High **EGFR** gene copy number and skin rash as predictive markers for EGFR tyrosine kinase inhibitors in patients with advanced squamous cell lung carcinoma

Youngjoo Lee¹, Hyo Sup Shim², Moo Suk Park³, Joo-Hang Kim³,⁴, Sang-Jun Ha⁵, Se Hoon Kim², and Byoung Chul Cho³,⁴*

¹Center for Lung Cancer, National Cancer Center, Goyang, Korea; ²Department of Pathology, Yonsei University College of Medicine, Seoul, Korea; ³Department of Internal Medicine, Yonsei University College of Medicine, Seoul, Korea; ⁴Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea, and ⁵Department of Biochemistry, College of Life Science and Biotechnology, Yonsei University, Seoul, Korea.

*Both authors contributed equally to this work.

**Corresponding authors:**

Byoung Chul Cho, MD, PhD; Yonsei Cancer Center, Division of Medical Oncology, Yonsei University College of Medicine, 250 Seongsanno, 134 Shinchon-Dong, Seodaemun-Gu, Seoul 120-752, Korea; Fax: 82-2-393-3562; cbc1971@yuhs.ac

Se Hoon Kim, MD, PhD; Department of Pathology, Yonsei University College of Medicine, 250 Seongsanno, 134 Shinchon-Dong, Seodaemun-Gu, Seoul 120-752, Korea; Fax: 82-2-362-0860; paxco@yuhs.ac
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CONFLICT OF INTEREST DISCLOSURES

The authors indicated no potential conflicts of interest.

Keywords: Epidermal growth factor receptor, Non-small-cell lung cancer, Predictive biomarker, Squamous Cell carcinoma
Translational Relevance

An EGFR tyrosine kinase inhibitor (TKI) has been used in practice to treat a wide range of NSCLC patients, including subsets that have a lower likelihood of EGFR mutation. However, there have been no molecular and clinical predictors for the efficacy of EGFR-TKIs, other than EGFR mutations. Therefore, this study was conducted to investigate predictive markers for EGFR-TKI efficacy in previously treated patients with squamous cell lung carcinoma in which activating EGFR mutations are very rare. Consequently, the EGFR-TKI treatment showed the clinical benefit to squamous cell carcinoma patients with EGFR/FISH positivity or a modest or severe skin rash in our study. When the combined criteria of EGFR/FISH and skin rash severity were analyzed, this study supports the lack of benefit conferred by EGFR-TKIs on squamous cell carcinoma patients with neither EGFR/FISH positivity nor skin rash.
Abstract

Purpose: This study is aimed to search for predictors of EGFR tyrosine kinase inhibitors (TKI) efficacy in previously treated patients with advanced squamous cell lung carcinoma in which EGFR mutations are very rare.

Experimental Design: EGFR gene copy numbers was assessed by fluorescence in situ hybridization (FISH) and was evaluated as predictors of EGFR-TKI efficacy in 71 patients with advanced squamous cell lung cancer who received gefitinib or erlotinib as a second-line or higher therapy. The tumors were classified into EGFR/FISH-positive (high polysomy/gene amplification) and EGFR/FISH-negative (other) groups.

Results: EGFR/FISH was positive in 19 (26.7%) patients. Only EGFR/FISH positive status was correlated with the EGFR-TKIs response (EGFR/FISH+ vs EGFR/FISH−, 26.3% vs 2.0%; P = 0.005). In a multivariate analysis, the risk of progression was lower in EGFR/FISH-positive patients (hazard ratio [HR] of EGFR/FISH+ vs EGFR/FISH−, 0.57; P = 0.057) or patients experiencing grade ≥2 rash (HR for rash grade ≥2 vs <2, 0.54; P = 0.042), compared with EGFR/FISH-negative patients or those experiencing grade <2 rash, respectively. When the combined criteria of EGFR/FISH and skin rash severity were analyzed, EGFR/FISH-negative patients with grade <2 rash had poorer clinical outcomes than patients with positive EGFR/FISH or grade ≥2 rash, apparent as a lower response rate (0.0% vs 21.4%; P = 0.003) and a shorter median progression-free survival (1.13 months vs 3.90 months; P = 0.0002).

