Epidermal Growth Factor Receptor Gene Amplification Predicts Worse Outcome in Patients With Surgically Resected Nonadenocarcinoma Lung Cancer

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Abstract

The epidermal growth factor receptor (*EGFR*) gene copy number was analyzed with fluorescent in situ hybridization (FISH) to examine the prognostic role in surgically resected nonadenocarcinoma of non–small-cell lung cancer (NA-NSCLC). Patients with *EGFR* gene amplification and high polysomy, who underwent curative-intent surgical resection, showed significantly shorter overall survival (hazard ratio, 1.36; 95% confidence interval, 1.040-1.782; $P = .025$). *EGFR* FISH evaluation of surgical tumor tissue, in addition to clinicopathologic factors, might better predict for the prognosis of early-stage or locally advanced NA-NSCLC patients.

Purpose: The aim of the present study was to examine the prognostic role of amplification and increased expression of the epidermal growth factor receptor (*EGFR*) gene in surgically resected non-adenocarcinoma of non-small cell lung cancer (NA-NSCLC). **Materials and Methods:** The present retrospective study included 114 consecutive NA-NSCLC patients with available tumor tissue and survival data. *EGFR* gene copy number and protein expression were evaluated using fluorescent in situ hybridization (FISH) and immunohistochemistry in tissue microarray sections, respectively. **Results:** Among 114 patients, 99 (86.8%) had squamous cell carcinoma histologic features. *EGFR* gene amplification and high polysomy (*EGFR* FISH$^+$) were observed in 7.9% and 31.6% of cases, respectively. Patients with *EGFR* FISH$^+$ had significantly shorter overall survival ($P = .011$). A multivariate model confirmed that patients with *EGFR* FISH$^+$ had a significantly greater risk of death than *EGFR* FISH$^-$ patients after adjusting for pathologic stage, presence of pleural invasion, venous invasion, and surgical margins (hazard ratio, 1.36; 95% CI, 1.040 to 1.782; $P = .025$). EGFR protein expression by immunohistochemistry was not associated with overall survival in the same group. Neither *EGFR* gene amplification nor EGFR immunohistochemistry expression was associated with relapse-free survival. **Conclusion:** An increased *EGFR* gene copy number in surgically resected NA-NSCLC was associated with worse survival.

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Introduction

Non–small-cell lung cancer (NSCLC) accounts for ~80% of lung cancers and is the leading cause of cancer-related death worldwide.[1] Emerging information on the molecular pathogenesis of lung cancer has made possible personalized anticancer treatments.[1] Molecularly targeted treatments with inhibitors of epidermal growth factor receptor (EGFR) and ALK (anaplastic lymphoma kinase) have led to a dramatic revolution in the

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treatment of patients with lung adenocarcinoma.[2,3] However, activating *EGFR* mutations and *ALK* fusions are naturally not present in nonadenocarcinoma of NSCLC (NA-NSCLC), including lung squamous cell carcinoma (the most common subtype of NA-NSCLC), large cell carcinoma, and sarcomatoid carcinoma. Targeted drugs for lung adenocarcinoma are generally unsuccessful in the treatment of NA-NSCLC, suggesting that these subtypes have different biologic characteristics.[1]

EGFR signaling affects proliferation, invasion, metastasis, and angiogenesis of tumor cells in NA-NSCLC and adenocarcinoma of the lung.[4-6] EGFR protein is expressed in > 60% of NSCLC tumors.[7-9] High *EGFR* polysomy and amplification using fluorescence in situ hybridization (FISH) were discovered in 20% to 40% of NSCLC patients.[4,8,10] The prognostic roles of EGFR protein and *EGFR* gene copy number variation have been studied but remain controversial.[4,8] In addition, most patients in previous studies had adenocarcinoma of the lung, which is more likely to harbor activating *EGFR* mutations compared with NA-NSCLC. Therefore, a comprehensive study of NA-NSCLC is needed to investigate the biologic influences of *EGFR* gene copy status and protein expression.

The present study was designed to examine the clinical and biologic characteristics and prognostic importance of *EGFR* gene status assessed by FISH and EGFR protein expression assessed by immunohistochemistry in Korean patients with NA-NSCLC.

