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ORIGINAL ARTICLE

# Erlotinib for Patients with EGFR Wild-Type Metastatic NSCLC: a Retrospective Biomarkers Analysis

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## Abstract

Erlotinib is approved for the treatment of patients with *EGFR* mutation positive, metastatic NSCLC. It is also approved as second/third line therapy for *EGFR* mutation negative patients, but in this setting the benefit of erlotinib is modest and there is no validated biomarker for selecting *EGFR* wild-type patients who may benefit the most from the treatment. We retrospectively assessed *EGFR* and *K-RAS* mutational status, and EGFR, c-MET and IGF1-R expression in tumor samples of 72 patients with metastatic NSCLC treated with erlotinib after at least one prior line of chemotherapy, from 2008 to 2012. We analyzed the association between biomarkers and outcome (RR, PFS, and OS). *EGFR* mutated patients achieved a better RR (56% vs 8%, $p = .002$), PFS (10 vs 3 months, HR 0.53, $p = 0.48$) and OS (20 vs 6 months, HR 0.55, $p = .07$), compared to *EGFR* wild-type patients. Among 63 *EGFR* wild-type patients, those with EGFR high-expression had a better outcome in terms of RR (40% vs 2%, $p = .002$), PFS (7.5 vs 2 months, HR 0.45, $p = .007$) and OS (30 vs 5 months, HR 0.34, $p < .001$) compared to patients with EGFR intermediate or low/negative-expression. IGF1-R expression, c-MET expression and *K-RAS* mutational status did not significantly affect the outcome; however, no patients with *K-RAS* mutation or c-MET high-expression achieved an objective response. In patients with metastatic, chemo-refractory *EGFR* wild-type NSCLC, EGFR high-expression may represent a positive predictor of activity for erlotinib, whereas *K-RAS* mutation and c-MET high-expression may predict lack of activity. These findings deserve further prospective evaluation.

**Keywords** *EGFR* wild-type · NSCLC · Erlotinib · *K-RAS* mutation · IGF1-R expression · C-MET expression

## Introduction

Non-small cell lung cancer (NSCLC) still remains the leading cause of cancer death worldwide [1], but over the last 5 to 10 years some encouraging improvement in the treatment of metastatic disease has been achieved, mainly due to a deeper understanding of cancer biology, the development of targeted agents [2] and, more recently, immune checkpoint inhibitors [3–6].

Activating mutations in exons 18–21 of the tyrosine kinase domain of epidermal growth factor receptor (*EGFR*) are the oncogenic drivers of a minority of NSCLCs and they occur in about 10–15% of Caucasian patients [7]. In the front-line setting, EGFR tyrosine kinase inhibitors (TKIs) erlotinib, gefitinib and afatinib are more effective than standard chemotherapy for patients with metastatic NSCLC harboring *EGFR* activating mutations, while they are basically ineffective for *EGFR* wild type tumors [8–10].

However, erlotinib has demonstrated some activity also in patients with *EGFR* mutation negative tumors previously treated with chemotherapy [11, 12]. In fact, it is approved not only for patients with metastatic, *EGFR*-mutated NSCLC but also for patients with *EGFR* wild-type, metastatic NSCLC as second or third line treatment. Unfortunately, no validated

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biomarker is available to identify *EGFR* wild-type patients who may benefit the most from erlotinib in this setting.

In the present study, we retrospectively evaluated the role of EGFR, c-MET and IGF1R expression and *K-RAS* mutational status as potential predictive biomarkers in *EGFR* wild type NSCLC patients receiving erlotinib after at least one previous line of chemotherapy.

## Patients and Methods

From 2008 to 2012, 117 consecutive patients with metastatic NSCLC who had already received at least one line of chemotherapy were treated at Department of Medical Oncology, Università Cattolica del Sacro Cuore in Rome, Italy, with erlotinib 150 mg daily. Tissue samples from 72 patients were available for biomarkers analysis. Only patients with tissue sample available for biomarkers analysis were included in the study, and their clinical data were retrospectively collected. Study protocol was reviewed and approved by the local institutional review board and conducted according to Good Clinical Practice guidelines and to the Declaration of Helsinki. Patients enrolled in the study signed a written informed consent for biomarkers analysis and clinical data collection at the beginning of therapy. Patients data were anonymized before analysis.