Conclusions: EGFR/FISH and skin rash severity may be used to identify which patients are likely to gain a benefit from EGFR-TKIs in this population.
Introduction

The utility of molecular biomarkers in identifying the appropriate patients for anti-cancer treatments has been emphasized since the introduction of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (EGFR-TKIs) into the treatment of non-small-cell lung cancer (NSCLC). Several large randomized phase III trials have verified that EGFR mutations are the strongest predictive biomarker of the efficacy of EGFR-TKIs as a first-line therapy (1-3). The first-line EGFR-TKI treatment has produced greater benefits in terms of progression-free survival (PFS), toxicity profiles, and quality of life than standard chemotherapy in advanced NSCLC patients with mutant EGFR, but not in those with wild-type EGFR (1-3). Therefore, if activating mutations in the EGFR gene are identified at the time of diagnosis, EGFR-TKI treatment is strongly recommended as a first-line therapy. However, other randomized clinical studies have shown that even in patients with wild-type EGFR, EGFR-TKIs are either superior to placebo or not inferior to docetaxel chemotherapy as a second- or third-line therapy (4, 5). These results suggest that a substantial subset of the population without EGFR mutations can derive clinical benefit from EGFR-TKIs as a second-line or higher treatment. In fact, these drugs have been used in practice to treat a wide range of NSCLC patients, including subsets that do not include a high proportion of EGFR-mutation-positive patients. Therefore, there is an urgent need to identify molecular or clinical predictors of the efficacy of EGFR-TKIs, other than EGFR mutations, in members of this population who are unlikely to carry EGFR mutations.

The rapid tumor cell death that occurs after treatment with EGFR-TKIs means that
the tumor is dependent on the EGFR signaling pathway for its survival and proliferation (6, 7). Both the mutation and amplification of the EGFR gene can fully activate EGFR tyrosine kinase and trigger downstream oncogenic pathways. Therefore, it seems reasonable to assume a correlation between an abnormality in the EGFR copy number and EGFR-TKI sensitivity. Although a number of studies have investigated the EGFR copy number as a predictive biomarker for EGFR-TKI sensitivity, its predictive role remains controversial. Early studies by Hirsch et al. and Cappuzzo et al. demonstrated that high polysomy or amplification of the EGFR gene was associated with significantly greater erlotinib sensitivity and longer survival (8-11). However, other researchers failed to replicate these findings (12, 13). Unlike EGFR mutations, which are more frequently found in specific patient subsets, such as East Asian, female, and never-smoker patients, and those with adenocarcinoma histology type, the distribution of high EGFR copy numbers is mostly independent of ethnicity, sex, smoking status, and histology (9-11, 14, 15). Based on these findings, we assumed that an increased EGFR copy number might be a good candidate marker for high sensitivity to EGFR-TKIs in EGFR-wild-type tumors.

This study was designed to investigate the molecular and clinical factors that predict EGFR-TKI efficacy in previously treated patients with squamous cell lung carcinoma in which activating EGFR mutations are less than 5% (11, 16, 17). We especially focused on whether the EGFR copy number, assessed by fluorescence in situ hybridization (FISH), can identify patients with a greater likelihood of clinical benefit from EGFR-TKIs.
Materials and Methods

Patient selection

We first identified 102 consecutive patients who had received gefitinib (250 mg/day) or erlotinib (150 mg/day) monotherapy for metastatic squamous cell lung carcinoma at the Severance Hospital (Seoul, Korea) from February 2007 to December 2010. Two pathologists (S.H. Kim and H.S. Shim) confirmed their squamous cell carcinoma of the lung by hematoxylin and eosin staining. The tumor samples of 71 patients were available for the examination of alterations in the \textit{EGFR} gene copy number. All the tissues had been obtained at the time of the primary diagnosis by biopsy (n = 46; 64.8%) or surgical resection (n = 25; 35.2%). The sampling sites were the primary tumor (n = 61; 85.9%) or metastatic sites (n = 10; 14.1%). The patients’ medical records and radiographic images were then reviewed to evaluate their clinicopathological characteristics, tumor responses, adverse effects, and survival outcomes using a predesigned data collection format. The study was approved by the Institutional Review Board of the Severance Hospital.

