Original Study

PTEN Loss as a Predictor of Tumor Heterogeneity and Poor Prognosis in Patients With *EGFR*-mutant Advanced Non–small-cell Lung Cancer Receiving Tyrosine Kinase Inhibitors

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Abstract

Rapid disease progression in epidermal growth factor receptor (*EGFR*) non–small-cell lung cancer treated with tyrosine kinase inhibitorss has been associated with concomitant mutations. The status of 4 genes (*PTEN*, *TP53*, *c-MET*, *IGFR*) was evaluated by immunohistochemistry in 51 tumor blocks, and it was correlated with overall response rate, overall survival, and progression-free survival. We point out that immunohistochemistry could be a valid tool to identify *PTEN* loss. Moreover, our results have shown worse outcomes in terms of progression-free survival, overall survival, and objective response rate in patients with concomitant *EGFR* mutation and *PTEN* loss. Furthermore, the coexistence of *PTEN* loss and *IGFR* overexpression identifies a potential prognostic 'signature' for a subgroup of patients with particularly poor prognosis.

Background: Rapid disease progression of patients with advanced epidermal growth factor receptor (*EGFR*)-mutant non–small-cell lung cancer (NSCLC) has been recently associated with tumor heterogeneity, which may be mirrored by coexisting concomitant alterations. The aim of this analysis was to investigate the correlation between loss of function of *PTEN* and the efficacy of tyrosine kinase inhibitors in this population. **Materials and Methods:** Archival tumor blocks from patients with *EGFR*-mutant NSCLC who were administered upfront tyrosine kinase inhibitors were retrospectively collected. The status of 4 genes (*PTEN*, *TP53*, *c-MET*, *IGFR*) was evaluated by immunohistochemistry, and it was correlated with overall response rate, overall survival (OS), and progression-free survival (PFS). **Results:** Fifty-one patients were included. In multivariate analysis, *PTEN* loss (hazard ratio [HR], 3.46; 95% confidence interval [CI], 1.56-7.66; $P = .002$), *IGFR* overexpression (HR, 2.22; 95% CI, 1.03-4.77; $P = .04$), liver metastases (HR, 3.55; 95% CI, 1.46-8.65; $P = .005$), and Eastern Cooperative Oncology Group performance status (ECOG PS) ≥ 1 (HR, 2.57; 95% CI, 1.04-6.34; $P = .04$) were significantly associated with shorter PFS. Patients with *PTEN* loss had a median PFS of 6 months (2-year PFS, 11.6%), whereas patients without *PTEN* loss had a median PFS of 18 months (2-year PFS, 43.6%) (log-rank $P < .005$). In the multivariate analysis, *PTEN* loss (HR, 5.92; 95% CI, 2.37-14.81; $P < .005$), liver metastases (HR, 2.63; 95% CI, 1.06-6.51; $P = .037$), and ECOG PS ≥ 1 (HR, 2.80; 95% CI, 1.15-6.81; $P = .024$) were significantly associated with shorter OS. Patients with *PTEN* loss had a median OS of 6 months (2-year OS, 12.2%), whereas in patients without *PTEN* loss, OS was not reached (2-year OS, 63.9%) (log-rank $P < .0005$).

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Conclusions: A low-cost and reproducible immunohistochemistry assay for *PTEN* loss analysis represents a potential tool for identifying tumor heterogeneity in patients with advanced *EGFR*-mutant NSCLC.

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Introduction

Treatment of advanced non–small-cell lung cancer (NSCLC) has historically consisted of systemic cytotoxic chemotherapy. However, randomized clinical trials conducted in patients carrying activating mutations of the epidermal growth factor receptor (*EGFR*) gene have shown that tyrosine-kinase inhibitors (TKIs) significantly improve prognosis and quality of life.[1,2] In such peculiar tumors, EGFR acts as an oncogenic driver, thus forcing cancer cells to depend (almost) exclusively on this genomic abnormality (ie, the phenomenon of oncogene addiction). Once constitutively activated, EGFR undergoes auto-phosphorylation of tyrosine residues in its intracellular domain, recruits different adaptors and signal-transducers, and activates downstream pathways that promote cell proliferation.[3] This molecular background represents the ideal genomic vulnerability to be specifically targeted by TKIs. *EGFR* mutations (exons 18-21) occur in approximately 10% to 15% of lung adenocarcinoma, more frequently in nonsmokers and female patients, although young patients with squamous histology are currently recommended to be screened for such alterations as well.[4] In addition to the common (sensitizing) mutations (ie, deletion exon 19 and point L858R exon 21), other uncommon alterations in exons 18 to 21 have been reported with variable sensitivity to EGFR TKIs, including the insertion in exon 20 that is currently considered as a resistance mutation in which TKIs are not effective.[5] Oncogene-addicted disease is generally sensitive to targeted treatment. The expected median progression-free survival (PFS) with first- and second-generation agents ranges from 8.4 to 14.7 months. In the second line, patients with a T790M mutant achieve a median PFS and overall survival (OS) of 10.1 and 26.8 months, respectively.[6-10] Nevertheless, a proportion of patients do not respond to treatment ('primary resistance') and, among responders, progression inevitably occurs because of the development of 'acquired resistance' mechanisms, requiring a shift to second-line options.[11] Although many mechanisms are involved for acquired resistance (and many are currently unknown), the most frequent include the appearance of resistance mutations (ie, *EGFR T790M*, *MET*, *PIK3CA*, *HER2*, *BRAF*, *RET*, *KRAS*) or the epithelial-mesenchymal transition.[12,13] In some cases (almost 25%), progression rapidly occurs during first-line treatment ('primary resistance').[14] Although the mechanisms of acquired resistance to first- and second-generation TKIs have been more extensively investigated, and in some cases (ie, *EGFR T790M*), acquired abnormalities represent additional treatment opportunities (ie, *EGFR T790M* for osimertinib), those factors that determine or influence the primary resistance to TKIs are less recognized to date. Because osimertinib has become the first-line treatment for patients with *EGFR*-mutant NSCLC,[15] several efforts have been made to identify mechanisms of primary and acquired resistance to this drug. The most common acquired resistance mechanisms detected are *MET* amplification and *EGFR C797S* mutation (about 15% each); other mechanisms included human epidermal growth factor receptor 2 (*HER2*) amplification, *PIK3CA*, *KRAS*, *NRTK*, *RET*, and *FGFR3* mutations (2%-7%).[16,17] Furthermore, Bcl-2-like protein 11 (BIM) upregulation is a pro-apoptotic molecule that belongs to the Bcl-2 family; low BIM protein level (owing to deletion polymorphism occurring in about 20% of the Asiatic population) is associated with resistance to first-, second-, and third-generation EGFR-TKIs.[18] After the initial consideration that driver mutations 'mutually exclude' others, a series of large-scale genome analyses have shown that other genetic alterations might commonly co-occur in *EGFR*-mutated lung adenocarcinoma and that they function as co-drivers, contributing to tumor progression and drug resistance.[19] The occurrence of additional mutations with proliferative effects (for example, the coexistence of *EGFR* mutations with mutations of onco-suppressor genes) in the tumor may mirror the tumor heterogeneity, whereas different subclones of cancer cells co-exist, and they may grow or not according to the specific (drug) selective pressure to which they are exposed. PTEN acts as a tumor suppressor and metabolizes PIP_3, the lipid product of PI3-Kinase, directly opposing the activation of the oncogenic PI3K/AKT/mTOR signaling. Loss of *PTEN* results in the lack of regulation of PIP3 levels, which in turn promote the PI3K/Akt pathway, leading to cellular proliferation and growth.[20] PTEN is commonly downregulated in many types of solid tumors, including NSCLC, and several studies have shown that *PTEN* loss is associated with poorer prognosis in patients with lung cancer.[21] *PTEN* loss can coexist in *EGFR*-mutated NSCLC and can negatively affect the prognosis of these patients. Given that the PI3K pathway is downstream of the EGFR-signaling pathway, it is likely that *PTEN* inactivation plays an important role in progression and/or therapeutic resistance in patients treated with EGFR TKIs.[22] The *TP53* gene provides instructions for making a protein called tumor protein p53 (or p53). This protein acts as a tumor suppressor, and it is called 'guardian of the genome' for its ability to regulate cell replication and proliferation.[23] The tumor suppressor gene *TP53* is frequently mutated in human cancers, and this alteration has been found in nearly one-half of all patients with lung cancer.[24] Without functioning p53, cell proliferation is not effectively regulated, and DNA damage can accumulate in cells. When *p53* mutation occurs in oncogene-addicted disease, the course is more aggressive, and the prognosis is poorer.[25-29] c-MET acts as an oncogene that binds hepatocyte growth factor. As a result, MET stimulates downstream signaling pathways, such as the extracellular signal-regulated kinase/mitogen-activated protein kinase and PI3K pathways. These pathways are known to involve cell growth, migration, angiogenesis, and survival. *MET* amplification or splice mutations can lead to EGFR TKI

