Clinical Cancer Research

# Impact of *TP53* Mutations on Outcome in *EGFR*-Mutated Patients Treated with First-Line Tyrosine Kinase Inhibitors

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## Abstract

**Purpose:** To analyze the impact of *TP53* mutations on response to first-line tyrosine kinase inhibitors (TKI) in patients with *EGFR*-mutated non–small cell lung cancer (NSCLC).

**Experimental Design:** 136 *EGFR*-mutated NSCLC patients receiving first-line TKIs were analyzed. *TP53* mutations were evaluated in 123 patients in relation to disease control rate (DCR), objective response rate (ORR), progression-free survival (PFS), and overall survival (OS).

**Results:** *TP53* mutations were observed in 37 (30.1%), 10 (27.0%), 6 (16.2%), 9 (24.3%), and 12 (32.4%) patients in exons 5, 6, 7, and 8, respectively. DCR was 70% in *TP53*-mutated patients compared with 88% in *TP53*-wild type (wt) patients [relative risk, RR, of disease progression: 3.17 (95% CI, 1.21–8.48), $P = 0.019$]. In particular, a 42% DCR was observed in patients with *TP53* exon 8 mutation versus 87% in exon 8 wt patients [RR of disease progression 9.6 (2.71–36.63), $P < 0.001$]. Shorter median PFS and OS were observed in patients with *TP53* exon 8 mutations compared with others (4.2 vs. 12.5, $P = 0.058$, and 16.2 vs. 32.3, $P = 0.114$, respectively); these differences became significant in the subgroup with *EGFR* exon 19 deletion (4.2 vs. 16.8, $P < 0.001$, and 7.6 vs. not reached, $P = 0.006$, respectively), HR 6.99 (95% CI, 2.34–20.87, $P < 0.001$) and HR 4.75 (95% CI, 1.38–16.29, $P = 0.013$), respectively.

**Conclusions:** *TP53* mutations, especially exon 8 mutations, reduce responsiveness to TKIs and worsen prognosis in *EGFR*-mutated NSCLC patients, mainly those carrying exon 19 deletions. *Clin Cancer Res; 23(9); 2195–202. ©2016 AACR.*



## Introduction

Patients with non–small-cell lung cancer (NSCLC) carrying specific sensitizing mutations at exons 18, 19, and 21 of the *EGFR* gene are usually sensitive to treatments with tyrosine kinase inhibitors (TKI) such as gefitinib, erlotinib, or afatinib (1–3). However, not all patients respond equally to TKI treatments, despite the presence of an *EGFR* mutation, and approximately 20%–30% show primary resistance to TKIs (1, 4), inducing early progressive disease (PD) after the first month of treatment.

Although the mechanisms responsible for acquired resistance are well known (5), those responsible for primary resistance are not understood. Some studies have demonstrated that different *EGFR* mutations have different impacts on the outcome of TKI response (6–9). Moreover some primary resistance mechanisms have been hypothesized, such as *BIM* polymorphisms, *MET* amplification, *PIK3CA* mutations, and mutations of the PIK3CA/AKT/mTOR pathway (10–12).

Mutations of the tumor suppressor *TP53* gene occur in about 30%–40% of NSCLC patients and are highly correlated with smoking habits (13, 14). Exons 5 to 8 of the gene encoded for the DNA-binding domain and recognize a consensus sequence in the promoter of several genes involved in DNA repair, cell-cycle arrest, and apoptosis (15, 16). On the basis of the *TP53* mutation classification performed by Poeta and colleagues (17), a recent study (18) has demonstrated that nondisruptive *TP53* mutations are independent markers of shorter overall survival (OS) in patients with advanced NSCLC, regardless of *EGFR* and *KRAS* mutation status. However, no differences were observed in terms of response and progression-free survival (PFS) in patients treated with either erlotinib or chemotherapy.

There is preclinical evidence of a link between *TP53* mutations and responsiveness to TKIs, in particular, gefitinib (19–21). Specifically, it has been shown that p53 wt is needed for gefitinib-induced apoptosis in NSCLC cell lines and leads to increased sensitivity to TKIs through the induction of FAS

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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## Translational Relevance

*EGFR*-mutated patients are usually responsive to tyrosine kinase inhibitor (TKI) treatments. However, about 20%–30% of patients show primary resistance to TKIs, the underlying mechanisms of which are still unknown. In a case series of *EGFR*-mutated NSCLC patients treated with TKIs in a first-line setting, we found that patients with *TP53* mutations showed a worse prognosis than *TP53* wild-type patients. In particular, *TP53* exon 8 mutations were associated with a significantly lower disease control rate (DCR) and shorter progression-free survival (PFS). These results were particularly significant in the subgroup of patients with *EGFR* exon 19 deletion, in which the presence of *TP53* exon 8 mutation was associated with significantly lower DCR, and shorter PFS and overall survival. Once confirmed in an independent and larger case series, these results could have a decisive impact on the selection of *EGFR*-mutated NSCLC patients to be treated with first-line TKIs.

and caspase-dependent cell death. Conversely, mutated p53 reduces gefitinib-induced apoptosis (19). Moreover, our previous results obtained on a case series of *EGFR*-wt NSCLC patients treated with second- or further-line TKIs showed that *TP53* mutations were more frequent in nonresponders than in responders, in line with our hypothesis of their potential role as resistance biomarkers to TKIs (22).