### Biomarkers Analysis

#### DNA Extraction and *K-RAS* and *EGFR* Mutational Analysis

DNA was extracted from formalin fixed, paraffin-embedded tissue samples using the QIAamp FFPE Tissue Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. Pathologic areas selected for DNA extraction contained at least 70% cancer cells. *K-RAS* codons 12, 13, and 61 and *EGFR* codons 18, 19, 20 and 21 were amplified using the same primers and polymerase chain reaction (PCR) conditions already described elsewhere [13, 14].

#### Immunohistochemical Assay for EGFR, IGF1R and c-MET

Immunohistochemical (IHC) analysis of c-MET and IGF1R was performed on 3-mm tissue slides using the antihuman rabbit polyclonal antibody c-MET (clone C28, dilution 1:250; membranous and cytoplasmic staining) (Santa Cruz Biotechnology, Milan, Italy), and antihuman goat polyclonal antibody IGF1R (dilution 1:30; membranous staining) (R&D Systems, Milan, Italy), as described elsewhere [15]. Immunohistochemical analysis of EGFR was performed using the antihuman rabbit monoclonal antibody EGFR (dilution 1:150; Spring Bioscience, USA) [16]. EGFR expression was scored using the semi-quantitative method proposed by Hirsch et al. [17]. According to this method, the percentage of positive tumor cells per slide (0% to 100%) was multiplied by the dominant intensity pattern of staining (1, negative or trace; 2, weak; 3, moderate; 4, intense); therefore, the overall score ranged from 0 to 400. Specimens with scores 0 to 200, 201 to 300, and 301 to 400 were respectively classified as having negative or low, intermediate, and high levels of expression. C-Met and IGF1-R expression were scored based on fraction of cells showing membranous staining, as follows: 0 = fraction of positive cells ≤25%, 1 + = 25–50%, 2 + = 50–75%, 3+ ≥ 75%. For statistical analyses, scores of 3+ were considered high-expression.

### Assessment of Response

Tumor assessment was done with CT scan or MRI approximately every 2 months as for routine local clinical practice, and response was evaluated according to RECIST 1.0 criteria. Progression-free survival (PFS) was defined as the time from starting treatment to disease progression or death for any cause, and overall survival (OS) as the time from starting treatment to death for any cause.

### Statistical Analysis

The association of clinicopathologic characteristics with response rate (RR) and disease control rare (DCR) was evaluated by Fisher's exact test or $\chi^2$ test, as appropriate. Estimates of survival times (PFS and OS) were calculated according to the Kaplan Meier method and compared with log-rank test. The multivariate analysis was performed using a Cox regression model. The $p$ values are 2-sided and considered statistically significant when less than 0.05. Data were analyzed using SPSS 20 (Armonk, NY: IBM Corp.).

## Results

### Characteristics of Patients and Efficacy Data

Characteristics of patients are reported in Table 1. Median age was 62 years (range 33–85), 40 patients (56%) were males, 51 (71%) were current or former smokers, ECOG PS was 0–1 for 55 (76%) patients. Histology was adenocarcinoma in 62 (86%), squamous in 5 (7%), adeno-squamous in 2 (3%) and not specified in 3 (4%) patients. All patients had received a platinum-based doublet as front line therapy. Erlotinib was administrated as second line in 50 (69%) patients and as third or fourth line in 22 (31%) patients. Skin toxicity was observed in 39 (54%) patients.

Response rate (RR) and disease-control rate (DCR) were 14% and 46%, respectively. After a median follow-up of 12 months, median PFS and OS were 4 and 7 months, respectively. Some clinical characteristics were associated with outcome. Females had a longer median OS compared with males

(HR 0.53, 95% CI 0.32–0.88, $p = .014$). Patients with ≤2 metastatic sites had longer PFS and OS compared to patients with >2 metastatic sites (PFS: HR 0.56, 95% CI 0.34–0.93, $p = .024$; OS: HR 0.55, 95% CI 0.34–0.90, $p = .019$). Skin toxicity (any grade) was significantly associated with a better outcome in terms of DCR (58% vs 28%, $p = .016$), PFS (HR 0.55, 95% CI 0.25–0.80, $p = .007$) and OS (HR 0.51, 95% CI 0.25–0.76, $p = .004$). There was no significant difference in terms of PFS and OS according to age, tumor histology, performance status, smoking history, number of prior lines therapy, and presence of brain metastases (Table 2).

