's correlation coefficient test was used to determine P-values. TNM, Tumor-Node-Metastasis.

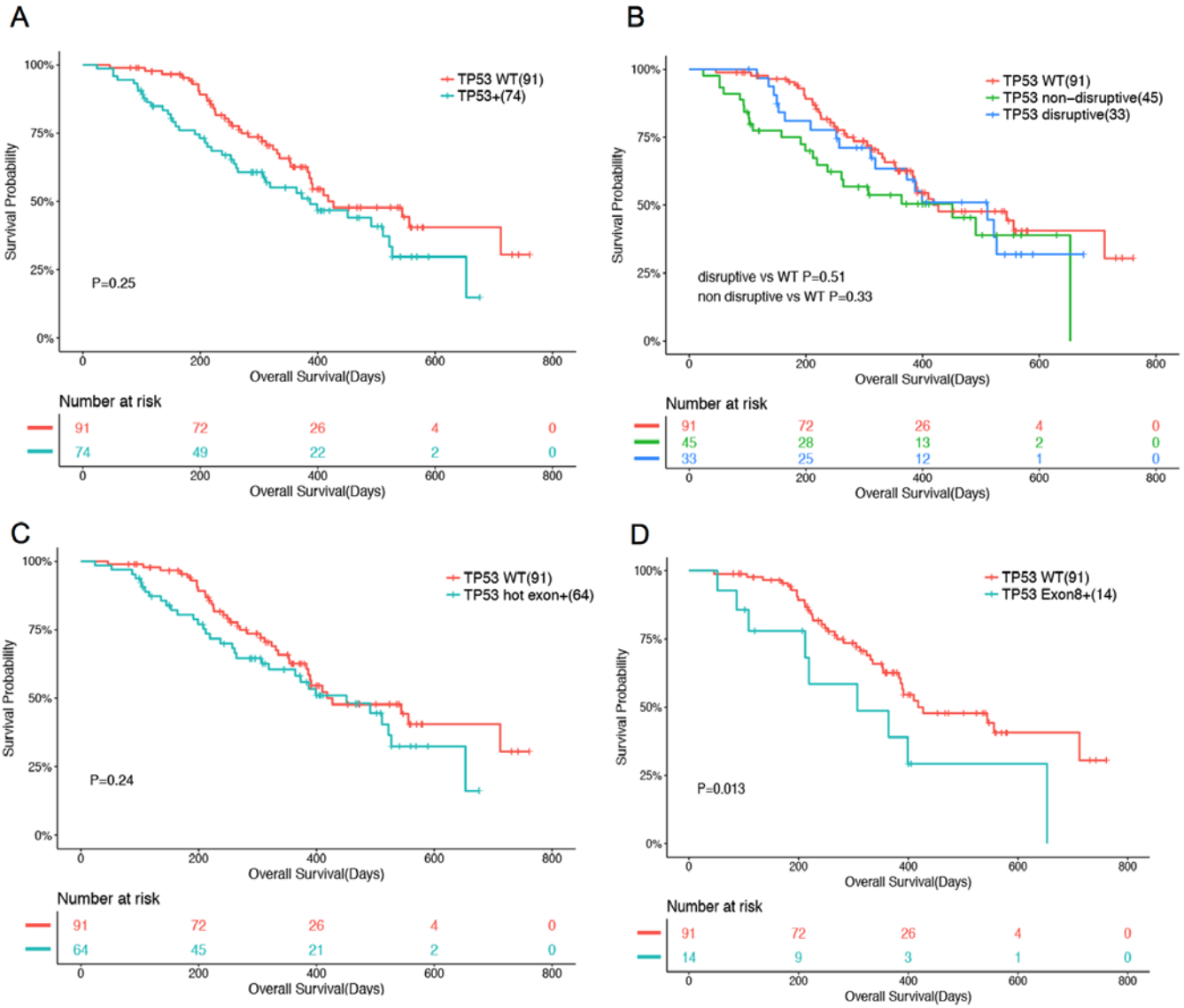

Figure 2. Associations between *TP53* mutations and survival outcomes in TKI-naïve patients. Kaplan-Meier curves comparing OS in (A) patients with WT and mutant *TP53*, (B) patients with WT *TP53* and disruptive and non-disruptive *TP53* mutations, (C) patients with WT *TP53* and hotspot exon mutations, and (D) patients with WT *TP53* and mutations occurring on exon 8. TKI, tyrosine kinase inhibitor; OS, overall survival; WT, wild-type.