Analysis of \textit{EGFR} copy number

The \textit{EGFR} gene copy number was determined with FISH testing using the LSI \textit{EGFR} SpectrumOrange/CEP 7 SpectrumGreen Probe (Vysis, Abbott Laboratories, IL), according to a published protocol (15). At least 50 cells were evaluated for each tumor by two pathologists (S.H. Kim and H.S. Shim). According to previously published criteria, the \textit{EGFR} gene copy number was classified into six FISH strata: disomy (≤ 2 copies in < 90% of cells), low trisomy (≤ 2 copies in ≥ 40% of cells, 3
copies in 10%–40% of cells, ≥ 4 copies in < 10% of cells), high trisomy (≤ 2 copies in ≥ 40% of cells, 3 copies in ≥ 40% of cells, ≥ 4 copies in < 10% of cells), low polysomy (≥ 4 copies in 10%–40% of cells), high polysomy (≥ 4 copies in ≥ 40% of cells), and gene amplification (defined by the presence of tight \textit{EGFR} gene clusters and a ratio of \textit{EGFR} genes to chromosome of ≥ 2, or ≥ 15 copies of \textit{EGFR} per cell in ≥ 10% of the cells analyzed) (11). Tumors were considered \textit{EGFR}/FISH positive (FISH\textsuperscript{+}) if they showed high polysomy or amplification of the \textit{EGFR} gene with FISH.

\textbf{Analysis of \textit{EGFR} and \textit{KRAS} mutations}

A mutation analysis was performed in the 37 patients (52.1%) for whom adequate tissue was available. DNA was extracted from areas of paraffin-embedded tissue samples containing more than 70% tumor. Mutation analysis of \textit{EGFR} exons 18–21 and \textit{KRAS} exons 12–13 was performed using a PCR-based assay described previously (18, 19). Among the 37 patients, 13 patients were \textit{EGFR}/FISH-positive.

\textbf{Assessment}

The tumor response was assessed by a computed tomography scan generally performed every eight weeks, in accordance with the guidelines established by the Response Evaluation Criteria in Solid Tumors (20). Any treatment-related event was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0.

\textbf{Statistical Analysis}

A $\chi^2$ test or Fisher’s exact test was used to analyze the association between
EGFR copy number changes and the radiological tumor response and skin rash. PFS was measured from the first day of EGFR-TKI treatment until the first documentation of disease progression or death. Overall survival (OS) was calculated from the first day of EGFR-TKI treatment until death or the most recent follow-up. For the survival analysis, patients were censored at the last date at which they were known to be alive. All time-to-event outcomes were estimated using the Kaplan–Meier method and compared across groups with the log-rank test or the Cox proportional hazards model. All statistical tests were two-sided, and statistical significance was defined as $P < 0.05$. 
Results

Patient and treatment characteristics

The median age was 64 years (range, 46–79 years). The proportions of males and ever smokers were 78.9% and 81.7%, respectively. Patients had received a median of two prior chemotherapy regimens (range, 1–4 regimens) for advanced disease before treatment with EGFR-TKIs. Half the patients (n = 35; 49.3%) received gefitinib treatment and the other half (n = 36; 50.7%) received erlotinib. Survival data were collected until June 2011 and the median follow-up time was 10.1 months (95% confidence interval [CI], 9.5–15.7). At the time of analysis, 61 patients (85.9%) had died and eight (11.3%) had survived. Three of the survivors were receiving EGFR-TKI treatment. For the entire patient population, the median PFS and OS were 2.0 months (95% CI, 1.6–2.4) and 9.5 months (95% CI, 7.3–11.8), respectively.

According to the EGFR/FISH analysis, disomy was present in 46 patients (64.8%), low trisomy in 1 (1.4%), high trisomy in 3 (4.2%), low polysomy in 2 (2.8%), high polysomy in 11 (15.5%), and gene amplification in 8 (11.3%). Therefore, 19 patients (26.7%) were categorized in the EGFR/FISH-positive group and 52 patients (73.3%) in the EGFR/FISH-negative group. The prevalence of EGFR/FISH positivity in this patient population did not differ according to age, sex, Eastern Cooperative Oncology group (ECOG) performance status (PS), or smoking status (Table 1). There was no difference in the distribution of EGFR/FISH positivity according to the time from diagnosis to EGFR-TKI treatment or number of previous chemotherapy regimens. EGFR/FISH positivity was found at similar frequencies in primary tumors and at metastatic sites. The tissue sampling method had no effect on the pattern of FISH for
the EGFR gene.