In this study, we aimed to assess the role of *TP53* mutation in a cohort of *EGFR*-mutated advanced NSCLC patients that received TKI in first-line treatment. We analyzed and evaluated the status of *TP53* gene and the different types of mutation, in relation to the different *EGFR* mutations and outcomes of patients in terms of overall response rate (ORR), disease control rate (DCR), duration of therapy, PFS, and OS.

## Materials and Methods

We retrospectively analyzed a total of 136 patients with advanced *EGFR*-mutated NSCLC treated with a TKI (gefitinib, erlotinib, or afatinib) in the first-line setting from January 2012 to April 2015. Patients were treated at the Medical Oncology Units of the Romagna catchment area (Area Vasta Romagna, AVR) and the S. Maria della Misericordia Hospital of Perugia, Italy. Patient characteristics were obtained using medical and radiographic records and included age, gender, smoking history, histology, and information on death and response to treatment. *EGFR* status had been routinely determined at the Biosciences Laboratory of IRST-IRCCS and the Laboratory of Molecular Biology of the S. Maria della Misericordia Hospital, Perugia, by MassARRAY, pyrosequencing, or direct sequencing methodologies.

The study was approved by the AVR Institutional Review Board.

### *TP53* mutation analysis

Both cytologic and histologic specimens were used for DNA extraction and *TP53* mutation analysis. The same samples were used for the *EGFR* mutation analysis. All specimens were accurately selected by a dedicated pathologist from each center at the time of routine *EGFR* molecular analysis. Moreover, periodic quality controls were performed during the course of the study to verify concordance of molecular results. An area comprising at least 50% of tumor cells was scraped off the slides for DNA extraction. Cells were lysed in 50 mmol/L KCl, 10 mmol/L Tris-HCl pH 8.0, 2.5 mmol/L $MgCl_2$, and Tween-20 0.45%, with the addition of Proteinase K at a concentration of 1.25 mg/mL, overnight at 56°C. Proteinase K was inactivated at 95°C for 10 minutes, after which samples were centrifuged twice to eliminate debris. Supernatant was assessed for DNA quality and quantity by Nanodrop (Celbio) and then underwent PCR amplification.

Exon 5–8 of *TP53* gene were amplified by PCR and sequenced by Direct Sequencing using 3130 Genetic Analyzer (Applied Biosystems).

### Response evaluation

Best clinical response to treatment with TKI was classified on the basis of interval CT scans as complete response (CR), partial response (PR), stable disease (SD), or PD using standard Response Evaluation Criteria in Solid Tumors criteria version 1.1 (23). Patients with both baseline imaging and at least one repeated evaluation after continuous EGFR TKI monotherapy were evaluable for radiographic response. The same criteria for response evaluation and follow-up times were used by the centers taking part in the study.

### Statistical analysis

Descriptive statistics were reported as frequencies and percentages for categorical variables and as means ± SD or median and range, when appropriate, for continuous variables.

DCR was defined as the sum of complete response (CR), partial response (PR), and stable disease (SD), whereas ORR considered. Duration of therapy was calculated from the date of initiation of first-line treatment to the last documented visit date during *EGFR* TKI monotherapy or time of disease progression.

The association between mutational status and response was tested by the Pearson's $\chi^2$ test or Fisher exact test, when appropriate. Association between categorical and continuous variables was tested by the Wilcoxon–Mann–Whitney test or the Kruskal–Wallis test, when appropriate.

The association between ORR or DCR and mutational status was assessed by logistic regression models. Results from logistic regression are reported as relative risk (RR) and 95% confidence interval (CI) in square brackets.

Patients were arbitrarily divided into nonresponders (PD or CR/PR/SD <3 months), short-term responders (CR/PR/SD ≥3 months and ≤10 months), or long-term responders (CR/PR/SD > 10 months) to analyze the association between the mutations and the duration of response to TKIs.

The survival endpoints examined were PFS and OS. PFS was defined as the time from start of first-line treatment to disease progression or death, whichever occurred first. Patients who were alive and progression-free were censored at the date of the last follow-up update (September 30, 2015). OS was defined as the time from start of first-line treatment to death. Observation was censored at the date of the last follow-up update or at the date of disease progression if the patient was still alive. PFS and OS functions were estimated using the Kaplan–Meier method, and In the text were reported the median values and 95% CI in square brackets. The log-rank test was used to assess differences between molecularly defined groups. Cox proportional hazards regression models were used to evaluate the

association between type of mutation and survival endpoints. Results are reported as HR and 95% CI in square brackets. The proportional hazards assumption was evaluated by the Schoenfeld residuals.