## Biomarkers and Outcome

### *EGFR* Mutational Status

An *EGFR* activating mutation was found in 9 (12.6%) patients. All patients with *EGFR* mutations had adenocarcinoma histology. Patients with *EGFR* activating mutations had higher RR compared with *EGFR* wild-type patients (56% vs 8%, $p = .002$). *EGFR*-mutated patients also had a longer PFS (10 vs 3 months, HR 0.53, 95% CI 0.27–0.99, $p = .048$) and OS (20 vs 6 months, HR 0.55, 95% CI 0.30–1.05, $p = .07$) in comparison with *EGFR* wild-type patients (Fig. 1).

### *K-RAS* Mutational Status

None of the *EGFR* mutated patients carried a *K-RAS* mutation. Among *EGFR* wild-type patients, *K-RAS* mutations were identified in 11 patients and none of them achieved an objective response. No significant difference in PFS and OS was observed according to *K-RAS* mutational status (Table 3).

### EGFR Expression

EGFR high level of expression was detected in 3 of the 9 patients with EGFR activating mutation. Among the 63 *EGFR* wild-type patients, EGFR high-expression was

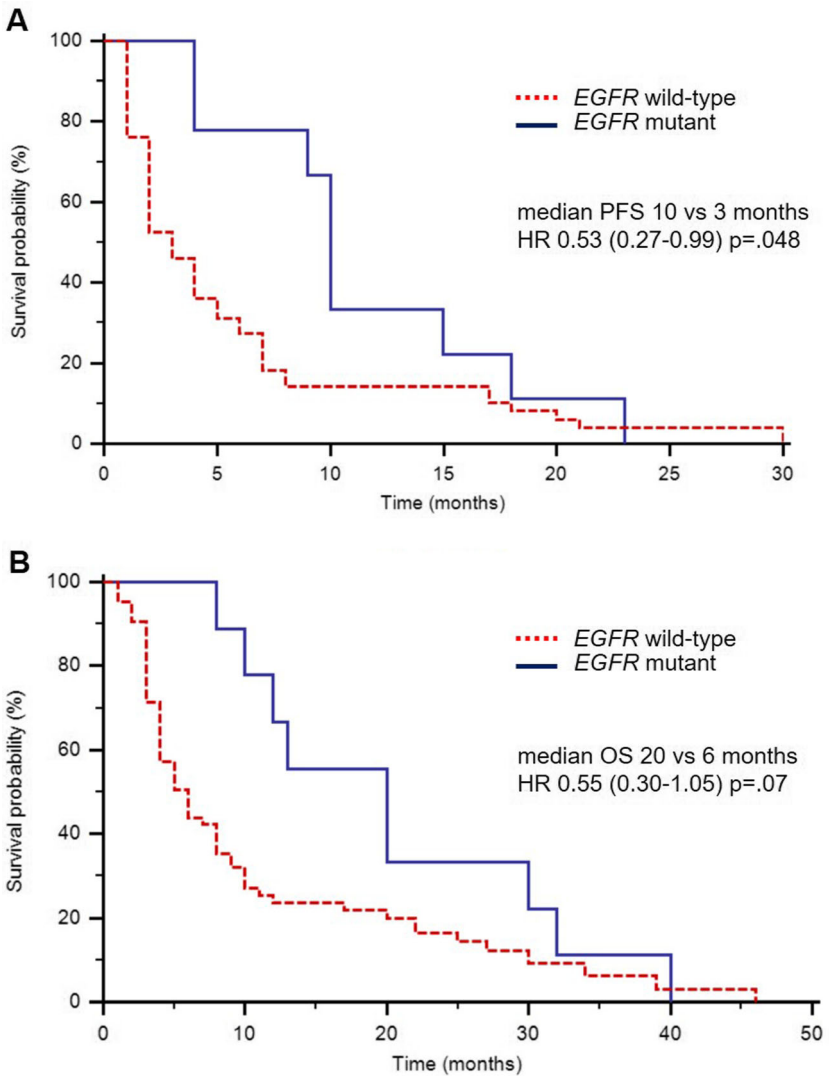


**Fig. 1** PFS (**a**) and OS (**b**) according to *EGFR* mutational status

**Table 1** Characteristics of patients

| | No. Patients | % |
|---|---|---|
| Overall | 72 | 100 |
| Sex | | |
| Male | 40 | 56 |
| Female | 32 | 44 |
| Age (yr) | | |
| Median (Range) | 62 (33–85) | – |
| < 70 | 51 | 71 |
| ≥ 70 | 21 | 29 |
| Smoking status | | |
| Never smoked | 21 | 29 |
| Current or former smoker | 51 | 71 |
| ECOG PS | | |
| 0–1 | 55 | 76 |
| > 1 | 17 | 24 |
| Histology | | |
| Adenocarcinoma | 62 | 86 |
| Squamous-cell carcinoma | 5 | 7 |
| Adeno-squamous carcinoma | 2 | 3 |
| NOS/Poorly differentiated carcinoma | 3 | 4 |
| Number of metastatic sites | | |
| ≤ 2 | 37 | 51 |
| > 2 | 35 | 49 |
| Brain metastases | | |
| Yes | 25 | 35 |
| No | 47 | 65 |
| PFS after 1st line chemotherapy | | |
| > 6 months | 37 | 51 |
| ≤ 6 months | 35 | 49 |
| Number of prior chemotherapy lines | | |
| 1 | 50 | 69 |
| 2 or more | 22 | 31 |
| Skin toxicity | | |
| Yes | 39 | 54 |
| No | 33 | 46 |

ECOG, Eastern Coast Oncology Group; NA, not applicable; NOS, not otherwise specified; PFS, progression-free survival; PS, Performance Status