Materials and Methods

Patients

The medical histories of 274 consecutive NSCLC patients who had undergone curative-intent surgical resection (lobectomy or pneumonectomy) from May 2003 to July 2006 at Seoul National University Bundang Hospital were studied. Subsequently, 160 patients were disqualified from the study owing to the following exclusion criteria: (1) lung adenocarcinoma or (2) synchronous extrapulmonary malignancy. The remaining 114 patients with NA-NSCLC who were treated with complete surgical resection—either lobectomy or pneumonectomy with mediastinal lymph node sampling—were registered in the study. Two pathologists individually examined the hematoxylin and eosin−stained slides of all cases in line with the 2004 World Health Organization classification.[11]

Pathologic TNM classification and staging according to the American Joint Committee on Cancer, 7th edition, were performed for all 114 cases.[12] None of the patients received EGFR-targeted treatment throughout the follow-up period. The Seoul National University Bundang Hospital institutional review board approved the present study (approval no. B-1412-280-303).

Tissue Microarray

Representative core tumor tissue sections (2 mm in diameter) were obtained from paraffin blocks and settled in new tissue microarray blocks using a trephine apparatus (Superbiochips Laboratories, Seoul, Korea). In cases with various histologic features, the most representative area was designated for tissue microarray construction.

Fluorescence In Situ Hybridization

EGFR gene copy number was examined by FISH, as previously reported.[4,8,13] In brief, 4-μm-thick sections from 5 tissue microarray blocks were deparaffinized, dehydrated, immersed in 0.2 N HCl, heated in a microwave in citrate buffer (pH 6.0), and incubated in 1 M NaSCN for 35 minutes at 80° C. The sections were then immersed in pepsin solution, and the tissues were fixed in 10% neutral buffered formalin. Dual probe hybridization was performed using the LSI EGFR Spectrum Orange/CEP7 Spectrum Green probe set (Abbott Molecular, Abbott Park, IL). The probe mixture was applied to the slides, which were then incubated in a humidified atmosphere with Hybrite (Abbott Molecular) at 73° C for 5 minutes to concurrently denature the probe and target DNA and subsequently at 37° C for 19 hours for hybridization. The slides were then immersed in 0.4 × SSC/0.3% NP-40 for 2 minutes at room temperature, followed by 2 × SSC/0.1% NP-40 for 5 minutes at 73° C. The nuclei were counterstained with 4,6-diamidino-2-phenylindole and anti-fade compound (phenylenediamine). The entire area of the tissue microarray core was assessed in each case, and all nonoverlapping nuclei were measured for orange (marker) and green (reference) signals by a pathologist who was unaware of the clinical data. *EGFR* gene status was classified into 6 categories according to the frequency of tumor cells with specific numbers of copies of the *EGFR* gene and chromosome 7 centromere as defined previously[4]: disomy (≤ 2 copies in > 90% of cells); low trisomy (≤ 2 copies in ≥ 40% of cells, 3 copies in 10%-40% of cells, and ≥ 4 copies in < 10% of cells); high trisomy (≤ 2 copies in ≥ 40% of cells, 3 copies in ≥ 40% of cells, and ≥ 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 (presence of tight *EGFR* gene clusters and ratio of the *EGFR* gene to chromosome 7 of ≥ 2, or ≥ 15 copies of *EGFR* per cell in ≥ 10% of cells). According to the *EGFR* gene status, the patients were categorized into 2 sets: *EGFR* $FISH^-$ or low gene copy (disomy, low trisomy, high trisomy, and low polysomy) and *EGFR* $FISH^+$ or high gene copy (high polysomy and gene amplification).

Immunohistochemistry and Scoring

We deparaffinized 4-μm sections from the tissue microarray blocks in xylene and rehydrated in graded alcohol. Immunohistochemical staining of the tissue microarray samples was performed using the Zymed monoclonal antibody (clone 31G7; 1:100; Zymed Laboratories, San Francisco, CA) according to the manufacturer's recommendations.[13]

Semiquantitative estimation of the immunohistochemical staining results was performed by a pathologist who was not informed of the clinicopathologic information or the gene amplification results. Only membranous (partial or complete) staining was termed as positive. The staining intensity of tumor cells was ranked as weak (1+), moderate (2+), or strong (3+). The modified Colorado scoring was performed by multiplying the percentage of positive cells (0%-100%) by the staining intensity (1+ to 3+). Tumors were then categorized as follows: grade 1, score of 0 to 100; grade 2, score of 101 to 200; and grade 3, score of 201 to 300. Tumors with grade ≥ 2 staining were considered positive for EGFR expression.[13]

Statistical Analysis

Fisher's exact or χ^2 test was applied for comparisons of patient groups, and a 2-tailed *P* value ≤ .05 was considered statistically significant. Relapse-free survival (RFS) was calculated from surgery