A two-sided $P < 0.05$ was considered statistically significant. This was an exploratory study in which data were retrieved with a specific aim but not with a predefined key hypothesis. Any adjustment would have not resolved the problem of making valid statistical inferences as hypotheses would have been generated by the data.

All statistical analyses were performed using R version 3.2.3.

## Results

### Patient characteristics

Clinicopathologic characteristics of patients, type of *EGFR* mutation, TKIs received, and type of response to TKIs are reported in Table 1. The majority of patients were female (75.0%) and never smokers (59.0%). Almost all patients had a diagnosis of adenocarcinoma (98.5%). Six patients (4.4%) had an exon 18 point mutation (3 G719A, 2 G719S, 1 G719D), 74 patients (54.4%) showed an exon 19 deletion, and 56 (41.2%) had an exon 21 mutation (49 L858R and 7 L861Q). Among patients with exon 19 deletion, 2 had also an exon 20 T790M mutation. All patients received a TKI in the first-line treatment. In particular, 104 patients (76.5%) received gefitinib, 27 patients (19.8%) erlotinib, 3 patients (2.2%) afatinib, and 2 patients (1.5%) dacomitinib.

**Table 1.** Clinicopathologic characteristics of patients ($n = 136$)

| | *n* (%) |
|---|---|
| Gender | |
| Female | 102 (75.0) |
| Male | 34 (25.0) |
| Age at start of first-line of therapy, years | |
| Mean ± sd | 70.4 ± 10.7 |
| Smoking status | |
| Never smoker | 62 (59.0) |
| Former smoker | 28 (26.7) |
| Current smoker | 15 (14.3) |
| Missing | 31 |
| Histology | |
| ADC | 134 (98.5) |
| Poorly differentiated carcinoma | 2 (1.5) |
| EGFR Mutation | |
| Exon 18 point mutation | 6 (4.4) |
| Exon 19 deletion | 74[a] (54.4) |
| Exon 21 point mutation | 56 (41.2) |
| L858R | 49 (36.0) |
| L861Q | 7 (5.1) |
| Type of first-line therapy | |
| Gefitinib | 104 (76.5) |
| Erlotinib | 27 (19.8) |
| Afatinib | 3 (2.2) |
| Dacomitinib | 2 (1.5) |
| Therapy response | |
| CR | 4 (3.0) |
| PR | 71 (52.6) |
| SD | 37 (27.4) |
| PD | 23 (17.0) |
| Missing | 1 |
| *TP53* status | |
| Wt | 86 (69.9)[b] |
| Mutated | 37 (30.1)[b] |
| Exon 5 | 10 (27.0)[c] |
| Exon 6 | 6 (16.2)[c] |
| Exon 7 | 9 (24.3)[c] |
| Exon 8 | 12 (32.4)[c] |

Abbreviations: CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.
[a]Two patients also harbored an exon 20 T790M mutation.
[b]Percentage refers to the 123 patients in whom *TP53* analysis was performed.
[c]Percentage refers to the total number of *TP53*-mutated patients.

### Outcome of patients in relation to EGFR mutations

ORR and DCR for TKIs in the entire case series were 56.0 % and 83.6%, respectively (Table 2). Exon 19 deletion was found to be significantly associated with a higher ORR and DCR to TKIs with respect to other *EGFR* mutations. In particular, an ORR of 68.9% and a DCR of 91.9% were observed in patients with this alteration compared with 45.8% and 77.1%, respectively, in patients with exon 21 L858R mutation, and 16.7% and 58.3%, respectively, in the group with the other *EGFR* mutations (Table 2). Two patients with exon 19 deletion also had an exon 20 T790M mutation and both obtained a PR, one for 3 months and the other for 4 months.

Median treatment duration in patients with objective response (CR and PR) or disease control (CR, PR and SD) was 14.3 months (range: 2.0–57.5) and 11.7 months (range: 2.0–57.5), respectively. Table 2 summarizes the distribution of nonresponders, short-term responders, and long-term responders in relation to the different *EGFR* mutations. Among patients with exon 19 deletion, 52.7% were long-term responders compared with 31.2% and 41,7% of patients with exon 21 L858R mutation or those with the other *EGFR* mutations, respectively ($P = 0.008$).