observed in 8 patients (13%), with higher incidence in females than in males (20% vs 6%, $p = .13$). RR and DCR were higher for patients with EGFR high-expression than for those with intermediate or low/negative expression (50% vs 2%, $p = .002$; 75% vs 36%, $p = .33$). Patients with EGFR high-expression also achieved a significantly longer PFS (12.5 vs 2 months, HR 0.40, 95% CI 0.17–0.73, $p = .005$) and OS (30 vs 5 months, HR 0.32, 95% CI 0.15–0.63, $p = .001$), compared to those with EGFR intermediate or low/negative expression (Fig. 2).

### C-MET Expression

c-MET high-expression was observed in 13 (21%) out of 63 *EGFR* wild-type patients. The level of c-MET expression did not significantly affect PFS and OS (Table 3). Interestingly, no patients with c-MET high-expression achieved an objective response to erlotinib.

### IGF-1R Expression

IFG-1R high-expression was observed in 10 (16%) out of 63 *EGFR* wild-type patients. No significant association between IGF-1R high-expression and outcome was observed (Table 3).

### Multivariate Analysis

A Cox regression model analysis for OS was performed on the overall population. *EGFR* mutational status, EGFR expression, number of metastatic sites, gender, skin toxicity, smoking history and PFS obtained with first-line treatment were included in multivariate analysis. Multivariate analysis showed that *EGFR* mutation, EGFR high-expression and skin toxicity were independent predictive factors of better OS (Table 4).

## Discussion

The efficacy of EGFR TKIs for the treatment of metastatic NSCLC with *EGFR* activating mutation has been clearly demonstrated. Based on the results of the BR.21 study, erlotinib is the only EGFR TKI approved also for patients without *EGFR* activating mutations in the second or third line setting. BR.21 was a randomized phase 3 trial on 731 patients with metastatic NSCLC unselected for *EGFR* mutations, previously treated with at least one line of chemotherapy, and ineligible for further chemotherapy. This study demonstrated a significant OS benefit for erlotinib compared with placebo (6.7 versus 4.7 months; HR 0.70, $p < 0.001$) [11]. A retrospective analysis on the subgroup of patients with known *EGFR* status, suggested that erlotinib was beneficial even for *EGFR* wild-type patients (HR for survival 0.74). A subgroup analysis of the SATURN trial further supported a survival benefit with erlotinib as maintenance therapy compared with placebo for patients with stable disease after induction chemotherapy, irrespective of *EGFR* mutational status [18].