resistance in *EGFR*-mutated NSCLC.[30,31] MET alterations are also considered as potential predictive biomarker, druggable with sundry compounds. In the PROFILE 1001 trial, a MET exon 14 skipping (METex14) cohort reported an overall response rate (ORR) of 44%, and a global retrospective series demonstrated a PFS of 7 months, both with crizotinib.[32] More recently, based on the Geometry-1 mono trial, the United States Food and Drug Administration approved capmatinib for patients with METex14 advanced NSCLC.[33] Insulin-like growth factor receptor 1 (IGFR-1) is a transmembrane protein located on chromosome 15q25−q26. It is implicated in promoting oncogenic transformation, growth, and survival of cancer cells. IGFR-1 high expression leads to activation of Ras, Raf and PI3K/Akt pathways. Previous studies have demonstrated that IGFR-1 mediates resistance to anti-EGFR therapy.[31] From what is discussed above, it is increasingly established that *EGFR*-mutated NSCLC treated with TKIs may become TKI-resistant by selecting pre-existing clones carrying resistance mutations or possessing the ability to depend on alternative oncogenic pathways for growth and survival, even from the beginning of the treatment.[34]

With these perspectives, and with the aim to generate the hypothesis of a prognostic effect of tumor heterogeneity mirrored by co-occurring mutations, we analyzed the immunohistochemical baseline expression of a series of key concomitant mutations (*PTEN*, *TP53*, *c-MET*, and *IGFR*) in the tumor cells of *EGFR*-mutated patients treated with TKIs.

Materials and Methods

Patients

Patients with the following characteristics were considered eligible for the current analysis: (1) patients carrying a known sensitizing mutation of the *EGFR* gene (ie, exon 19 deletion or L858R exon 21-point mutation); (2) patients receiving upfront line TKIs (afatinib, gefitinib, or erlotinib) referred to the Medical Oncology of the Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma (Italy) from 2015 to 2017; (3) patients with available tissue (formalin-fixed and paraffin-embedded [FFPE]) as tumor excisional/trans-bronchial biopsy (core biopsy was performed for 80% of patients) at the Department of Pathology and Diagnostics of the Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma (Italy); (4) clinical and pathologic annotations available from clinical charts and pathology reports; and (5) at least 12 months of follow-up (for endpoint maturity).

DNA Extraction and EGFR Mutational Analysis

All samples were processed at the Molecular Pathology Unit of the Department of Pathology and Diagnostics of the Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma (Italy). DNA was extracted from three 10-microm slides from paraffin-embedded tissues using QIAamp DNA FFPE Tissue Kit (QIAGEN, Milan, Italy), following the manufacturer's protocol. To minimize contamination by normal cells, the tumor areas dissected for DNA extraction contained at least 80% of tumor cells and at least 250 to 300 neoplastic viable cells. Before EGFR mutational analysis, extracted DNA concentration and purity were determined by QIAxpert. EGFR mutational analysis was carried out using a Therascreen EGFR RGQ PCR Kit (Qiagen) in a real-time Rotor-Gene Q (Qiagen). DNA samples were first assessed for total amplifiable DNA using the control mix, and if this was adequate, samples were then tested for the detection of *EGFR* mutations. Each test was run with an internal control, a positive control, and a no-template control, all included in the kit.

Immunohistochemical Assay for TP53, PTEN, IGFR-1, and c-MET and Staining Evaluation Score

FFPE sections (4 microns thick) were mounted on positive charged glass slides. For antigen retrieval to detect TP53, PTEN, IGFR-1, and c-MET protein, deparaffinized and rehydrated sections were boiled in citric acid solution (pH 6) for 20 minutes. The slides were cooled, and endogenous peroxidase was blocked with peroxidase block buffer (citric acid 0.04 M, Na2HPO4x2H2O 0.12 M, NaN3 0.03 M, and H2O2 at 1.5% v/v) for 15 minutes at room temperature. Then, the sections were incubated for 1 to 3 hours at room temperature with the following antibodies: p53 (clone Bp-53-11, Ventana-Roche, Milan, Italy), for PTEN (clone sp218, Ventana-Roche), for c-MET (clone C28, Santa Cruz Biotechnology, Milan, Italy), and for IGF1R (R&D Systems, Milan, Italy), following the manufacturers' protocol. The primary antibodies were visualized using the avidin-biotin-peroxidase complex method (UltraTek HRP Anti-polyvalent, ScyTek, Logan, UT) according to the instruction manual. 3,3' diaminobenzidine (DAB) was used as the enzyme substrate to observe the specific antibody localization, and Mayer hematoxylin was used as a nuclear counterstain. The staining intensity of tissue slides was evaluated independently by 2 observers (M.B. and M.M.) who were blinded to the patients' characteristics and survival. Cases with disagreement were discussed using a multiheaded microscope until agreement was achieved. The agreement indices (Cohen's K) between the 2 pathologists were very good: $k = 0.88$, $k = 0.81$, $k = 0.85$, and $k = 0.86$ for TP53, PTEN, c-MET, and IGFR-1 expression, respectively. To assess differences in staining intensity, an immunoreactivity scoring system was applied. c-MET and IGFR-1 expression in each specimen was scored according to the extent (percent of stained cells) and intensity of nuclear expression staining. IHC score for C-MET was defined as follows: 0, absence of staining or any intensity staining in less than 50% of tumor cells; 1, weak to moderate intensity staining in more than 50% of tumor cells; 2, moderate to strong intensity staining in more than 50% of tumor cells; and 3, strong intensity staining in more than 50% of tumor cells.[35] An IHC score of 2 or 3 was defined as positivity. IHC for IGFR-1 was considered as positive only when a distinct cell membrane staining was evident. The analysis was performed using a semiquantitative grading system based on 4 stages: 0, no staining; 1, staining in 1% to 10% of considered cells; 2, staining in 11% to 25% of considered cells; and 3, staining in more than 25% of considered cells. A cutoff value of 10% positive cells was used in order to avoid inclusion of scattered positivity of the same intensity found in normal lung tissue.[36]