Overall, median PFS was 11.6 months [7.9–14.6], (Fig. 1A). A significantly longer PFS was observed in patients with *EGFR* exon

**Table 2.** Response to TKIs in *EGFR*-mutated NSCLC patients

| Response | Overall ($n = 136$) | Exon 19 deletion ($n = 74$) | Exon 21 L858R ($n = 49$) | Exon 21 L861Q and exon 18 mutations ($n = 13$) | *P* |
|---|---|---|---|---|---|
| Best response | | | | | |
| CR | 4 | 3 | 1 | — | |
| PR | 71 | 48 | 21 | 2 | |
| SD | 37 | 17 | 15 | 5 | |
| PD | 22 | 6 | 11 | 5 | |
| Missing | 2 | — | 1 | 1 | |
| ORR | 56.0% | 68.9% | 45.8% | 16.7% | <0.001 |
| DCR | 83.6% | 91.9% | 77.1% | 58.3% | 0.001 |
| Duration of response | | | | | |
| Non responders or responders for <3 months | 25 | 7 (9.5%) | 13 (27.1%) | 5 (41.7%) | 0.008 |
| Short-term responders | 50 | 28 (37.8%) | 20 (41.7%) | 2 (16.6%) | |
| Long-term responders | 59 | 39 (52.7%) | 15 (31.2%) | 5 (41.7%) | |

Abbreviations: CR, complete response; DCR, disease control rate; ORR, objective response rate; PD, progressive disease; PR, partial response; SD, stable disease.

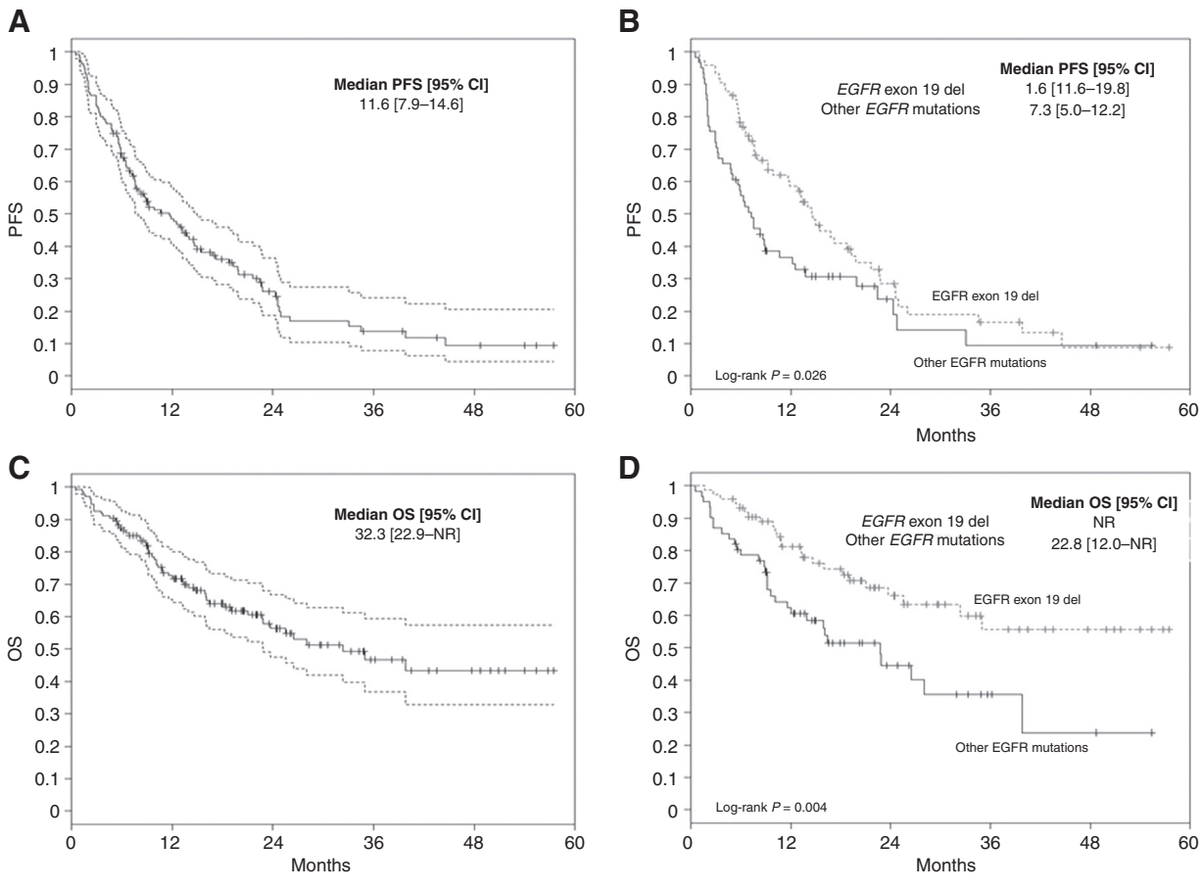


**Figure 1.**

Overall PFS (**A**), PFS of *EGFR* exon –9 deleted patients compared with other *EGFR*-mutated patients (**B**), overall OS (**C**), OS of *EGFR* exon 19–deleted patients compared with other *EGFR*-mutated patients (**D**). NR, not reached.

19 deletions with respect to patients with other types of mutation ($P = 0.026$; Fig. 1B).