Table 1 Correlations Between Demographic Characteristics and Molecular Status

Characteristics	Patients, n (%)	*EGFR* FISH$^+$ (n = 45), n/Subgroup (%)	EGFR IHC$^+$ (n = 70), n/Subgroup (%)
Total	114 (100)		
Sex			
Male	104 (91.2)	43/104 (41.3)	64/104 (61.5)
Female	10 (8.8)	2/10 (20.0)	6/10 (60.0)
Age, y			
< 65	46 (40.4)	15/46 (32.6)	23/46 (50.0)
≥ 65	68 (59.6)	30/68 (44.1)	47/68 (69.1)
Smoking			
Never	10 (8.8)	5/10 (50)	4/10 (40.0)
Ever	104 (91.2)	40/104 (38.4)	66/104 (63.5)
Histologic type			
Squamous	99 (86.8)	38/99 (38.3)	62/99 (62.6)
Other	15 (13.2)	7/15 (46.6)	8/15 (53.5)
Stage			
I	41 (36.0)	16/41 (39.0)	28/41 (68.3)
II	49 (42.9)	22/49 (44.8)	29/49 (59.2)
III	23 (20.1)	7/23 (30.4)	12/23 (52.2)
IV	1 (0.9)	0/1 (0)	1/1 (100)
T stage			
T1	37 (32.5)	11/37 (29.7)	21/37 (56.8)
T2	51 (44.7)	23/51 (45.1)	35/51 (68.6)
T3	23 (20.2)	10/23 (43.5)	12/23 (52.2)
T4	3 (2.6)	1/3 (33.3)	2/3 (6.7)
N stage			
N0	60 (52.6)	26/60 (43.3)	38/60 (63.3)
N1	36 (31.6)	14/36 (38.9)	22/36 (61.1)
N2-N3	18 (15.8)	5/18 (27.8)	10/18 (55.6)
Pleural invasion			
Negative	77 (67.5)	27/77 (35.0)	46/77 (59.7)
Positive	37 (32.5)	18/37 (48.6)	24/37 (64.9)
Venous invasion			
Negative	91 (79.8)	36/91 (39.6)	59/91 (64.8)
Positive	23 (20.2)	9/23 (39.1)	11/23 (47.8)
Lymphatic invasion			
Negative	57 (50.0)	20/57 (35.1)	38/57 (66.7)
Positive	57 (50.0)	25/57 (43.9)	32/57 (56.1)
Perineural invasion			
Negative	101 (88.6)	42/101 (41.6)	65/101 (64.4)
Positive	13 (11.4)	3/13 (23.1)	5/13 (38.5)
Surgical margin			
Negative	105 (92.9)	43/105 (40.9)	65/105 (61.9)
Positive	9 (7.1)	2/9 (22.2)	5/9 (55.6)

Abbreviations: EGFR = epidermal growth factor receptor; FISH = fluorescent in situ hybridization; IHC = immunohistochemistry.

until the initial tumor relapse or death from any cause, and overall survival (OS) was estimated from surgery until death from any cause. The Kaplan-Meier method was applied to generate survival curves, and differences between groups were examined using the log-rank test. Multivariate analysis was performed by Cox regression analysis with a significance level of $P < .05$. Factors with significance level of $P < .05$ on univariate analyses were entered into the Cox proportional hazards model. All statistical analyses were performed with SPSS software, version 16.0 (IBM Corp, Armonk, NY).

Table 2 Univariate and Multivariate Analyses of Overall Survival

Variable	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
EGFR FISH						
Negative	1	NA	NA	1	NA	NA
Positive	1.40	1.08-1.83	.011[a]	1.36	1.04-1.78	.025[a]
Stage						
I	1	NA	NA	NA	NA	NA
II-IV	1.89	1.04-3.42	0.036[a]	NA	NA	NA
T stage						
1	1	NA	NA	NA	NA	NA
2-4	2.58	1.33-5.0	.005[a]	NA	NA	NA
Pleural invasion						
Negative	1	NA	NA	1	NA	NA
Positive	2.69	1.57-4.61	< .001[a]	2.04	1.10-3.74	.022[a]
Vascular invasion						
Negative	1	NA	NA	NA	NA	NA
Positive	2.08	1.14-3.78	.016[a]	NA	NA	NA
Surgical margin						
Negative	1	NA	NA	1	NA	NA
Positive	8.52	3.86-18.8	< .001[a]	7.09	2.63-19.1	< .001[a]

Abbreviations: CI = confidence interval; FISH = fluorescent in situ hybridization; HR = hazard ratio; NA = not applicable.
[a]$P < .05$.