Tumor response

Of the 69 patients available for response evaluation, 6 patients (8.7%) had a partial response, 34 (49.3%) had stable disease, and 29 (42.0%) had progressive disease as their best tumor response. Therefore, the objective response rate (ORR) was 8.7% and the disease control rate was 58.0%. EGFR/FISH status was the only factor identified as predicting the response to EGFR-TKI treatment (Figure 1). The EGFR/FISH-positive patients showed a significantly higher response rate than the EGFR/FISH-negative patients (26.3% vs 2.0%, respectively; \( P = 0.005 \)). When the analysis was limited to the 37 patients with EGFR mutation results, the difference in response rate between two groups remained unchanged (33.3% vs 4.2%, respectively; \( P = 0.034 \)). The similar trend was observed when the only EGFR wild-type patients were analyzed (27.3% vs 4.2%, respectively; \( P = 0.082 \)).

Overall, 56 patients (78.9%) experienced skin rash of any grade during EGFR-TKI treatment; 39 (54.9%) grade 1, 14 (19.7%) grade 2, and three (4.2%) grade 3. The incidence of grade ≥ 2 rash was higher in the patients with good ECOG PS (30.3% vs 5.6% for ECOG PS 2 and 3; \( P = 0.053 \)), ever smokers (29.3% vs 0.0% for never smokers; \( P = 0.029 \)), and those receiving erlotinib (33.3% vs 14.3% for those receiving gefitinib; \( P = 0.060 \)) (Figure 2). The EGFR/FISH-positive patients also experienced grade ≥ 2 rash more frequently than the EGFR/FISH-negative patients (42.1% vs 17.3%, respectively; \( P=0.056 \)).

Survival outcomes
The analysis of PFS showed that both EGFR/FISH status and skin rash severity were possible predictors of PFS after EGFR-TKI treatment (Table 2). In the multivariate analysis, the EGFR/FISH-positive patients had a lower risk of progression than the EGFR/FISH-negative patients, even though the difference was borderline statistically significant (HR of EGFR/FISH+ vs EGFR/FISH−, 0.57; 95% CI, 0.32–1.02; P = 0.057). The median PFS of the EGFR/FISH-positive patients was higher than that of the EGFR/FISH-negative patients [3.87 (95% CI, 2.78-4.96) vs. 1.93 (95% CI, 1.11-2.76); P = 0.058]. When the analysis was limited to the 37 patients with EGFR mutation results, the difference in median PFS remained numerically unchanged but statistically decreased [4.10 (95% CI, 1.66-6.54) vs. 2.10 (95% CI, 1.21-2.99); P = 0.110]. The same trend was observed when the only EGFR wild-type patients were analyzed [4.10 (95% CI, 3.55-4.65) vs. 2.10 (95% CI, 1.21-2.99); P = 0.201]. The risk of progression was significantly higher in patients with grade < 2 rash than in those with grade ≥ 2 rash (HR for rash grade ≥ 2 vs < 2, 0.54; 95% CI, 0.30-0.98; P = 0.042; Figure 3B). Similar results were observed when the analysis was limited to rash-evaluable population who received the drug for at least four weeks.

The univariate analysis of OS showed that ECOG PS, smoking history, and skin rash were significant predictors of OS (Table 2). In the multivariate analysis, ECOG PS remained an independent predictor of OS. The patients with ECOG PS of 0 or 1 had a significantly lower risk of death than those with ECOG PS of 2 or 3 (HR of ECOG PS 0–1 vs PS 2–3, 0.34; 95% CI, 0.19–0.62; P < 0.001). Interestingly, never smokers with squamous cell carcinomas had poorer survival outcomes than ever smokers with the same histology type (HR of never smokers vs ever smokers, 1.85;
The risk of death was numerically lower in patients experiencing grade ≥ 2 rash than in those experiencing grade < 2 rash (HR for rash grade ≥ 2 vs < 2, 0.59; 95% CI, 0.30-1.13; \( P = 0.111 \)).