Median OS was 32.3 months [22.9–not reached (NR); Fig. 1C]. A significantly longer OS was observed in patients with exon 19 deletions with respect to patients with other types of mutation ($P = 0.004$; Fig. 1D).

At univariate survival analysis from Cox model *EGFR* exon 19 deletion resulted a significant predictor of better PFS and OS [HR 0.64 [0.43–0.95], $P = 0.027$ and HR 0.47 [0.27–0.79], $P = 0.005$, respectively; results not shown]. Neither confounding nor modifying effect by age, gender, and smoking habits was observed.

### *TP53* mutation

Out of the 136 *EGFR*-mutated patients, 123 had biological material available for *TP53* mutation analysis. Overall, 37 (30.1%) patients showed a *TP53* mutation: 27.0% were on exon 5, 16.2% on exon 6, 24.3% on exon 7, and 32.4% on exon 8 (Table 1). Following a previous report's differentiation of *TP53* mutations into disruptive and nondisruptive (16), we found 13 disruptive and 24 nondisruptive mutations. Worthy of note is that all exon 6 mutations were disruptive, whereas all except one exon 8 mutations were nondisruptive (Supplementary Table S1).

Percentage of *TP53* mutation was higher in males than in females, with no statistically significant difference ($P = 0.07$). The difference was statistically significant when considering exon 5 mutations only: 18.2% of mutation was observed in males, whereas 4.4% in females ($P = 0.02$). No statistically significant association was observed between any type of *TP53* mutation and smoking habit and age (Supplementary Table S2).

Finally, percentage of nondisruptive mutations was higher in males (33.3%) than in females (14.5%; Supplementary Table S2).

### *TP53* status in relation to *EGFR* mutation

No statistically significant association was observed among *TP53* mutation (considering all types together) and different *EGFR* mutations (Supplementary Table S3). With regard to the distribution of *TP53* gene mutations, a significant association was observed for *TP53* exon 5 and exon 7 mutations with respect to *EGFR* mutations. In particular, *TP53* exon 5 mutations were more frequently seen in patients with *EGFR* exon 21 L861Q mutation, whereas *TP53* exon 7 mutations were more common in those with *EGFR* exon 19 deletion. Moreover, frequency of *TP53* nondisruptive mutations was lower in *EGFR* exon 19–deleted patients than in other *EGFR*-mutated patients, although this difference was not statistically significant. Finally,

**Table 3.** Disease control rate (DCR) in relation to the different types of *TP53* mutation

| | DCR, *n* (%) | | Unadjusted | |
|---|---|---|---|---|
| ***TP53* mutation** | **No ($n = 22$)** | **Yes ($n = 101$)** | **RR [95% CI]** | ***P*** |
| All mutations[a] | | | | |
| Wt | 10 (11.8) | 75 (88.2) | 1 | |
| Mut | 11 (29.7) | 26 (70.3) | 3.17 [1.21–8.48] | 0.019 |
| Exon 5[a] | | | | |
| Wt | 18 (16.1) | 94 (84.0) | 1 | |
| Mut | 3 (30.0) | 7 (70.0) | 2.24 [0.45–8.92] | 0.2745 |
| Exon 6[a] | | | | |
| Wt | 20 (17.2) | 96 (82.8) | 1 | |
| Mut | 1 (16.7) | 5 (83.3) | 0.96 [0.05–6.39] | 0.971 |
| Exon 7[a] | | | | |
| Wt | 21 (18.4) | 93 (81.6) | 1 | |
| Mut | — | 9 (100) | — | — |
| Exon 8[a] | | | | |
| Wt | 14 (12.7) | 96 (87.3) | 1 | |
| Mut | 7 (58.3) | 5 (41.7) | 9.6 [2.71–36.63] | <0.001 |
| Disruptive/nondisruptive[a] | | | | |
| Wt/disruptive | 13 (13.2) | 85 (86.7) | 1 | |
| Nondisruptive | 8 (33.3) | 16 (67.7) | 3.27 [1.14–9.12] | 0.024 |

[a]Sum does not add up to the total due to missing values.

all *TP53* mutations found in patients with *EGFR* exon 21 L861Q mutation were nondisruptive ($P = 0.02$; Supplementary Table S3).

### *TP53* mutation and response to TKIs

The presence of *TP53* mutation was significantly associated with a lower DCR in relation to TKI treatment (Table 3). In particular, patients with any of the *TP53* mutations had a 3-fold risk of disease progression than *TP53* wt patients ($P = 0.019$). Considering the different *TP53* mutations, *TP53* exon 8–mutated patients were associated with the worst response. In particular, 41.7% DCR was observed in *TP53* exon 8–mutated patients with respect to 87.3% DCR in *TP53* exon 8 wt patients ($P < 0.001$). RR of disease progression was almost 10-fold in exon 8–mutated patients than in wt patients, despite the poor precision of the estimated effect given by the limited number of patients carrying *TP53* exon 8 mutation, $P < 0.001$; Table 3).