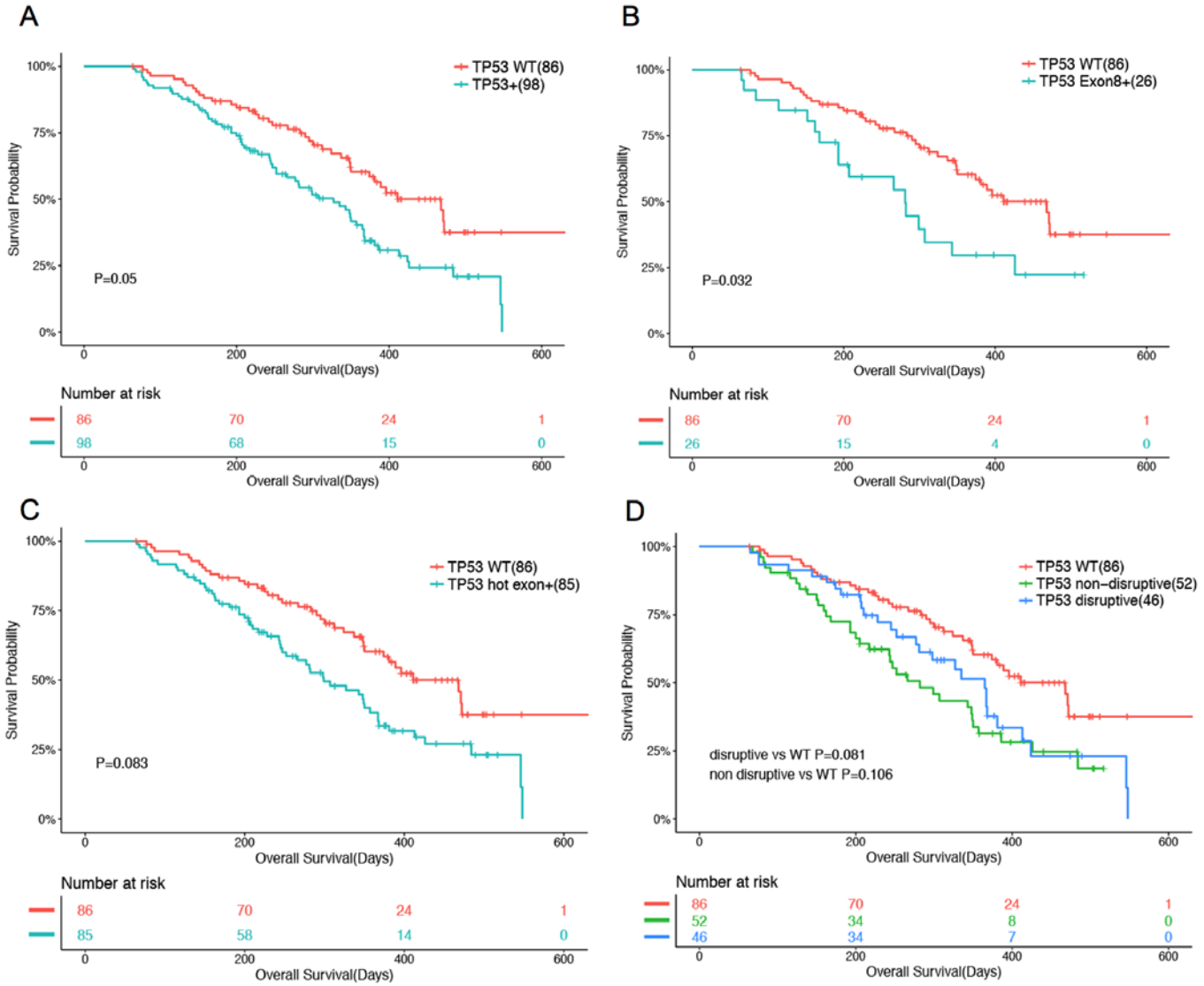

Figure 3. Associations between *TP53* mutations and survival outcomes in patients who had one course of TKI treatment. Kaplan-Meier curves comparing OS in (A) patients with WT and mutant *TP53*, (B) patients with WT *TP53* and mutations occurring on exon 8, (C) patients with WT *TP53* and hotspot exon mutations, and (D) patients with WT *TP53*, and disruptive and non-disruptive *TP53* mutations. TKI, tyrosine kinase inhibitor; OS, overall survival; WT, wild-type.