The phase 3 randomized TAILOR trial, which compared erlotinib with docetaxel as second line treatment for patients with *EGFR* wild-type NSCLC, showed superiority of docetaxel over erlotinib in terms of RR, PFS and OS [17]. Furthermore, several clinical trials showed a clear superiority of immune checkpoint inhibitors over docetaxel in the second-line setting [4–6]. However, erlotinib may still represent a therapeutic option for some *EGFR*-wild type patients who failed previous chemotherapy and immunotherapy [19–21]. The CONFERMER study

**Table 2** Clinical characteristics of patients and outcome (univariate analysis)

| Characteristics | RR | p | PFS HR (95% CI) | p | OS HR (95% CI) | p |
|---|---|---|---|---|---|---|
| Sex | | | | | | |
| Female vs Male | 22% vs 7,5% | NS | 0.80 (0.43–1.29) | NS | 0.54 (0.29–0.83) | **0.008** |
| Age (yr) | | | | | | |
| ≤65 vs >65 | 13.7% vs 15% | NS | 1,18 (0.68–2.17) | NS | 1.08 (0.61–1.93) | NS |
| ECOG PS | | | | | | |
| 0–1 vs ≥2 | 16.4% vs 6% | NS | 0.84 (0.43–1.29) | NS | 0.70 (0.33–1.24) | NS |
| Histology | | | | | | |
| Adenocarcinoma vs Other | 14.5% vs 10% | NS | 0.76 (0.30–0.58) | NS | 0.79 (0.34–1.63) | NS |
| No of metastatic sites | | | | | | |
| ≤2 vs >2 | 13.5% vs 14.3% | NS | 0.58 (0.28–0.85) | **0.01** | 0.56 (0.29–0.86) | **0.01** |
| Brain Metastases | | | | | | |
| No vs Yes | 17% vs 8% | NS | 0.75 (0.38–1.26) | NS | 0.77 (0.40–1.32) | NS |
| Smoking history | | | | | | |
| Never smoked vs current/former smoker | 28.6% vs 7.8% | NS | 0.86 (0.48–1.47) | NS | 0.63 (0.35–1.03) | NS |
| PFS after 1st line chemotherapy | | | | | | |
| >6 vs ≤6 months | 16.2% vs 11.4% | NS | 0.54 (0.23–0.75) | **0.004** | 0.60 (0.32–0.93) | **0.03** |
| No. of previous lines | | | | | | |
| 1 vs ≥2 | 16% vs 9% | NS | 0.82 (0.43–1.82) | NS | 0.67 (0.33–1.10) | NS |
| Skin toxicity | | | | | | |
| Yes vs No | 18.4% vs 9.4% | NS | 0.55 (0.25–0.80) | **0.007** | 0.51 (0.25–0.76) | **0.004** |

Statistically significant *p* values are indicated in bold

CI, confidence interval; ECOG, Eastern Coast Oncology Group; HR, hazard ratio; NS, non-significant; OS, overall survival; PFS, progression-free survival; PS, Performance Status; RR, response rate

(EudraCT 2014–001207-42), a randomized phase 3 clinical trial comparing monochemotherapy with gemcitabine or vinorelbine versus erlotinib as third line for patients with *EGFR* wild-type NSCLC is currently ongoing, and its results will further clarify the possible role of erlotinib in pretreated NSCLC patients without *EGFR* activating mutations.

In the overall population of our study, we reported a median OS of 7 months with erlotinib, which is comparable to that reported by the BR.21 study (6.7 months) and also by a large phase 4 study (7.9 months) [12] in NSCLC patients unselected for EGFR mutational status. In our study, *EGFR* mutant patients clearly derived the most benefit from the treatment, with 56% of RR, 10 months of median PFS and 30 months of median OS. However, also 8% of *EGFR* wild-type patients achieved an objective response, suggesting that erlotinib may have activity in a minority of patients with NSCLC not harboring *EGFR* activating mutations. In the multivariate analysis, besides *EGFR* mutational status, EGFR expression was the only other biomarker which was significantly associated with the outcome. Our finding adds to the data of BR.21 and

**Table 3** Molecular biomarkers and outcome in EGFR wild-type patients (univariate analysis)

| Characteristics | RR | p | PFS HR (95% CI) | p | OS HR (95% CI) | p |
|---|---|---|---|---|---|---|
| EGFR | | | | | | |
| High vs intermediate or low/negative | 50% vs 2% | 0.002 | 0.40 (0.17–0.73)ù | 0.005 | 0.32 (0.15–0.63) | 0.001 |
| K-RAS | | | | | | |
| wt vs mut | 9.6% vs 0% | NS | 1.14 (0.55–2.57) | NS | 0.96 (0.45–2.02) | NS |
| IGF1-R IHC | | | | | | |
| Low/Normal vs High | 7.5% vs 10% | NS | 1.01 (0.46–2.19) | NS | 1.12 (0.53–2.45) | NS |
| c-MET IHC | | | | | | |
| Low/Normal vs High | 10% vs 0% | NS | 0.97 (0.47–1.94) | NS | 0.72 (0.32–1.38) | NS |