Programmed Death-Ligand 1 (PD-L1) Assessment and Staining

PD-L1 expression was assessed during screening at our molecular pathology laboratory by means of the PD-L1 immunohistochemical 22C3 pharmDx assay (Dako, REF: SK006) in formalin-fixed tumor samples obtained by core needle or excisional biopsy or from tissue

resected at the time that metastatic disease was diagnosed. Expression was categorized according to the tumor proportion score (ie, the percentage of tumor cells with membranous PD-L1 staining).[37]

Study Design, Aim, and Endpoints

The aim of this single-center, retrospective study was to investigate the correlation between the expression abnormalities of PTEN, TP53, IGFR, and c-MET (potentially mirroring tumor heterogeneity) and the efficacy and activity of EGFR TKIs in patients affected by *EGFR*-mutant advanced NSCLC. The primary endpoint was PFS, defined as the time from treatment initiation and disease progression or death from any cause; secondary endpoints were OS, defined as time from treatment initiation and death from any cause, and ORR, according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 criteria. Tumor assessment was performed with computed tomography scan approximately every 3 months as per routine local clinical practice. The study was approved by the local Ethics Committee (Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma, Italy: Prot. n. 0010700/20, March 9th, 2020).

Statistics

Descriptive statistics for clinical and pathologic characteristics was used. The multivariate analysis for PFS and OS was performed using a Cox regression model. The *P*-values were 2-sided and considered statistically significant when less than .05. Estimates of survival times for PFS and OS were calculated according to the Kaplan-Meier method and compared with log-rank test. With regard to ORR, the association of clinicopathologic characteristics with ORR was evaluated by the Fisher exact test or the χ^2 test, as appropriate. Relative ORR frequencies and 95% confidence intervals (CIs) according to the expression abnormalities of PTEN, TP53, IGFR, and c-MET were derived. Potential expression signatures combining multiple abnormalities were explored. The odds ratio (OR) to derive the risk of ORR (with 95% CIs) according to each one of the expression abnormalities (*PTEN*, *TP53*, *IGFR*, and *c-MET*) was derived. Data were analyzed using licensed SPSS (version 21.0) and MedCalc (version 9.4.2.0).

Results

Patient Characteristics

Fifty-one patients were included in the study. Patient characteristics are reported in Table 1. The median age was 65 years (range, 40-86 years). Twenty-seven (52.9%) and 23 (45.0%) patients showed loss (intended as protein low expression) of p53 and PTEN, respectively; c-MET was overexpressed in 32 (63.0%) patients, and IGFR-1 was overexpressed in 22 (43.1%) patients. Eastern Cooperative Oncology Group performance status (ECOG PS) was 0 in 14 (27%) patients and $\geq$ 1 in 34 (67%) patients. With a median follow-up of 15 months (range, 1-64 months), 38 (74.8%) events of progression were recorded.

Efficacy and Activity

In the multivariate analysis for PFS, the presence of liver metastases, $\geq$ 2 metastatic sites, and ECOG PS $\geq$ 1 were found to be independent predictors of shorter PFS (Table 2). Kaplan-Meier curves of independent predictors of PFS are shown in

Table 1 Demographics and Clinical, Pathologic, and Molecular Characteristics of the 51 Patients

Patient Characteristics	n (%)
Gender	
Male	24 (47.1)
Female	27 (52.9)
Smoking status	
Current	6 (11.8)
Former	18 (35.3)
Never	24 (47.1)
ECOG PS	
0	14 (27.5)
1-2	33 (64.7)
Other	4 (7.9)
Histology	
Adenocarcinoma	47 (92.2)
Squamous	2 (3.9)
Other	2 (3.9)
Stage	
IIIB	4 (7.8)
IV	47 (92.2)
No. metastatic sites	
≤2	24 (47.1)
>2	27 (52.9)
Liver metastases	
Yes	10 (19.6)
No	41 (80.4)
EGFR mutation	
Exon 19 deletion	37 (72.5)
L858R exon 21 point mutation	14 (27.5)
Type of treatment	
Erlotinib	2 (3.9)
Gefitinib	36 (70.6)
Afatinib	13 (25.5)
PTEN IHC	
Normal expression	28 (54.9)
Loss of expression	23 (45.1)
TP53 IHC	
Normal expression	24 (47.1)
Loss of expression	27 (52.9)
IGFR IHC	
Normal expression	29 (56.9)
High expression	22 (43.1)
MET IHC	
Normal expression	19 (37.3)
High expression	32 (62.7)
PD-L1 IHC	
<50%	15 (29.4)
>50%	2 (3.9)
Unknown	34 (66.7)

Abbreviations: ECOG PS = Eastern Cooperative Oncology Group Performance status; IHC = immunohistochemistry; PD-L1 = programmed death-ligand 1.

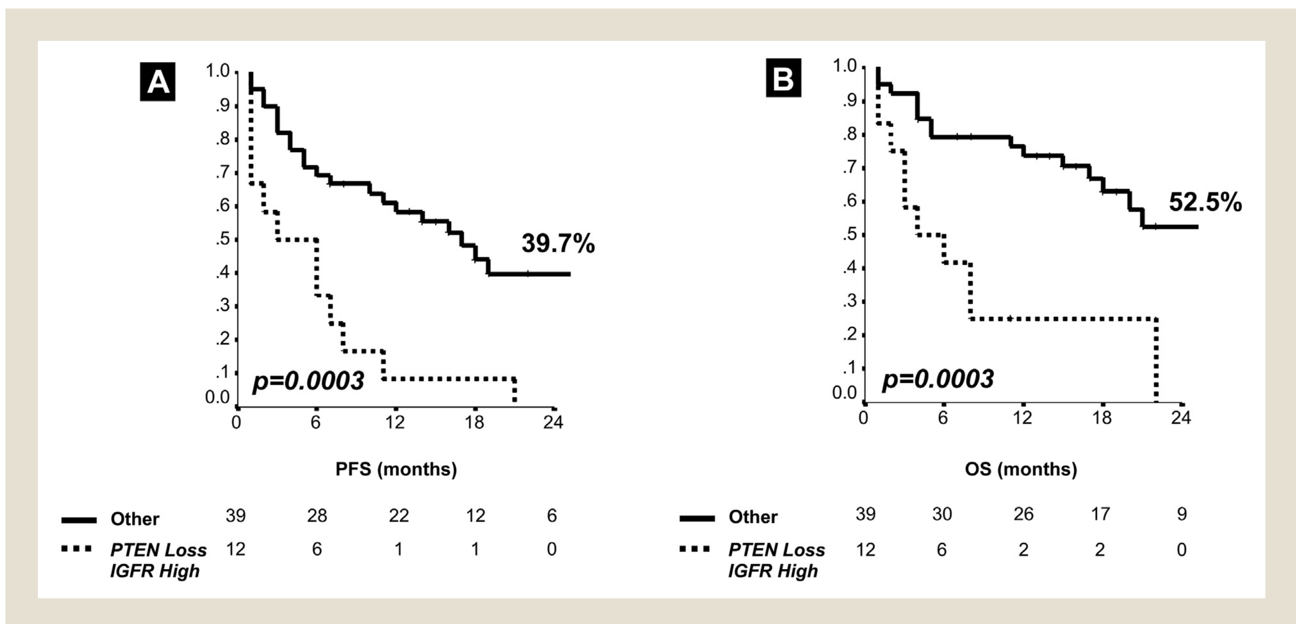
Table 2 Univariate and Multivariate Analysis for Progression-free Survival