In the cohort, 99 patients were undergoing osimertinib treatment, a third-generation EGFR TKI (43). The impact of *TP53* mutations on OS in osimertinib-treated patients was subsequently analyzed. In this cohort, 32 patients had WT *TP53* and 67 patients had *TP53* mutations (data not shown). No association was observed between *TP53* status and OS, regardless of the classification system (data not shown). In the examined cohort of patients, 62 patients possessed *EGFR* 19 del and 37 possessed *EGFR* L858R (data not shown). No association was observed between *TP53* status and OS, regardless of classification system, in patients harboring *EGFR* L858R (data not shown). In patients harboring *EGFR* 19 del concurrent to T790M treated with osimertinib, non-disruptive mutations (P=0.031) were found to be associated with OS (Fig. 4A). A total of 14 patients with non-disruptive *TP53* mutations exhibited a significantly shorter OS compared with patients with WT *TP53*. All *TP53* mutations (P=0.156), particularly disruptive mutations (P=0.690) as well as all hotspot exon mutations (P=0.128) including exon 8 (P=0.075) did not exhibit an association with OS (Fig. 4).

In patients treated with two lines of TKI, the analysis revealed that *TP53* mutations, when considered collectively, were identified to be associated with OS (P=0.037; Fig. 5A). Mutations occurring on exon 8 (P=0.079) as well as all hot exon mutations (P=0.052) also exhibited an association with OS (Fig. 5B and C). In contrast, disruptive mutations (P=0.086) did not exhibit an association with OS, whereas non-disruptive mutations (P=0.048) exhibited a marginal association with OS (Fig. 5D). All analyses were controlled for smoking status. Collectively, the data provided evidence supporting the hypothesis that *TP53* mutations are not equal. Furthermore, by comparing multiple *TP53* mutation classification systems, it was identified that mutations in exon 8 may serve as prognostic biomarkers across all patients.

Discussion

In the present study, the association between *TP53* mutations, analyzed using two classification methods (based on location