**Combined criteria of EGFR/FISH status and skin rash grade**

The treatment outcomes in the subgroups classified according to the combined criteria of EGFR/FISH status and grade of skin rash are shown in Table 3 and Figure 3C. The group of EGFR/FISH-negative patients with low-grade skin rash had poorer clinical outcomes than the groups with EGFR/FISH positivity or high-grade skin rash: with lower ORR (0.0% vs 21.4%, respectively; \( P = 0.003 \)), shorter median PFS (1.13 months [95% CI, 0.40–1.86] vs 3.90 months [95% CI, 3.45–4.35], respectively; \( P = 0.0002 \)), and shorter median OS (4.20 months [95% CI, 0.61–7.79] vs 9.43 months [95% CI, 5.67–13.20], respectively; \( P = 0.007 \)).

**EGFR and KRAS mutations**

One patient (2.7%) had a mutant-type EGFR gene, which was deleted at exon 19. The tumor sample was from resection of primary tumor at T3N1M0 stage. The other patient (2.7%) had a KRAS mutation (G12D type). The sample of this patient was from biopsy of primary tumor at the diagnosis of advanced stage NSCLC. The EGFR gene was also amplified in the patient harboring the activating EGFR mutation. Therefore, the proportion of patients with activating EGFR mutations among the EGFR/FISH-positive group was 8.3%.

The patient with the EGFR-mutant tumor was a 71-year-old woman who had never smoked in her life. She achieved a partial response to gefitinib treatment and her
PFS was 19.0 months. However, the patient carrying the KRAS mutation, a 73-year-old woman with no smoking history, was EGFR/FISH negative. Her best response to gefitinib was PD and her PFS was 2.0 months.
Discussion

Squamous cell carcinoma accounts for about 25% of NSCLC (21), but recent advances in personalized treatments with targeted biological agents, including agents that target mutant kinases such as EGFR, ALK, and HER2, the antiangiogenic agent bevacizumab, and the multitargeting antifolate pemetrexed, are not applicable to this histological type of NSCLC. However, in the BR.21 trial, erlotinib showed a survival advantage over the best supportive care as salvage therapy in the subgroup of patients with nonadenocarcinoma histology (HR 0.81; 95% CI, 0.64–1.02) (22). The INTEREST study also reported that there was no survival difference between gefitinib and docetaxel treatments in patients with nonadenocarcinoma who had failed 1–2 previous regimens (median OS, 6.4 months vs 6.9 months, respectively) (5). The placebo-controlled phase III SATURN study also showed that maintenance erlotinib after first-line chemotherapy had OS benefits in the stable group, even among squamous cell carcinoma patients (HR, 0.67; 95% CI, 0.48–0.92) (23). These findings provide some evidence of the clinical utility of EGFR-TKIs as salvage therapy in patients with squamous cell carcinoma. However, there is no predictive marker for EGFR-TKI sensitivity other than EGFR mutations, in patients with this histological type. Moreover, this population has very low frequency of activating mutations of EGFR gene (about 5%) (11, 16, 17). To the best of our knowledge, the present study is the first designed to look for predictive markers appropriate for squamous cell carcinoma patients.

In this study, we have demonstrated that a significant proportion of squamous cell lung carcinoma have genomic gain in the EGFR gene without mutational event. The
EGFR/FISH-positive patients showed a significantly higher response rate than the EGFR/FISH-negative patients. Moreover, the patients with EGFR/FISH-positive tumors had longer PFS than those with EGFR/FISH-negative tumors, although this difference was marginally significant after the appropriate adjustments. A modest or severe skin rash after EGFR-TKI treatment was also a good predictor of longer PFS. Therefore, this study supports EGFR-TKIs may give the clinical benefit to squamous cell carcinoma patients with EGFR/FISH positivity or a modest or severe skin rash.