Characteristics	Univariate		Multivariate	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Smoking status	1.62 (0.82-3.22)	.16	—	—
ECOG PS	2.67 (1.17-6.08)	.02	2.56 (1.04-6.33)	.04
Stage	1.88 (0.45-7.9)	.38	—	—
No. metastatic sites	2.05 (1.02-4.11)	.04	1.82 (1.00-3.33)	.50
Liver metastases	2.13 (0.99-4.6)	.05	3.55 (1.46-8.65)	.005
Gender	1.03 (0.53-1.98)	.93	—	—
PD-L1	0.99 (0.95-1.01)	.40	—	—
TP53	1.25 (0.65-2.37)	.50	—	—
PTEN	2.54 (1.31-4.89)	.05	3.46 (1.56-7.76)	.002
MET	1.21 (0.59-2.45)	.60	—	—
IGFR	2.29 (1.18-4.44)	.01	2.22 (1.03-4.77)	.041

Abbreviations: CI = confidence intervals; ECOG PS = Eastern Cooperative Oncology Group performance status; HR = hazard ratio; IHC = immunohistochemistry.

Figure 1A-D. The median PFS was 3 months in patients with liver metastasis (24-month PFS, 13.3%), compared with 12 months in patients without (24-month PFS, 31.5%; log-rank test $P = .01$). The median PFS was 2 months in patients with ECOG PS ≥ 1 (24-month PFS, 11.1%) in comparison with 10 months in patients with ECOG PS = 0 (24-month PFS, 25.4%; log-rank test $P = .01$). Patients with ≥ 2 metastatic sites had shorter PFS compared with patients with < 2 metastatic sites (HR, 2.06; 95% CI, 1.03-4.12; $P = .042$). The presence of *PTEN* low expression ('loss') and *IGFR* overexpression were found to be independent predictors of worse PFS. The median PFS was 6 months in patients with *PTEN* loss (24-month PFS, 11.6%) in comparison with 18 months in patients without (24-month PFS, 43.6%; log-rank test $P = .003$). The median PFS was 6 months in patients with *IGFR* overexpression (24-month PFS, 13.6%) in comparison with 18 months in patients without (24-month PFS, 41.4%; log-rank test $P = .01$). No significant differences for PFS were found according to PD-L1 expression (HR, 0.99; 95% CI, 0.95-1.02; $P = .402$). In the multivariate analysis for OS, the presence of liver metastasis and ECOG PS ≥ 1 were independent predictors of shorter OS (Table 3). Kaplan-Meier curves of independent predictors of OS are shown in Figure 1E-G. The median OS was 5 months in patients with liver metastasis (24-month OS, 13.3%) in comparison with 22 months in patients without (24-month OS, 44.6%; log-rank test

Figure 1 Progression-free Survival (A) and Overall Survival (B) according to the *PTEN/IGFR* Signature at Immunohistochemistry; *P*-Value: Log-Rank Analysis. Survival Rates (%) at 2 years are Reported



Abbreviations: OS = overall survival; PFS = progression-free survival.

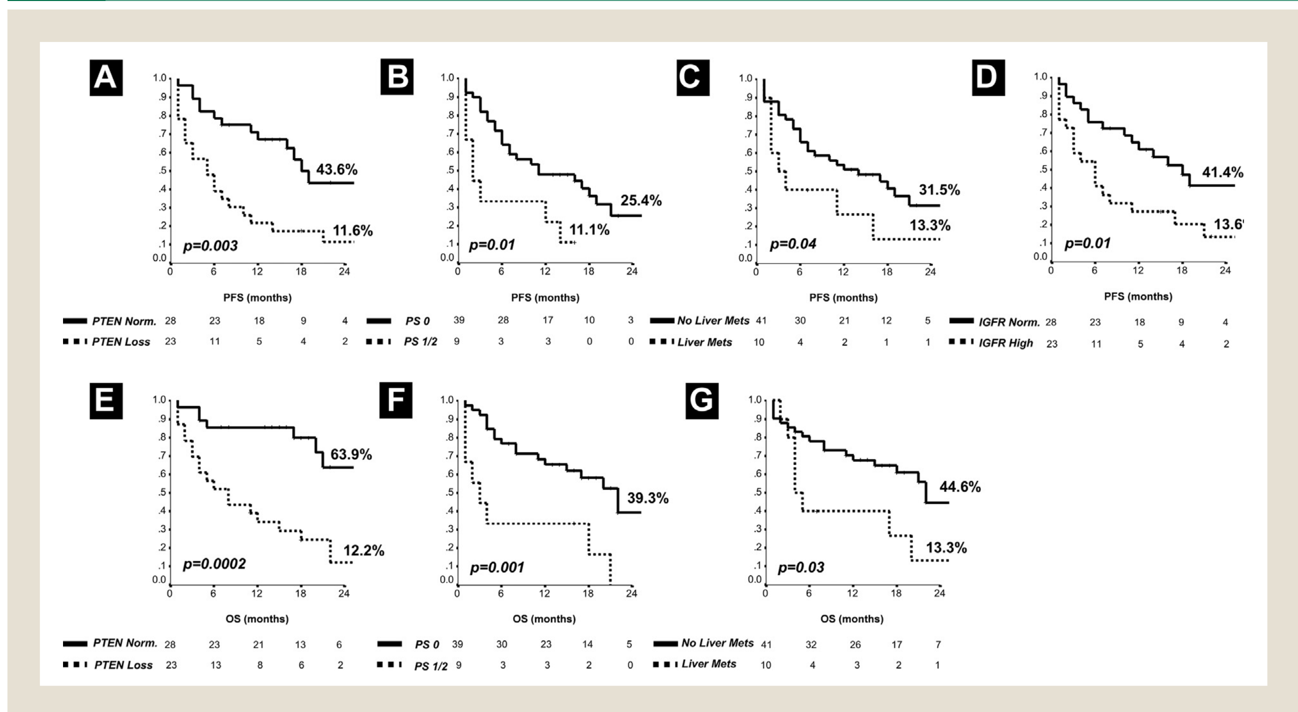
Table 3 Univariate and Multivariate Analysis for Overall Survival

Characteristics	Univariate		Multivariate	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Smoking status	1.02 (0.48-2.18)	.96	—	—
ECOG PS	3.71 (1.58-8.75)	.003	2.79 (1.15-6.81)	.024
Stage	2.29 (0.31-16.94)	.42	—	—
No. metastatic sites	1.93 (0.88-4.21)	.09	—	—
Liver metastases	2.47 (1.07-5.72)	.03	2.63 (1.06-6.51)	.037
Gender	1.14 (0.55-2.37)	.73	—	—
PD-L1	0.99 (0.95-1.02)	.43	—	—
TP53	1.50 (0.72-3.13)	.27	—	—
PTEN	3.92 (1.78-8.64)	< .005	5.92 (2.37-14.81)	<.005
MET	1.11 (0.51-2.40)	.80	—	—
IGFR	2.22 (1.03-4.77)	.04	—	—

Abbreviations: CI = confidence intervals; ECOG PS = Eastern Cooperative Oncology Group performance status; HR = hazard ratio; IHC = immunohistochemistry.