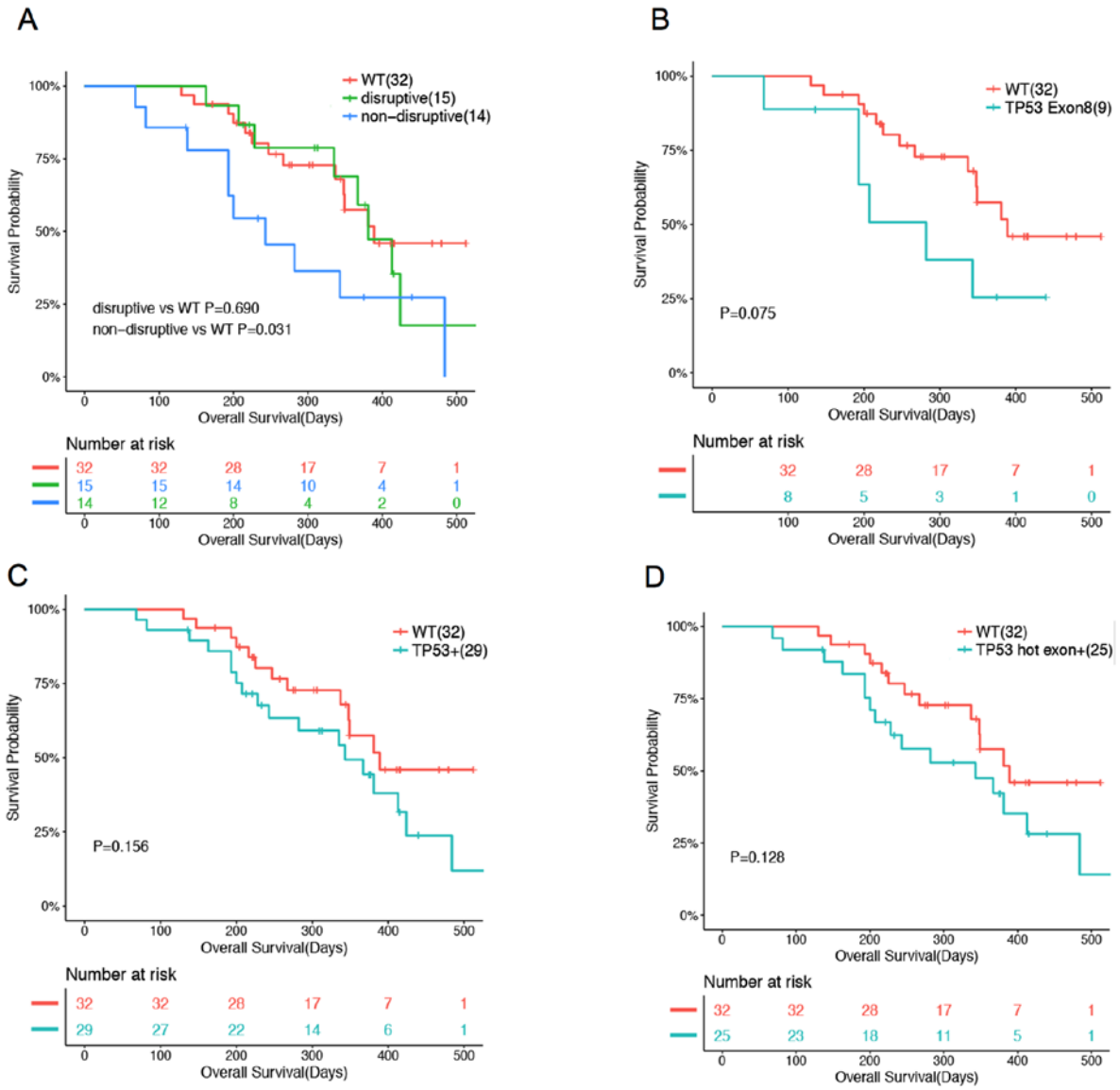

Figure 4. Associations between *TP53* mutations and survival outcomes in patients harboring *EGFR* 19 del in conjunction with *EGFR* T790M undergoing osimertinib treatment. Kaplan-Meier curves comparing OS in (A) patients with disruptive and non-disruptive *TP53* mutations, (B) patients with WT *TP53* and mutations occurring on exon 8, (C) patients with WT and mutant *TP53*, and (D) patients with WT *TP53* and hotspot exon mutations. TKI, tyrosine kinase inhibitor; OS, overall survival; WT, wild-type.

and function), and OS was investigated in a large cohort of patients with advanced lung cancer. It was demonstrated that mutations occurring on exon 8 may serve as prognostic biomarkers across all patients regardless of treatment history. The present results revealed that mutations occurring in exon 8 correlated with shorter OS in TKI-naïve and patients previously treated with one line of TKI. Such mutations also exhibited a slight association, although not significant, with shorter OS in patients previously treated with two lines of treatment. Therefore, *TP53* exon 8 mutations defined a distinct subset of patients with an unfavorable prognosis. The association between OS and *TP53* mutations categorized by function or considered collectively was not consistent across various treatment histories. In fact, *TP53* mutations considered collectively were only associated with OS in patients who received a certain treatment. *TP53* mutations were not associated with the prognosis in treatment-naïve patients. Such inconsistencies could be attributed to the following reasons: i) Not all mutations occurring on hotspot exons (exons 5-8) are functional; ii) treatment history of treated patients may vary among patients; iii) the number of patients were significantly fewer in patients treated with ≥2 lines of treatment; and iv) a number of studies have reported that *TP53* can serve as a resistance mechanism against the function of EGFR inhibitors (17,21,44-46). Therefore, the impact of mutations in *TP53* in patients treated with such inhibitors may be greater compared with patients treated with other therapies, such as chemotherapy. However, further examination is required as to why mutations occurring on exon 8 are associated with unfavorable prognoses. Therefore, exon 8 mutations that potentially serve as prognostic biomarkers require validation in larger cohorts.

Currently, all *TP53* mutations are considered equally in clinical settings, as well as during the development of therapeutic strategies, which primarily focuses on the restoration of