The association between the mutation and amplification of the EGFR gene has been studied exclusively in the adenocarcinoma subtype of tumors (24, 25). Many preclinical and clinical data have demonstrated the EGFR amplification usually coexists with mutations in lung adenocarcinomas (24, 25). This observation supports the idea that the mutation occurs first and then induces gene amplification during tumor progression and metastasis. The IPASS study was landmark study that showed the superiority of first-line gefitinib to chemotherapy in terms of PFS in never smoker or light ex-smoker Asian patients with lung adenocarcinoma (1). It reported a concordance between the mutation and high gene copy number of EGFR in 88.9% of patients and showed that the predictive value of the EGFR copy number was driven by coexisting EGFR mutations (26). In that study, patients with a high EGFR gene copy numbers had significantly longer PFS when treated with gefitinib than when treated with chemotherapy in the presence of an EGFR mutation (HR, 0.48; 95% CI, 0.34–0.67), but shorter PFS in the absence of an EGFR mutation (HR, 3.85; 95% CI, 2.09–7.09), whereas patients with mutant EGFR had longer PFS after gefitinib therapy irrespective of the EGFR copy number (26). However, in our study, a significant proportion of squamous cell carcinoma patients had an amplified EGFR
gene with no mutation, and yet a high EGFR gene copy number was correlated well with responsiveness to EGFR-TKI and PFS benefit. This finding suggests that an amplified wild-type EGFR tumor is also dependent on the EGFR signaling pathway for growth, although to a lesser extent than tumors with amplified mutant EGFR genes. Consequently, the number of EGFR gene copy could be considered as an alternative molecular predictive marker of EGFR-TKI efficacy in EGFR-wild type population.

Our analyses have also shown a relationship between the severity of the skin rash and survival improvement, which is consistent with previous studies reporting that the skin rash can be a surrogate marker of the clinical benefits of EGFR-TKIs, regardless of the EGFR status (27, 28). Moreover, the grade of skin rash appeared more significant in predicting the risk of progression than the EGFR/FISH status through the multivariate analysis. However, it should be carefully interpreted due to the relatively too small sample size to ensure the statistical significance. At the subgroup analysis, EGFR/FISH-negative patients with high-grade skin rash showed a lower ORR but similar PFS and longer OS time, compared to EGFR/FISH-positive patients with low-grade skin rash. This result means that the patients experiencing high-grade skin rash may have a better prognosis regardless of tumor shrinkage. Some previous studies also have explained that a significant skin reaction may indicate a patient’s ability to raise an immune-mediated inflammatory response to drug rather than more effective target receptor inhibition (29). Therefore, the EGFR/FISH status and skin rash severity are likely to play a role as predictive markers of EGFR-TKIs by different mechanisms. When we combined EGFR copy number and skin rash as a tumor- and host-related predictive factor, respectively,
either an amplified EGFR gene or a significant skin rash was shown a potential positive predictor of benefit from EGFR-TKI treatment.

There are conflicting results in the literature about the predictive role of EGFR gene copy number (8-13). These may be attributable to interobserver variability in FISH testing and interpretation. Alternatively, the EGFR gene copy status may be altered intrinsically or extrinsically during the course of the disease. Compared with EGFR mutations, EGFR amplification occurs as a late event during the progression of lung adenocarcinoma, in association with the invasive growth of the tumor (24). Therefore, there might be discrepancies in the EGFR gene copy number between time points because most studies, including our own, have analyzed tissue samples acquired at the time of the primary diagnosis rather than at the time of treatment. However, one study by Cappuzzo et al. reported the concordance between these time points in 88.9% of patients for both EGFR FISH and mutation biomarkers ($P = 0.01$ and $P = 0.001$, respectively) (8). In addition, it remains unknown whether EGFR amplification is homogeneously distributed within a primary tumor or both in a primary tumor and metastases. A lack of this information about the time and place concordance of EGFR gene amplification seems significant limitation for deciding on whether EGFR FISH can be used as a reliable biomarker in clinical setting.