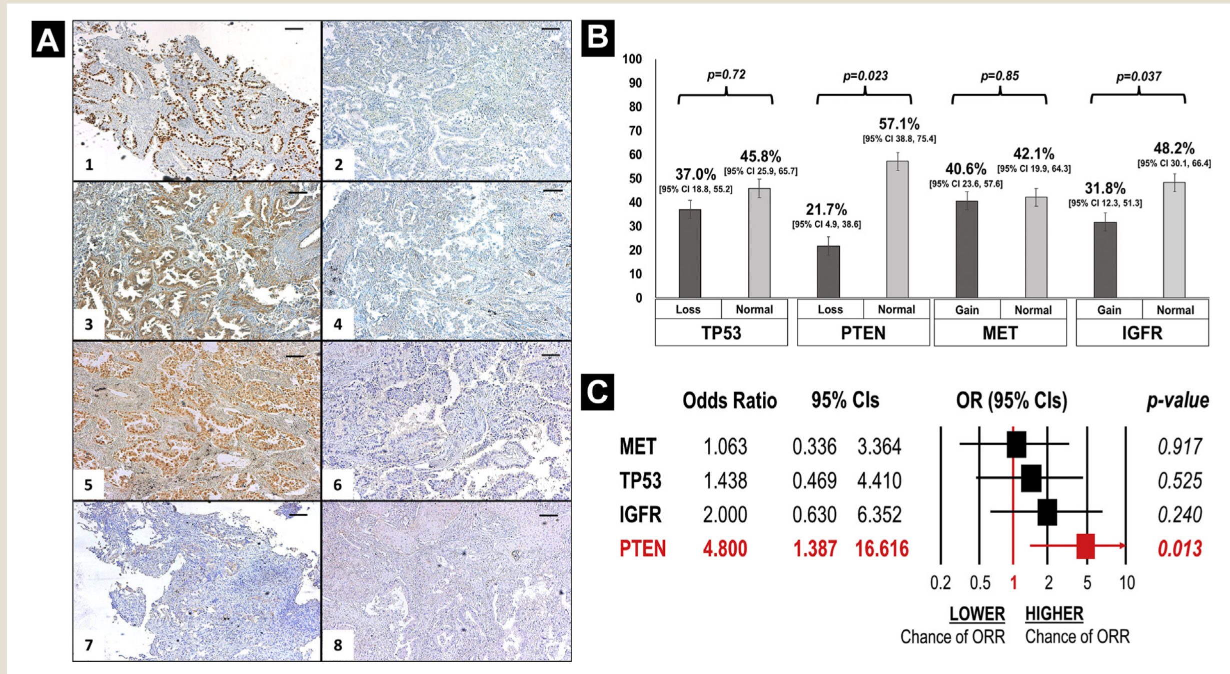
$P = .03$). The median OS was 3 months in patients with ECOG PS ≥ 1 (24-month OS, 0%) in comparison with 21 months in patients with ECOG PS = 0 (24-month OS, 39%; log-rank $P = .001$). The presence of *PTEN* loss was found to be an independent predictor of shorter OS. The median OS was 6 months in patients with *PTEN* loss (24-month OS, 12.2%) versus not yet reached in patients without *PTEN* loss (24-month OS, 63.9%; log-rank test $P = .0002$). PD-L1 expression does not significantly affect OS (HR, 0.99; 95% CI, 0.95-1.02; $P = .427$). As an exploratory finding, to maximize the identification of patients with very good prognosis, a molecular signature combining *PTEN* loss and *IGFR* overexpression (both independent predictors of poor PFS at the multivariate analysis) was considered for the survival analysis. Figure 2 shows PFS and OS data of patients with both *PTEN* loss and *IGFR* overexpression versus all other patients. The 12 patients harboring the molecular signature had a significantly lower PFS ($P = .0003$) and OS ($P = .0003$) in comparison with the other 39, with none of them surviving at 24 months. With regard to the activity, Figure 3 reports the observed ORR according to *p53* normal (Figure 3A-1) versus loss (Figure 3A-2), *PTEN* normal (Figure 3B-1) versus loss (Figure 3B-2), *MET* high (Figure 3C-1) versus normal (Figure 3C-2), and *IGFR* high (Figure 3D-1) versus normal (Figure 3D-2). As

Figure 2 Progression-free Survival (A-D) and Overall Survival (E-G) according to Independent Predictors at Multivariate Analyses; *P*-Value: Log-Rank Analysis. Survival Rates (%) at 2 years are Reported



Abbreviations: Mets = metastasis; Norm = normal; OS = overall survival; PFS = progression-free survival; PS = Eastern Cooperative Oncology Group performance status.

Figure 3 A, Immunohistochemistry Staining of TP53 (1: Loss; 2: Normal), PTEN (3: Loss; 4: Normal), MET (5: High; 6: Normal), and IGFR (7: High; 8: Normal) Status. B, Overall Response Rate (RECIST 1.1 Criteria) according to TP53, PTEN, MET, and IGFR Status (P-Value: χ^2 Test). C, Chance to Achieve a Response, according to TP53 (Normal vs. Loss), PTEN (Normal vs. Loss), MET (Normal vs. High), and IGFR (Normal vs. High) Status (P-Value: χ^2 Test). An Odds Ratio Higher than 1 Indicates a Higher Chance to Achieve Response



Abbreviations: CI = confidence interval; OR = odds ratio; ORR = overall response rate.

shown in Figure 2B, a significant difference ($P = .023$) in terms of ORR was found for patients without the *PTEN* loss (with an overall difference in response higher than 35%) and for patients overexpressing *IGFR* (with an overall difference in response higher than 16%; $P = .037$). At the univariate analysis for ORR, patients without a *PTEN* loss had a significantly higher change of ORR than patients with (Figure 3 [panel C]), with an OR of 4.8 (95% CI, 1.38-16.6; $P = .013$). None of the other analyzed genes (ie, *p53*, *IGFR*, and *MET*) or clinical and pathologic characteristics (PD-L1 expression included) significantly affected the change of response to TKIs.

Discussion

Although limited by the retrospective nature and the small sample size, our study points out that IHC could be a valid and low-cost tool to identify *PTEN* loss in *EGFR*-mutant patients. Moreover, we further validated that the presence of additional alterations impairs the efficacy of targeted therapy. In fact, our results have shown worse outcomes in terms of PFS, OS, and ORR in patients with concomitant *EGFR* mutation and *PTEN* loss. Furthermore, the co-existence of *PTEN* loss and *IGFR* overexpression identifies a potential prognostic ‘signature’ for a subgroup of patients with particularly poor prognosis. The main limitation of this study is that the IHC evaluation is focused on the expression of only 4 proteins; thus, it may not be fully informative about tumor complexity and heterogeneity. Actually, the most interesting perspective of our study proposed regards the application of IHC as a simple and reproducible tool to identify a subgroup of patients with poorer prognosis owing to co-alterations. Our next step will be to perform a confirmatory next-generation sequencing (NGS) analysis, to validate these study results and enlarge the available spectrum of candidate resistance mechanisms.