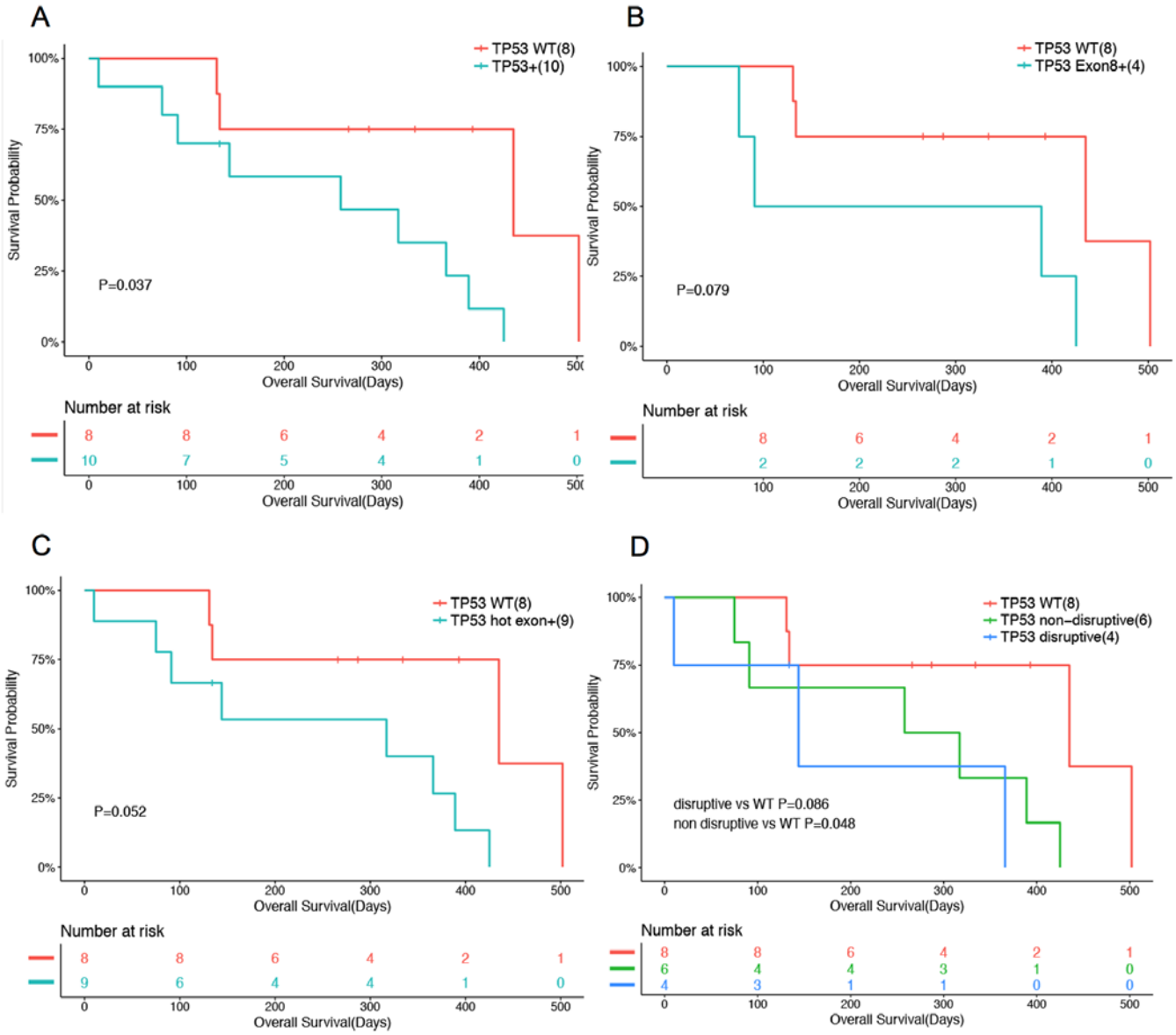

Figure 5. Associations between *TP53* mutations and survival outcomes in patients who received two lines of TKI treatment. Kaplan-Meier curves comparing OS in (A) patients with WT and mutant *TP53*, (B) patients with WT *TP53* and mutations occurring on exon 8, (C) patients with WT *TP53* and hotspot exon mutations, and (D) patients with WT *TP53*, and disruptive and non-disruptive *TP53* mutations. TKI, tyrosine kinase inhibitor; OS, overall survival; WT, wild-type.

the WT activity of *TP53* (47). Numerous studies investigating the prognostic value of *TP53* mutations, when all mutations were considered collectively, identified either no or slight associations, which was subsequently lost in the multivariate analysis (22,48). An increasing number of studies have been categorizing *TP53* mutations based on the multiple biological effects produced by different mutant proteins (15,49). Notably, the present study strongly followed the aforementioned approach. Several previous studies categorized *TP53* mutations and examined their prognostic value, presenting conflicting results, partially due to the lack of a unifying classification system (19,22,50). A number of studies reported shorter OS in the presence of specific mutations, including non-disruptive mutations (19), truncated, structural and DNA-binding mutations (51) or mutations occurring in certain exons (52,53). Other studies did not identify an association in patients with lung cancer (22-24,54). Some studies have demonstrated that non-disruptive mutations, allowing the maintenance of functional properties, are associated with gain-of-function properties (55,56). Furthermore, mutations occurring on different parts of the gene have different biological functions such as the AT domain, DBD and oligomerization domain (25-28). Studies have shown that mutations occurring in the L2 and L3 domains, providing for DNA contacts, are associated with poor prognosis (2,28,53). Due to the discrepancies identified in previous studies, the development of a clinically relevant unifying classification system is required. To the best of our knowledge, the present study is the first that compared the two classification systems commonly used (based on the position and the type of mutation) in a large cohort.