In conclusion, EGFR/FISH status can be used to select those patients with previously treated squamous cell lung cancer who are likely to gain a benefit from EGFR-TKIs before treatment. In addition, the high grade of skin rash development during EGFR-TKIs in this histologic group may help to support the continuous use of EGFR-TKIs. However, because of the limitations of our study, a single retrospective study with a relatively small number of patients, future prospective studies should be
conducted to validate our findings.
References


7. Sharma SV, Bell DW, Settleman J, Haber DA. Epidermal growth factor


**Figure legends**

**Figure 1.** Objective response rates by clinical and biomarker characteristics. Response was assessable in 67 patients. Statistical differences were tested with a $\chi^2$ test or Fisher's exact test.

**Figure 2.** Grade 2 or 3 skin rash rates according to clinical and biomarker characteristics. Statistical differences were tested with a $\chi^2$ test or Fisher's exact test.

**Figure 3.** Progression-free survival (PFS) curves for the two groups according to (A) $EGFR$/FISH status, (B) skin rash grade, and (C) the combined criteria of $EGFR$/FISH status and skin rash grade in 71 patients with advanced squamous cell lung cancer, receiving gefitinib or erlotinib as the second-line or higher therapy.
Table 1. Correlations between demographic characteristics and EGFR/FISH status

<table>
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<tr>
<th>Characteristics</th>
<th>Total (n = 71)</th>
<th>Positive EGFR/FISH (n= 19)</th>
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<tr>
<td></td>
<td>No. (%)</td>
<td>No./Subgroup (%)</td>
<td></td>
</tr>
<tr>
<td>Age, year</td>
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<td></td>
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<tr>
<td>≤ 65</td>
<td>40 (56.3)</td>
<td>9/40 (22.5)</td>
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<tr>
<td>&gt; 65</td>
<td>31 (43.7)</td>
<td>10/31 (32.3)</td>
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<tr>
<td>Sex</td>
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<tr>
<td>Female</td>
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<td>2/15 (13.3)</td>
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<tr>
<td>Male</td>
<td>56 (78.9)</td>
<td>17/56 (30.4)</td>
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<td>ECOG PS</td>
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<td>53 (74.6)</td>
<td>15/53 (28.3)</td>
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<td>2,3</td>
<td>18 (25.4)</td>
<td>4/18 (22.2)</td>
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<td>Ever</td>
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<td>18/58 (31.0)</td>
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<td>30 (42.3)</td>
<td>6/30 (20.0)</td>
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<td>2 or more</td>
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<td>Time from diagnosis to treatment</td>
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<td>&lt;12 months</td>
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<td>≥12 months</td>
<td>19 (26.8)</td>
<td>4/19 (21.1)</td>
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<td>14/46 (30.4)</td>
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Notes: *tested by χ² test or Fisher’s exact test. Abbreviations: ECOG, Eastern Cooperative Group; PS, performance status; EGFR-TKI, EGFR tyrosine kinase inhibitor.
Table 2. Survival analysis