The advent of TKIs for lung cancer care has radically changed the quantity and quality of life of patients affected by oncogene-addicted disease, showing a clear advantage compared with standard chemotherapy. Nevertheless, although *EGFR*-mutant NSCLC has long been considered as a single entity, EGFR inhibitor activity ranges from 56% to 84%.[38] Unfortunately, acquired resistance occurs within approximately 12 months from therapy initiation, requiring a treatment change. Mechanisms of secondary resistance have been clarified[39,40] and include development of secondary mutations as the main mechanism (*T790M*), gene amplification of the primary oncogene, epigenetic alterations, or histologic transformation.[41,42]

Mechanisms of primary resistance remain largely unknown. Although most of the patients receiving TKIs achieve an objective response, the duration of clinical benefit can vary, and approximately 20% to 30% of patients do not respond or respond only for a short period of time because of the presence of multiple mechanisms of resistance. Several studies carried out during these past

years have led to the characterization of *EGFR*-mutant NSCLC as a variety of tumors that may harbor an intrinsic heterogeneity, potentially influencing response rate and thus prognosis. After the initial belief regarding the mutual exclusivity of *EGFR* mutation with other driver alterations, recent reports have demonstrated that additional mutations can coexist.[43-45] Concomitant mutations can co-occur from the beginning and might lead to primary treatment resistance. This phenomenon validates the existence of a clinically relevant intratumoral heterogeneity (ITH) that has been associated with a poorer prognosis.[43,46-48] In this regard, the complexity of the lung cancer genome is particularly high, as shown by deep-sequencing analyses with the concomitant presence of subclones carrying different types of mutations (spatial ITH). Furthermore, molecular studies performed on lung cancers during treatment have shown the phenomenon of clonal evolution, supporting the occurrence of a temporal ITH.[49]

The clinical relevance of ITH is supported by the demonstration that concurrent mutations, identified through NGS, induce primary resistance to TKIs and shorter PFS in *EGFR*-mutated patients. This evidence confirms that *EGFR*-mutant NSCLC is a heterogeneous disease and that further molecular analysis might help to select patients experiencing major benefits of anti-EGFR agents.[24,41,50] We have previously contributed to further validate the prognostic impact of ITH, in terms of additional coexisting mutations (mainly *TP53*), in *EGFR*-mutant NSCLC.[27] In the current study, we observed a significantly shorter PFS in *EGFR*-mutant patients with concomitant *PTEN* loss, *IGFR* overexpression, liver metastasis at baseline, or ECOG PS $\geq$ 1. Furthermore, *PTEN* loss, *IGFR* overexpression, liver metastasis at baseline, and ECOG PS $\geq$ 1 are significantly associated with worse OS. Moreover, *PTEN* loss is the only molecular characteristic associated with worse ORR.

Although liver metastasis and ECOG PS are validated prognostic factors,[51,52] a limited number of data are available for *PTEN* loss.[22,53,54] Yu et al have performed NGS on 374 samples of *EGFR*-mutated NSCLC, finding that *EGFR*-mutant lung cancers harbor a spectrum of concurrent alterations affecting patient survival. In particular, they have identified an activating mutation in *mTOR* able to induce resistance to TKIs.[55] Another study has shown that micro RNA-21 (miR-21) promotes NSCLC by negatively regulating *PTEN* expression. Authors have demonstrated that high miR-21 and low *PTEN* expression predict poor prognosis and a worse objective response in patients treated with TKIs.[56]

Regarding IGFR-1, Al-Saad et al have investigated the prognostic significance of MET and IGFR-1 alteration in 326 *EGFR*-mutant patients. Their study has shown that IGFR-1 alteration correlates with worse survival, demonstrating a highly significant and independent negative prognostic impact, especially in males, maybe because of different sex hormone effects on this protein.[57]

In our study, patients were treated with first- and second-generation TKIs (erlotinib, gefitinib, or afatinib). Putting our results into the current treatment algorithm of *EGFR*-mutant NSCLC, further analyses are needed to understand the impact of *PTEN* loss (and our combined molecular 'signature') as a mechanism of primary resistance for osimertinib-treated patients, which currently represents the treatment of choice for *EGFR*-mutant patients. In this light, few data are available suggesting that *PTEN* loss could represent a potential mechanism of resistance to osimertinib.[42,58] Important studies are ongoing to further clarify mechanisms of resistance to first-line osimertinib. In particular, the TEMPLE-2 trial (EudraCT 2020-001879-33, going to start) is an efficacy study of osimertinib in treatment-naive patients with *EGFR*-mutant NSCLC according to *TP53* status.

Considering the rapidly changing treatment landscape of oncogene-addicted NSCLC, the fast identification of those patients behaving as primary resistant to first-line targeted therapy is crucial to optimize treatment strategies. This is rational, considering the continuous emergence of new approaches, as the combination of chemotherapy and TKI show significant improvement of PFS and OS outcomes at the expense of increased toxicity[59,60] or the quadruple approach combining atezolizumab, bevacizumab, and chemotherapy.[61] With regard to immunotherapy, *PTEN* loss is a common mechanism of resistance that may hinder treatment efficacy. Indeed, *PTEN* loss induces an immunosuppressive tumor microenvironment through secretion of immunosuppressive cytokines and MDSCs/Tregs chemoattractant molecules.[62] Moreover, lung cancer is extremely complex, but mechanisms can be similar between oncogene- and not oncogene-addicted NSCLC. An extensive research effort is ongoing to overcome resistance to immunotherapy, in which *PTEN* loss represents a crucial player. The ultra-stratification of *EGFR*-mutant patients in subgroups experiencing a differential clinical benefit with selective TKIs may improve the expected success deriving from precision medicine application in cancer care.

Clinical Practice Points

- The occurrence of additional mutations with proliferative effects (for example, the co-existence of *EGFR* mutations with mutations of onco-suppressor genes) in the tumor may mirror the tumor heterogeneity. Loss of *PTEN* results in the lack of regulation of PIP3 levels, which, in turn, promote the PI3K/Akt pathway leading to cellular proliferation and growth. Protein p53 is an onco-suppressor that acts as a tumor suppressor, and it is called 'guardian of the genome' for its ability to regulate cell replication and proliferation. c-MET acts as an oncogene that, when altered, stimulates cell proliferation. IGFR-1 is a transmembrane protein implicated in growth and survival of cancer cells.
- Although limited by the retrospective nature and the small sample size, our study points out that IHC could be a valid and low-cost tool to identify *PTEN* loss in *EGFR*-mutant patients as a mirror of tumor heterogeneity. Moreover, we further validate, as the presence of additional alterations impair the efficacy of targeted therapy. In fact, our results have shown worse outcomes in terms of PFS, OS, and ORR in patients with concomitant *EGFR* mutation and *PTEN* loss. Furthermore, the co-existence of *PTEN* loss and *IGFR* overexpression identifies a potential prognostic 'signature' for a subgroup of patients with particularly poor prognosis.
- The ultra-stratification of *EGFR*-mutant patients in subgroups experiencing a differential clinical benefit with selective TKIs may improve the expected success deriving from precision medicine application in cancer care.