Nondisruptive *TP53* mutation was also associated to a lower DCR. In particular, 86.7% DCR was observed in patients with *TP53* wt or *TP53*-disruptive mutations, with respect to 67.7% DCR observed in patients with nondisruptive mutations. These latter patients had almost a 4-fold risk of disease progression than wt patients (Table 3). Interestingly, 92% of *TP53* exon 8 mutations were nondisruptive.

Regarding the association between *TP53* mutations and response to TKIs according to the type of *EGFR* mutation, interesting results were observed in the subgroup of patients with exon 19 deletion. Among 74 *EGFR* exon 19–deleted patients, 66 carried information on the *TP53* mutational status. DCR was 98% in *EGFR* exon 19–deleted *TP53* wt patients, whereas it was 70% in patients carrying a *TP53* mutation (Supplementary Table S4).

No significant association was observed between *TP53* mutations and response in patients with other types of *EGFR* mutation (data not shown).

The difference in ORR and DCR was even more marked considering *TP53* exon 8 mutations (Supplementary Table S4).

Moreover, ORR and DCR were lower in *EGFR* exon 19–deleted patients with *TP53* nondisruptive mutations (37.5% and 75%) than in patients with *TP53*-disruptive mutations or wt gene (70.7% and 93.1%, respectively; $P = 0.079$ and $P = 0.151$, respectively).

With regard to clinicopathologic characteristics of patients and response, no significant associations were observed between gender, age, or smoking habits and DCR or ORR. Moreover, no significant differences in terms of response were observed between patients treated with either gefitinib or erlotinib.

The frequency of *TP53* exon 8 mutation was higher in non-responders and responders of <3 months (58%) than in short- or long-term responders (4% and 5%, respectively, $P = 0.004$).

### *TP53* mutation and survival

No statistically significant difference was observed in terms of PFS and OS between *TP53*-mutated and wt patients. However, shorter PFS and OS were observed in patients with *TP53* exon 8 mutations compared with all the other patients (Fig. 2A and B). No significant difference was observed in patients grouped by *TP53* mutation type: *TP53* exon 8 mutation versus *TP53* nondisruptive mutations (other than exon 8) versus *TP53* wt or *TP53*-disruptive mutations (Supplementary Fig. S1A and S1B).

In patients with *EGFR* exon 19 deletions, shorter, nonsignificant PFS and OS were observed in *TP53*-mutated patients with respect to *TP53* wt patients ($P = 0.081$ and $P = 0.317$, respectively; Table 4). *TP53* exon 8 mutations were significantly associated with shorter PFS and OS. In particular, patients with *TP53* exon 8 mutations showed a median PFS and OS of 4.2 and 7.6 months, respectively, with respect to a median PFS of 16.8 months ($P < 0.001$) and a median OS not reached ($P = 0.006$) in the other patient groups (Fig. 2C and D). Cox regression model showed that *TP53* exon 8 mutated patients had an almost 7-fold higher risk of disease progression or death than wt patients [HR 6.99 [2.34–20.87], $P = 0.006$] and death HR 4.75 [1.38–16.29], $P = 0.013$; Table 4]. The negative prognostic value of *TP53* exon 8 mutation was not seen in patients with other *EGFR* mutations.

Finally, in *EGFR* exon 19–deleted patients, we observed that exon 8–mutated patients had a poorer prognosis than *TP53* nondisruptive mutated (other than exon 8) patients and *TP53* wt/*TP53*-disruptive–mutated patients. In particular, *TP53* exon 8-disruptive–mutated patients had a higher risk of progression (HR 7.05 [2.35–21.17], $P < 0.001$) than *TP53* wt patients, whereas findings were not significant for patients with other nondisruptive *TP53* mutations (Supplementary Fig. S1C). Similar results were found for OS (Supplementary Fig. S1D).

These data remained significant after adjusting for age, gender, and smoking habits.

No statistically significant difference in PFS and OS was observed in relation to *TP53* mutations in patients treated with either gefitinib or erlotinib.

## Discussion

In the current study, we analyzed the impact of *TP53* mutations in relation to the outcome of advanced *EGFR*-mutated NSCLC patients treated with a TKI in the first-line setting. To the best of our knowledge, this was the first study to have analyzed the association between *TP53* mutations and response to TKI, in a relatively large case series of *EGFR*-mutated patients. We found that *TP53* mutation resulted as a primary resistance mechanism