CI, confidence interval; HR, hazard ratio; IHC, immunohistochemistry; mut, mutant; NS, non-significant; OS, overall survival; PFS, progression-free survival; RR, response rate; wt, wild type

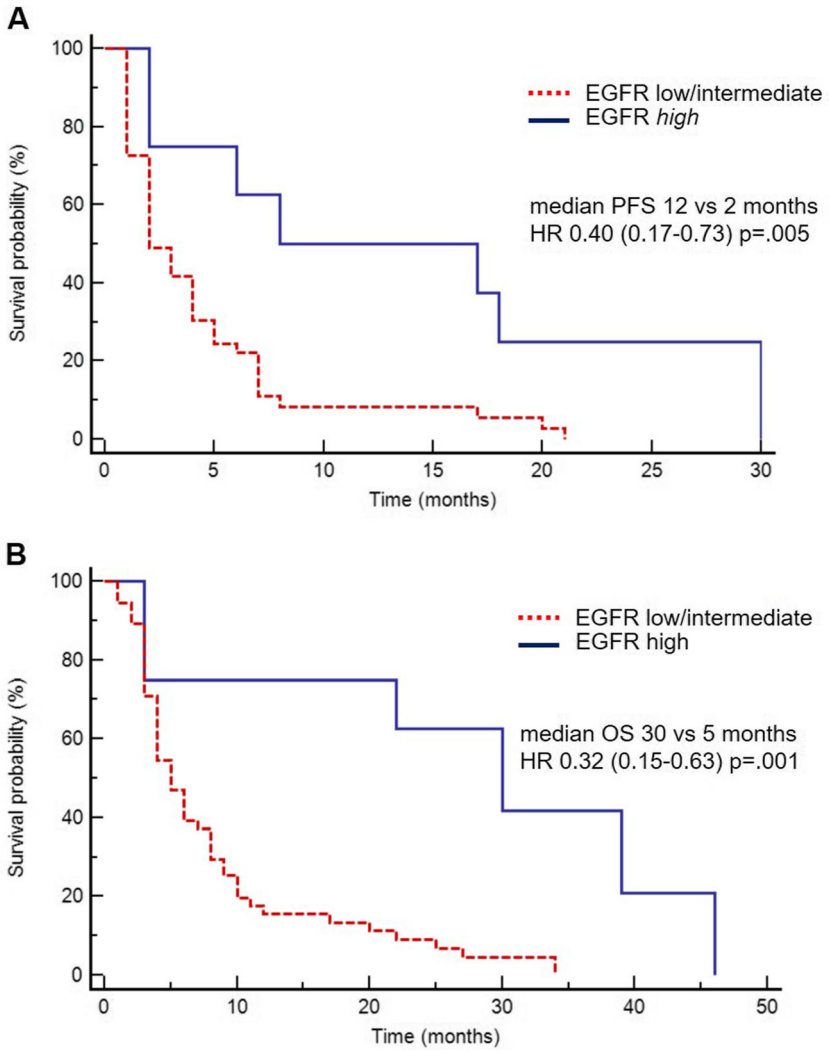


**Fig. 2** PFS (**a**) and OS (**b**) according to EGFR immunohistochemistry expression in EGFR wild-type patients

ISEL trials. In fact, the retrospective subgroup analysis of BR.21 suggested a significant survival benefit for EGFR IHC positive patients (HR for death 0.68, $p = 0.02$) and not for negative patients (HR for death 0.93, $p = 0.70$). The ISEL trial compared gefitinib with placebo in 1692 patients with chemorefractory NSCLC. A retrospective, biomarker analysis showed that EGFR protein expression was related to clinical outcome, and patients with EGFR positive tumors had longer overall survival (HR for death, 0.77; $p = .049$) [22].

However, other studies did not show a significant association between EGFR protein expression and outcome of patients treated with EGFR-TKIs [23]. A possible explanation for these conflicting results may be represented by the heterogeneity of the studies and, above all, by the lack of standardization in staining procedures and cut-off levels for defining EGFR positive tumors. A cut-off of 10% of positive cells was used for the IHC analysis in the ISEL trial [22], whereas other authors used a 20% cut-off level [23]. Different cut-off levels were investigated in the BR.21 trial, but an optimal level was not identified [24]. In our analysis, we used the Hirsch score that, combining the percentage of positive cells and the intensity of staining, may provide an accurate evaluation of protein expression in order to identify those tumors highly dependent on EGFR pathway for cell proliferation and survival.