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References

1. Schiller JH, Harrington D, Belani CP, et al. Eastern Cooperative Oncology Group. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002; 346:92-8.
2. Tan CS, Gilligan D, Pacey S. Treatment approaches for EGFR-inhibitor-resistant patients with non-small-cell lung cancer. *Lancet Oncol* 2015; 16:e447-59.
3. Pao W, Chmielecki J. Rational biologically based treatment of EGFR-mutant non-small-cell lung cancer. *Nat Rev Cancer* 2010; 10:760-74.
4. Zhang YL, Yuan JQ, Wang KF, et al. The prevalence of EGFR mutation in patients with non-small cell lung cancer: a systematic review and meta-analysis. *Oncotarget* 2016; 7:78985-93.
5. Liu X, Wang P, Zhang C, Ma Z. Epidermal growth factor receptor (EGFR): a rising star in the era of precision medicine of lung cancer. *Oncotarget* 2017; 8: 50209-20.
6. Han JY, Park K, Kim SW, et al. First-SIGNAL: first-line single-agent iressa versus gemcitabine and cisplatin trial in never-smokers with adenocarcinoma of the lung. *J Clin Oncol* 2012; 30:1122-8.
7. Rosell R, Carcereny E, Gervais R, et al. Spanish Lung Cancer Group in collaboration with Groupe Français de Pneumo-Cancérologie and Associazione Italiana Oncologia Toracica. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol* 2012; 13:239-46.
8. Sequist LV, Yang JC, Yamamoto N, et al. Phase III study of afatinib or cisplatin plus pemetrexed in patients with metastatic lung adenocarcinoma with EGFR mutations. *J Clin Oncol* 2013; 31:3327-34.
9. Wu YL, Cheng Y, Zhou X, et al. Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHER 1050): a randomised, open-label, phase 3 trial. *Lancet Oncol* 2017; 18: 1454-66.
10. Papadimitrakopoulou VA, Mok TS, Yan JY, et al. Osimertinib versus platinum-pemetrexed for patients with EGFR T790M advanced NSCLC and progression on a prior EGFR-tyrosine kinase inhibitor: AURA3 overall survival analysis. *Ann Oncol* 2020; 31:1536-44.
11. Yu HA, Arcila ME, Rekhtman N, et al. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin Cancer Res* 2013; 19:2240-7.
12. Lim SM, Syn NL, Cho BC, Soo RA. Acquired resistance to EGFR targeted therapy in non-small cell lung cancer: mechanisms and therapeutic strategies. *Cancer Treat Rev* 2018; 65:1-10.
13. Shi J, Hua X, Zhu B, et al. Somatic genomics and clinical features of lung adenocarcinoma: a retrospective study. *PLos One* 2016; 13:e1002162.
14. Lee JK, Shin JY, Kim S, et al. Primary resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in patients with non-small-cell lung cancer harboring TKI-sensitive EGFR mutations: an exploratory study. *Ann Oncol* 2013; 24:2080-7.
15. Soria JC, Ohe Y, Vansteenkiste J, et al. FLAURA Investigators. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N Engl J Med* 2018; 378:113-25.
16. Le X, Puri S, Negrao MV, et al. Landscape of EGFR-dependent and -independent resistance mechanisms to osimertinib and continuation therapy beyond progression in EGFR-mutant NSCLC. *Clin Cancer Res* 2018; 24:6195-203.
17. Leonetti A, Sharma S, Minari R, Perego P, Giovannetti E, Tiseo M. Resistance mechanisms to osimertinib in EGFR-mutated non-small cell lung cancer. *Br J Cancer* 2019; 121:725-37.
18. Tanimoto A, Takeuchi S, Arai S, et al. Histone deacetylase 3 inhibition overcomes BIM deletion polymorphism−mediated osimertinib resistance in EGFR-mutant lung cancer. *Clin Cancer Res* 2017; 23:3139-49.
19. Kim Y, Lee B, Shim JH, et al. Concurrent genetic alterations predict the progression to target therapy in EGFR-mutated advanced NSCLC. *J Thorac Oncol* 2018; 14:193-202.
20. Pérez-Ramírez C, Cañadas-Garre M, Molina MA, Faus-Dáder MJ, Calleja-Hernández MÁ. PTEN and PI3K/AKT in non-small-cell lung cancer. *Pharmacogenomics* 2015; 16:1843-62.
21. Xiao J, Hu CP, He BX, et al. PTEN expression is a prognostic marker for patients with non-small cell lung cancer: a systematic review and meta-analysis of the literature. *Oncotarget* 2016; 7:57832-40.
22. Yamamoto C, Basaki Y, Kawahara A, et al. Loss of PTEN expression by blocking nuclear translocation of EGR1 in gefitinib-resistant lung cancer cells harboring epidermal growth factor receptor−activating mutations. *Cancer Res* 2010; 70: 8715-25.
23. Aisner DL, Sholl LM, Berry LD, et al. LCMC2 Investigators. The impact of smoking and TP53 mutations in lung adenocarcinoma patients with targetable mutations − the Lung Cancer Mutation Consortium (LCMC2). *Clin Cancer Res* 2018; 24:1038-47.
24. Labbé C, Cabanero M, Korpanty GJ, et al. Prognostic and predictive effects of TP53 co-mutation in patients with EGFR-mutated non-small cell lung cancer (NSCLC). *Lung Cancer* 2017; 111:23-9.
25. Vaughan CA, Pearsall I, Singh S, et al. Addiction of lung cancer cells to GOF p53 is promoted by upregulation of epidermal growth factor receptor through multiple contacts with p53 transactivation domain and promoter. *Oncotarget* 2016; 7: 12426-46.
26. Molina-Vila MA, Bertran-Alamillo J, Gasco A, et al. Nondisruptive p53 mutations are associated with shorter survival in patients with advanced non-small cell lung cancer. *Clin Cancer Res* 2014; 20:4647-59.
27. Bria E, Pilotto S, Amato E, et al. Molecular heterogeneity assessment by next-generation sequencing and response to gefitinib of EGFR mutant advanced lung adenocarcinoma. *Oncotarget* 2015; 6:12783-95.
28. Canale M, Petracci E, Delmonte A, et al. Impact of TP53 mutations on outcome in EGFR-mutated patients treated with first-line tyrosine kinase inhibitors. *Clin Cancer Res* 2017; 23:2195-202.
29. Burke L, Flieder DB, Guinee DG, et al. Prognostic implications of molecular and immunohistochemical profiles of the Rb and p53 cell cycle regulatory pathways in primary non−small cell lung carcinoma. *Clin Cancer Res* 2005; 11:232-41.
30. Cappuzzo F, Marchetti A, Skokan M, et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. *J Clin Oncol* 2009; 27:1667-74.
31. Lai GGY, Lim TH, Lim J, et al. Clonal MET amplification as a determinant of tyrosine kinase inhibitor resistance in epidermal growth factor receptor-mutant non-small-cell lung cancer. *J Clin Oncol* 2019; 37:876-84.
32. Shaw AT, Riely GJ, Bang YJ, et al. Crizotinib in ROS1-rearranged advanced non-small-cell lung cancer (NSCLC): updated results, including overall survival, from PROFILE 1001. *Ann Oncol* 2019; 30:1121-6.
33. Wolf J, Seto T, Han JY, et al. GEOMETRY mono-1 Investigators. Capmatinib in MET exon 14-mutated or MET-amplified non-small-cell lung cancer. *N Engl J Med* 2020; 383:944-57.
34. Blakely CM, Watkins TBK, Wu W, et al. Evolution and clinical impact of co-occurring genetic alterations in advanced-stage EGFR-mutant lung cancers. *Nat Genet* 2017; 49:1693-704.
35. Park S, Koh J, Kim DW, et al. MET amplification, protein expression, and mutations in pulmonary adenocarcinoma. *Lung Cancer* 2015; 90:381-7.
36. Ludovini V, Bellezza G, Pistola L, et al. High coexpression of both insulin-like growth factor receptor-1 (IGFR-1) and epidermal growth factor receptor (EGFR) is associated with shorter disease-free survival in resected non-small-cell lung cancer patients. *Ann Oncol* 2009; 20:842-9.
37. Roach C, Zhang N, Corigliano E, et al. Development of a companion diagnostic PD-L1 immunohistochemistry assay for pembrolizumab therapy in non-small-cell lung cancer. *Appl Immunohistochem Mol Morphol* 2016; 24:392-7.
38. Rossi A, Pasquale R, Esposito C, Normanna N. Should epidermal growth factor receptor tyrosine kinase inhibitors be considered ideal drugs for the treatment of selected advanced non-small cell lung cancer patients? *Cancer Treat Rev* 2013; 39: 489-97.
39. Minari R, Bordi P, Tiseo M. Third-generation epidermal growth factor receptor-tyrosine kinase inhibitors in T790M-positive non-small cell lung cancer: review on emerged mechanisms of resistance. *Trans Lung Cancer Res* 2016; 5:695-708.
40. Schrock AB, Zhu VW, Hsieh WS, et al. Receptor tyrosine kinase fusions and BRAF kinase fusions are rare but actionable resistance mechanisms to EGFR tyrosine kinase inhibitors. *J Thorac Oncol* 2018; 13:1312-23.
41. Camidge DR, Pao W, Sequist LV. Acquired resistance to TKIs in solid tumours: learning from lung cancer. *Nat Rev Clin Oncol* 2014; 11:473-81.
42. Kim TM, Song A, Kim DW, et al. Mechanisms of acquired resistance to AZD9291: a mutation-selective, irreversible EGFR inhibitor. *J Thorac Oncol* 2015; 10:1736-44.
43. Yu HA, Jordan E, Ni A, et al. Concurrent genetic alterations identified by next-generation sequencing in pre-treatment, metastatic EGFR-mutant lung cancers. *J Clin Oncol* 2016; 34(15 Suppl):9053.
44. Li S, Li L, Zhu Y, et al. Coexistence of EGFR with KRAS, or BRAF, or PIK3CA somatic mutations in lung cancer: a comprehensive mutation profiling from 5125 Chinese cohorts. *Br J Cancer* 2014; 110:2812-20.
45. Ju L, Han M, Zhao C, Li X. EGFR, KRAS and ROS1 variants coexist in a lung adenocarcinoma patient. *Lung Cancer* 2016; 95:94-7.
46. Rachiglio AM, Fenizia F, Piccirillo MC, et al. The presence of concomitant mutations affects the activity of EGFR tyrosine kinase inhibitors in EGFR-mutant non-small cell lung cancer (NSCLC) patients. *Cancers (Basel)* 2019; 11:341.
47. Lim SM, Kim HR, Cho EK, et al. Targeted sequencing identifies genetic alterations that confer primary resistance to EGFR tyrosine kinase inhibitor (Korean Lung Cancer Consortium). *Oncotarget* 2016; 7:36311-20.
48. Scheffler M, Bos M, Gardizi M, et al. PIK3CA mutations in non-small cell lung cancer (NSCLC): genetic heterogeneity, prognostic impact and incidence of prior malignancies. *Oncotarget* 2015; 6:1315-26.
49. Testa U, Castelli G, Pelosi E. Lung cancers: molecular characterization, clonal heterogeneity and evolution, and cancer stem cells. *Cancers (Basel)* 2018; 10:248.