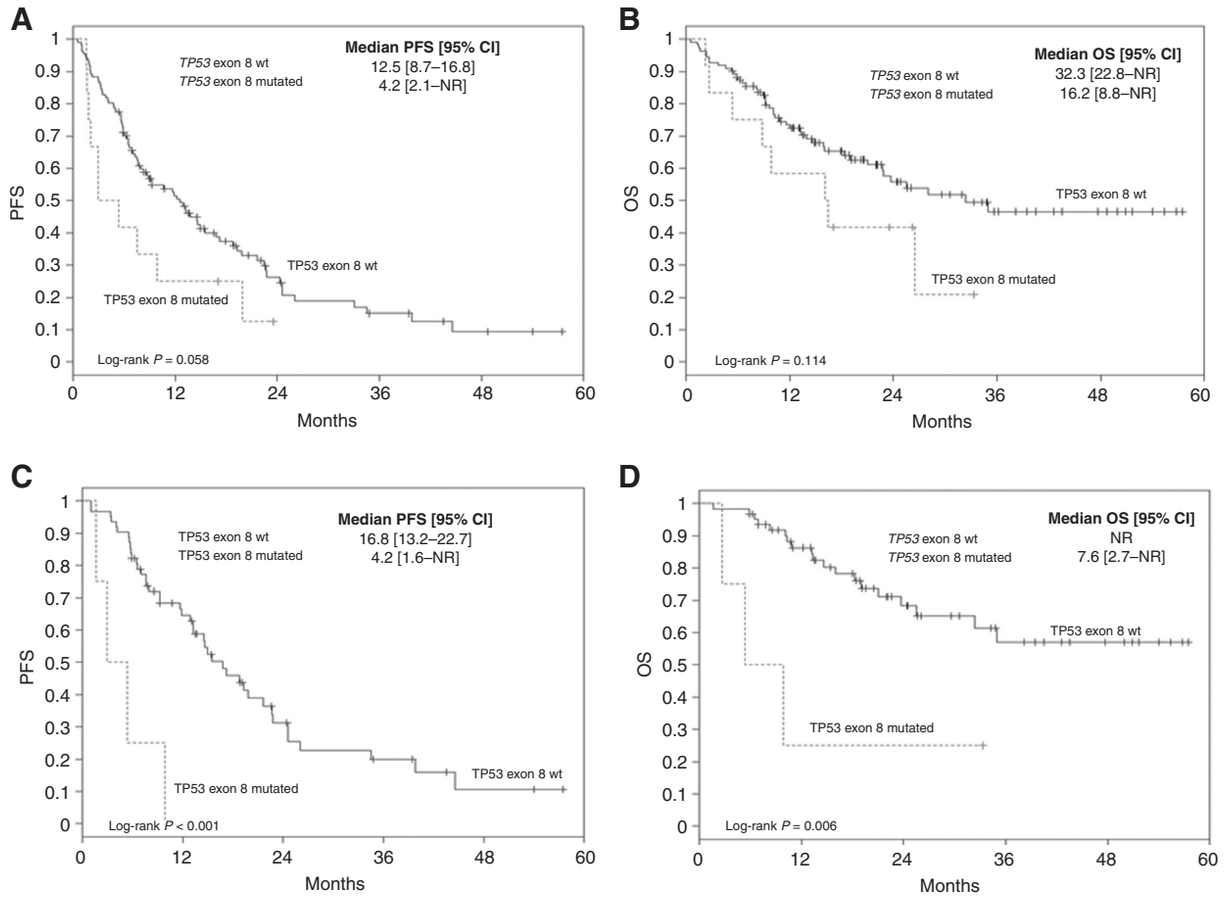


**Figure 2.**

PFS (**A**) and OS (**B**) of patients with *TP53* exon 8–mutated patients compared with *TP53* exon 8 wt patients; PFS (**C**) and OS of *TP53* exon 8–mutated patients compared with *TP53* exon 8 wt patients in the subgroup of *EGFR* exon 19–deleted patients (**D**). NR, not reached.

that significantly reduces response to TKI. In particular, *TP53* exon 8 mutations were associated with a significantly lower DCR with respect to *TP53* exon 8 wt. These data were even more significant for patients with *EGFR* exon 19 deletion, in which *TP53* exon 8 mutations were associated with a significantly lower DCR and shorter PFS and OS. Conversely, the predictive role of *TP53* mutation was not statistically significant in the subgroup of patients with other *EGFR* mutations.

*TP53* mutations occur in about 50% of lung cancer patients and are more frequent in squamous cell lung carcinoma than in lung ADC (13), with mutation rates ranging from 25% to 40% in the latter (24–26). We observed a mutation percentage of 30% in our case series of *EGFR*-mutated ADC patients, in agreement with another study in which *TP53* mutations analyzed separately in *EGFR* wt and *EGFR* mutated lung ADC patients showed mutation frequencies of 34.4% and 25.9%, respectively (18). The presence of a low number of smokers and high number of female among EGFR-mutated ADC patients contribute to a generally lower *TP53* mutation frequencies (25). The p53 protein regulates cellular response to a variety of cellular stress signals by inducing cell-cycle arrest, senescence, and/or apoptosis (27). Disruption of p53's normal function disrupts such cellular responses, leading to possible malignant cell transformation. Disruptive mutations lead to a complete, or almost complete, loss of p53 functions, whereas nondisruptive mutations can retain some of the p53 protein functional properties (17). Interestingly, there is some evidence that these maintained functional properties are associated with gain-of-function (GOF) activities (28–30).