Since a significant percentage of patients exhibiting mutations in *EGFR* have concurrent *TP53* mutations, numerous studies have also assessed the impact of *TP53* mutations on the clinical outcomes of patients with EGFR mutations treated

with EGFR-TKIs (17,21,44-46). Such studies also yielded conflicting results. A previous study revealed that the predictive and prognostic power of *TP53* status to first-generation EGFR-TKI treatment are more reliable in patients harboring *EGFR* exon 19 deletion (19 del) (17). Since *TP53* mutations have been confirmed as a primary resistance mechanism to EGFR-TKI, some studies reported diminished responses. Canale *et al* (17) revealed that *TP53* exon 8 mutations, especially in conjunction with *EGFR* 19 deletion, were associated with a significantly lower disease control rate. Labbé *et al* (21) reported a marginally lower response rate and shorter PFS in patients with concurrent *EGFR* and *TP53* mutations, where all TP53 mutations were considered collectively. Collectively, these previous reports and the present study suggest that the use of a unifying classification system may be important in clinical settings.

Furthermore, the majority of studies examining the clinical relevance of *TP53* were primarily conducted in patients with early stage lung cancer and resectable tumors (20,24,57). To the best of our knowledge, a few studies investigated patients with advanced lung cancer and a majority of them included a limited number of patients (17,19,21,45,54,58). To the best of our knowledge, the present study is the first study to investigate, in a large cohort, the clinical relevance of *TP53* mutations in Chinese patients with advanced lung cancer, who had received previous treatments. Furthermore, to the best of our knowledge, the present study is also the first one to investigate the association between *TP53* mutations and OS in patients treated with osimertinib, a TKI inhibitor. It was revealed that the prognostic power of *TP53* mutations only existed in patients with *EGFR* 19 del and T790M. In such patients, non-disruptive mutations were associated with shorter OS. The prognostic power was not statistically significant in patients harboring *EGFR* L858R. A previous study evaluated the impact of *TP53* mutations on the outcomes of patients with *EGFR* mutations treated with one course of EGFR-TKI and revealed similar results (17). Patients harboring concurrent mutations in the exon 8 of *TP53* and *EGFR* 19 del were associated with a shorter PFS and OS. The predictive and prognostic power was much weaker in subgroups containing patients with other *EGFR* mutations (17). One major limitation associated with the present study is that it only included patients with classic NSCLC driver mutations. Further examination is required in order to validate these findings in larger cohorts, including patients without NSCLC driver mutations.

The results of the present study suggested that not all *TP53* mutations are equal. Mutation in exon 8 can identify a subgroup of patients with unfavorable prognoses across diverse treatment group. To the best of our knowledge, the present study is the first one that compared different *TP53* mutation classification systems in a large cohort of patients with advanced lung cancer. Furthermore, to the best of our knowledge, the current study may be the first to reveal that the prognostic potential of *TP53* mutations, in patients treated with osimertinib, only exists in patients with *EGFR* 19 del mutation. Further studies are required to elucidate why *TP53* mutations determined significantly poor prognoses in patients harboring *EGFR* 19 del but not in patients presenting *EGFR* L858R. The present study may provide novel insights into the identification of the most optimal treatment strategy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RG, LZ, YL and FX conceived and designed the study. YL, FX, YW, QW, BW, YY, YZ, JL, ZZ, XM and LZ collected the data. JY performed the statistical analysis of the data. YL, FX, YW, QW, BW, YY, YZ, HHZ, JY and LZ analyzed and interpreted the data. HHZ, RG, LZ, YL, FX and LZ wrote the manuscript. All authors approved the final version of the manuscript and are accountable for all aspects of the work.

Ethics approval and consent to participate

All procedures performed involving human subjects were in accordance with the ethical standards of the Medical Ethics Committee of Jiangsu Province Hospital (Nanjing, China). All patients provided written informed consent for participating in the study.

Patient consent for publication

Not applicable.

Competing interests

HHZ, JZ, LZ, ZZ and JL are employees of Burning Rock Biotech. The other authors declare that they have no competing interests.

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