50. VanderLaan PA, Rangachari D, Mockus SM, et al. Mutations in TP53, PIK3CA, PTEN and other genes in EGFR mutated lung cancers: correlation with clinical outcomes. *Lung Cancer* 2017; 106:17-21.
51. Wu KL, Tsai MJ, Yang CJ, et al. Liver metastasis predicts poorer prognosis in stage IV lung adenocarcinoma patients receiving first-line gefitinib. *Lung Cancer* 2015; 88:187-94.
52. He Y, Wang Y, Boyle T, et al. Hepatic metastases is associated with poor efficacy of erlotinib as 2nd/3rd line therapy in patients with lung adenocarcinoma. *Med Sci Monit* 2016; 22:276-83.
53. Sos LM, Koker M, Weir BA, et al. PTEN loss contributes to erlotinib resistance in EGFR-mutant lung cancer by activation of Akt and EGFR. *Cancer Res* 2009; 69: 3256-61.
54. Jiang J, Gu Y. Homozygous deletion of PTEN and poor outcomes in EGFR mutant NSCLC patients with gefitinib therapy. *Eur Respir J* 2015; 46:PA4841.
55. Yu HA, Suzawa K, Jordan E, et al. Concurrent alterations in EGFR-mutant lung cancers associated with resistance to EGFR kinase inhibitors and characterization of MTOR as a mediator of resistance. *Clin Cancer Res* 2018; 24:3108-18.
56. Shen H, Zhu F, Liu J, et al. Alteration in Mir-21/PTEN expression modulates gefitinib resistance in non-small cell lung cancer. *PLoS One* 2014; 9:e103305.
57. Al-Saad S, Richardsen E, Kilvaer TK, et al. The impact of MET, IGF-1, IGF1R expression and EGFR mutations on survival of patients with non-small-cell lung cancer. *PLoS One* 2017; 12:e0181527.
58. Bordi P, Del Re M, Minari R, et al. From the beginning to resistance: study of plasma monitoring and resistance mechanisms in a cohort of patients treated with osimertinib for advanced T790M-positive NSCLC. *Lung Cancer* 2019; 131:78-85.
59. Norohna V, Patil VM, Joshi A, et al. Gefitinib versus gefitinib plus pemetrexed and carboplatin chemotherapy in EGFR-mutated lung cancer. *J Clin Oncol* 2020; 38: 124-36.
60. Hosomi Y, Morita S, Sugawara S, et al. Gefitinib alone versus gefitinib plus chemotherapy for non-small-cell lung cancer with mutated epidermal growth factor receptor: NEJ009 study. *J Clin Oncol* 2020; 38:115-23.
61. Reck M, Mok T, Nishio M, et al. IMpower150 Study Group. Atezolizumab plus bevacizumab and chemotherapy in non-small-cell lung cancer (IMpower150): key subgroup analyses of patients with EGFR mutations or baseline liver metastases in a randomised, open-label phase 3 trial. *Lancet Respir Med* 2019; 7:387-401.
62. Piro G, Carbone C, Carbognin L, et al. Revising PTEN in the Era of Immunotherapy: New Perspectives for an Old Story. *Cancers (Basel)* 2019; 11:1525.