<table>
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<th>Variables</th>
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</tr>
<tr>
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<td>HR (95% CI)</td>
<td>P*</td>
<td>HR (95% CI)</td>
<td>P*</td>
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<tr>
<td>Age ≤65 vs. &gt;65</td>
<td>1.67 (0.90-2.84)</td>
<td>0.106</td>
<td>1.49 (0.88-2.51)</td>
<td>0.139</td>
</tr>
<tr>
<td>Female vs. Male</td>
<td>1.09 (0.59-2.02)</td>
<td>0.782</td>
<td>1.25 (0.68-2.28)</td>
<td>0.468</td>
</tr>
<tr>
<td>ECOG PS 0,1 vs. 2,3</td>
<td>0.86 (0.48-1.55)</td>
<td>0.662</td>
<td>0.32 (0.18-0.58)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Never vs. Ever smoker</td>
<td>1.47 (0.75-2.88)</td>
<td>0.256</td>
<td>2.27 (1.14-4.13)</td>
<td>0.019</td>
</tr>
<tr>
<td>1 vs. ≥2 previous chemotherapy</td>
<td>0.95 (0.58-1.56)</td>
<td>0.849</td>
<td>0.99 (0.59-1.64)</td>
<td>0.941</td>
</tr>
<tr>
<td>Gefitinib vs. Erlotinib</td>
<td>1.19 (0.73-1.96)</td>
<td>0.488</td>
<td>1.20 (0.72-2.01)</td>
<td>0.488</td>
</tr>
<tr>
<td>EGFR/FISH+ vs. FISH−</td>
<td>0.59 (0.33-1.03)</td>
<td>0.064</td>
<td>0.65 (0.37-1.56)</td>
<td>0.144</td>
</tr>
<tr>
<td>Skin rash grade ≥2 vs. &lt;2</td>
<td>0.56 (0.32-0.99)</td>
<td>0.046</td>
<td>0.47 (0.25-0.87)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Notes: *tested with the Cox proportional hazards model. Abbreviations: HR, hazard ratio; CI, confidential interval; ECOG, Eastern Cooperative Group; PS, performance status.
<table>
<thead>
<tr>
<th>FISH&lt;sup&gt;+&lt;/sup&gt; and rash G &lt;2</th>
<th>FISH&lt;sup&gt;-&lt;/sup&gt; and rash G ≥2</th>
<th>FISH&lt;sup&gt;+&lt;/sup&gt; and rash G ≥2</th>
<th>FISH&lt;sup&gt;-&lt;/sup&gt; and rash G &lt;2</th>
<th>FISH&lt;sup&gt;-&lt;/sup&gt; or rash G ≥2</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of responder/ total</td>
<td>0/41</td>
<td>1/9</td>
<td>3/11</td>
<td>2/8</td>
<td>6/28</td>
</tr>
<tr>
<td>ORR, %</td>
<td>0.0</td>
<td>11.1</td>
<td>27.3</td>
<td>25.0</td>
<td>21.4</td>
</tr>
<tr>
<td>Median PFS, mo (95% CI)</td>
<td>1.13 (0.40-1.86)</td>
<td>4.30 (2.84-5.76)</td>
<td>4.10 (3.50-4.70)</td>
<td>3.07 (0.53-5.61)</td>
<td>3.90 (3.45-4.35)</td>
</tr>
<tr>
<td>HR of progression (95% CI)</td>
<td>1.00</td>
<td>0.30 (0.13-0.67)</td>
<td>0.32 (0.14-0.70)</td>
<td>0.53 (0.24-1.16)</td>
<td>0.36 (0.21-0.64)</td>
</tr>
<tr>
<td>Median OS, mo (95% CI)</td>
<td>4.20 (0.61-7.79)</td>
<td>11.03 (6.36-15.71)</td>
<td>7.07 (1.49-12.64)</td>
<td>9.17 (3.16-15.17)</td>
<td>9.43 (5.67-13.20)</td>
</tr>
<tr>
<td>HR of death (95% CI)</td>
<td>1.00</td>
<td>0.51 (0.22-1.18)</td>
<td>0.68 (0.33-1.42)</td>
<td>0.44 (0.19-1.009)</td>
<td>0.55 (0.32-0.94)</td>
</tr>
</tbody>
</table>

Notes: *statistical difference between two groups ([FISH<sup>+</sup> or rash G ≥2] and [FISH<sup>-</sup> and rash < G2]); †tested with Fisher’s exact test; ‡tested with the Cox proportional hazards model; §tested with the Cox proportional hazards model adjusted for ECOG performance status. Abbreviations: ORR, objective response rate; PFS, progression-free survival; mo, months; CI, confidential interval; HR, hazard ratio; OS, overall survival.
A Median PFS (months)

EGFR/FISH- (n= 52) 1.93
EGFR/FISH+ (n= 19) 3.87
Log-rank test, \( P = 0.058 \)
Median PFS (months)

Rash G $\geq 2$ (n=17) 3.80
Rash G <2 (n=54) 1.97
Log-rank test, P = 0.041
Median PFS (months)

EGFR/FISH+ or Rash G ≥ 2 (n=28) 3.90
EGFR/FISH- and Rash G < 2 (n=43) 1.13
Log-rank test, P = 0.0002

Probability of PFS

Time (months)

Median PFS (months)
Clinical Cancer Research

High EGFR gene copy number and skin rash as predictive markers for EGFR tyrosine kinase inhibitors in patients with advanced squamous cell lung carcinoma

Youngjoo Lee, Hyo Sup Shim, Moo-Suk Park, et al.


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