In our study, none of patients with *K-RAS* mutation achieved an objective response to erlotinib, thus suggesting a possible prognostic or predictive role for *K-RAS* mutational status. However, we did not observe an association between *K-RAS* mutational status and survival. In fact, despite some studies suggest that *K-RAS* mutations may be prognostic or predictive in NSCLC [25,26], data are not conclusive and therefore *K-RAS* is still not a validated biomarker [27]. In the TAILOR study, *K-RAS* mutational status did not affect the outcome of patients treated with erlotinib or chemotherapy [28]. Although a subgroup analysis of the BR.21 trial showed no survival benefit from erlotinib compared with placebo for patients with *K-RAS* mutations (HR 1.67, 95% CI 0.62–4.50, $p = .31$), the interaction test was not significant [29]. While *K-RAS* mutations represent a known mechanism of acquired resistance to EGFR TKIs [30], their role in primary resistance to EGFR TKIs has not been yet established.

**Table 4** Multivariate analysis for OS

| Variate | HR | 95% CI | p |
|---|---|---|---|
| EGFR mutational status | | | |
| mut vs wt | 0.38 | 0.16–0.86 | **0.02** |
| EGFR IHC expression | | | |
| High vs Low/absent | 0.39 | 0.16–0.86 | **0.016** |
| K-RAS mutational status | | | |
| wt vs mut | 1.20 | 0.56–2.59 | NS |
| No of metastatic sites | | | |
| ≤2 vs >2 | 0.62 | 0.33–1.14 | NS |
| PFS after 1st line chemotherapy | | | |
| >6 vs ≤6 months | 0.68 | 0.40–1.18 | NS |
| Sex | | | |
| Female vs Male | 0.55 | 0.29–1.02 | NS |
| Skin Toxicity | | | |
| Yes vs No | 0.53 | 0.31–0.91 | **0.02** |
| Smoking status | | | |
| Never smoked vs Current/Former smoker | 0.94 | 0.50–1.80 | NS |

Statistically significant *p* values are indicated in bold

CI, confidence interval; HR, hazard ratio; IHC, immunohistochemistry; NS, non-significant; mut, mutant OS, overall survival; wt, wild type

Therefore, taking into account our results together with available data from other studies, no definitive conclusion can be driven on the prognostic or predictive role of *K-RAS* mutations for patients with *EGFR* wild-type NSCLC treated with erlotinib.

Again, no patients with c-MET high-expression responded to erlotinib, but c-MET status did not affect survival in our study. The amplification of *c-MET*, which is often associated with c-MET high-expression [31], is a known mechanism of acquired resistance to EGFR-TKIs in patients with *EGFR* mutated NSCLC [32], but presently there are no data on its possible role in primary resistance to erlotinib in chemo-refractory patients with *EGFR* wild-type NSCLC [33]. Based on our results, no definitive conclusion can be driven on the role of c-MET expression as a biomarker for patients with *EGFR* wild-type NSCLC treated with erlotinib.

Our study has many limitations. First, being a retrospective trial, it can be only hypothesis-generating and our results should be confirmed in prospective, larger studies. Second, since all the patients in this study received erlotinib, EGFR expression might represent a general prognostic rather than predictive factor. However, given the negative impact of EGFR expression on the prognosis of NSCLC patients showed in other studies [34], the association of EGFR high-expression and a better outcome of patients treated with erlotinib observed in our study support the hypothesis of a positive predictive role of EGFR expression. Third, the tissue sample available for the biomarker analysis was collected before starting first-line treatment, and may be not representative of the molecular status of the tumor at the moment of the initiation of erlotinib.

## Conclusion

In conclusion, our results suggest a possible activity of erlotinib in a subgroup of *EGFR* wild-type chemo-refractory patients. EGFR high-expression may represent a positive predictive factor for erlotinib in this setting, whereas *K-RAS* mutations and c-MET high-expression might be associated with lack of response although their predictive role has been not clearly established.

## Compliance with Ethical Standards

**Ethical Approval** All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed Consent** For this type of study formal consent is not required.

**Conflict of Interest** All authors declare that they do not have any conflict of interest.

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