A recent study has showed that nondisruptive *TP53* mutations are associated with a worse prognosis in advanced NSCLC patients, in both *EGFR* wt and mutated patients (18). Moreover, a recent study has demonstrated that, in a small case series of *EGFR*-mutated patients treated with TKI, *TP53* mutation was associated with a worse prognosis (31).

**Table 4.** Risk of PFS and OS in relation to *TP53* exon 8 mutation in the subgroup of patients with *EGFR* exon 19 deletion

| | PFS | | OS | |
|---|---|---|---|---|
| | **HR [95% CI]** | ***P*** | **HR [95% CI]** | ***P*** |
| *TP53* mutation | | | | |
| Wt | 1 | | 1 | |
| Mut | 1.74 [0.92–3.29] | 0.086 | 1.58 [0.64–3.87] | 0.321 |
| *TP53* exon 8 mutation | | | | |
| Wt | 1 | | 1 | |
| Mut | 6.99 [2.34–20.87] | 0.006 | 4.75 [1.38–16.29] | 0.013 |

Our case series included all *EGFR*-mutated patients treated with a TKI in the first-line setting. *TP53* mutations were associated with a significantly lower DCR respect to wt patients, and the most significant result was that *TP53* exon 8 mutation were associated with the worst prognosis. In particular, more than half *TP53* exon 8–mutated patients showed PD within two months of start of therapy, with no CR recorded Moreover, all *TP53* exon 8 mutations except one were nondisruptive.

We observed that the predictive role of *TP53* mutations was even more relevant in the subgroup of patients with exon 19 deletion, especially considering *TP53* exon 8 mutation. In particular, patients with *EGFR* exon 19 deletion harboring a *TP53* wt had an almost absolute (95%) DCR. On the other hand, patients with *TP53* exon 8 mutation had a DCR of 25%. Interestingly, the impact of *TP53* mutation was not statistically significant in patients with other types of *EGFR* mutations. We can hypothesize that, as exon 19–deleted patients are usually more responsive to TKIs (also confirmed by our results), the negative effect caused by the presence of *TP53* mutations is more evident and significant in this subgroup than in those with other *EGFR* mutations. Of note, 2 patients with *EGFR* exon 19 deletion (*TP53* wt) also had an *EGFR* exon 20 T790M mutation at baseline, and both showed a relatively short-term response to TKIs. This confirms that T790M, although rarely present at baseline, represents a marker of poor response to TKIs.

Results from some *in vitro* studies seem to endorse our hypothesis that *TP53* is an important factor in determining TKI sensitivity. In particular, p53 wt has been found to be an important factor for gefitinib-induced apoptosis in NSCLC cell lines, whereas p53 mutant reduces gefitinib-induced apoptosis (19). The presence of p53-responsive elements on FAS promoter region (20) and the evidence that gefitinib treatment induces an upregulation of FAS on lung cancer cells (21) further suggest a link between TKI response and *TP53* status. The correlation between *TP53* mutation and TKI resistance has also been demonstrated in other *in vitro* tumor cell models, in particular in urothelial carcinoma (32, 33).

A recent study showed that GOF *TP53* mutations are associated with a higher expression of *EGFR*, leading to hypothesize a direct role of p53 protein on *EGFR* promoter (34) and corroborating our hypothesis of a direct connection between *TP53* mutations and response to TKIs. Recent results presented at the 2015 ASCO Annual Meeting would also seem to confirm our observations (35, 36).

As our case series included all *EGFR*-mutated patients treated with TKI, we cannot exclude that *TP53* mutations could have a role as prognostic, rather than predictive, factor. Nevertheless, a confirmation by an independent study of the very low response rate to TKIs in *TP53* exon 8–mutated patients could clarify whether these patients can be referred to other treatment strategies. As conflicting data are present in the literature on the prognostic role of *TP53* mutation in NSCLC (14, 18, 24), additional studies should be performed for clarification.

In conclusion, our results highlighted for the first time the involvement of *TP53* mutation in determining primary resistance to TKI in *EGFR*-mutated NSCLC patients. *TP53* exon 8 mutations seemed to identify a subgroup of *EGFR*-mutated patients with unlikely response to TKIs. These data are particularly relevant in patients with *EGFR* exon 19 deletion. Further studies are needed to clarify the reason why *TP53* exon 8 mutations determined a poor prognosis in patients carrying *EGFR* exon 19 deletions. Once confirmed by an independent and larger case series, these results could have an important impact on the selection of *EGFR*-mutated NSCLC patients to be treated with TKI in the first-line setting.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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