Results

Patient Characteristics

We investigated 114 patients with NA-NSCLC. The clinicopathologic features of the study patients are summarized in Table 1. Of the 114 patients, 104 were men and 10 were women, with a median age of 65 years (range, 35-84 years). Most patients had a history of smoking (91%), squamous cell carcinoma (87%), pathologic stage II (43%), and negative surgical margins (92%).

EGFR *Gene Copy Number Assessed by FISH*

EGFR gene disomy was present in 27 patients (23.7%), low trisomy in 11 (9.6%), low polysomy in 24 (21.1%), high trisomy in 7 (6.1%), high polysomy in 36 (31.6%), and gene amplification in 9 (7.9%). Total *EGFR* FISH positivity (high gene copy number) was detected in 39.5% of the NA-NSCLC patients. The distribution of *EGFR* FISH positivity was not related to age, sex, smoking status, histologic type, stage, tumor status, or presence of lymph node metastasis (Table 1).

EGFR Protein Expression

EGFR protein positivity (immunohistochemistry grades 2 to 3) was detected in 70 cases (61.4%). A significant difference was associated with patient age, with older patients expressing greater levels of EGFR than younger patients (72% vs. 50%; $P = .04$). However, EGFR protein expression was not associated with other clinicopathologic factors.

The EGFR protein expression level was associated with *EGFR* gene copy number; 73% (33 of 45) of *EGFR* FISH$^+$ cases and 54% (37 of 69) of *EGFR* FISH$^-$ cases expressed EGFR protein ($P = .035$).

Survival Analysis

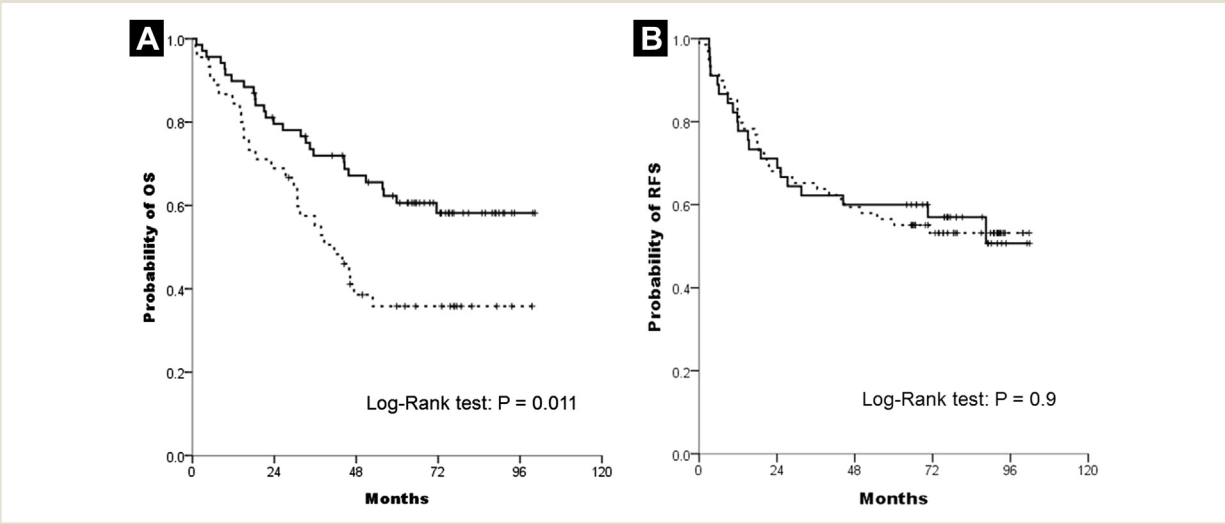
The 5-year OS and RFS rates for all patients were 50.8% and 57.9%, respectively. On univariate analysis, pathologic stage, tumor size, pleural invasion, venous invasion, positive surgical margin, and *EGFR* FISH positivity showed a significant association with survival (Table 2, Figure 1). The 75th percentile survival duration was 16.5 months for *EGFR* FISH$^+$ patients and 34.4 months for FISH$^-$ patients. On multivariate analysis for OS, *EGFR* FISH$^+$ patients had a greater risk of death than did *EGFR* FISH$^-$ patients (hazard ratio, 1.36; 95% confidence interval, 1.04-1.78; $P = .025$). In addition, pleural invasion and positive surgical margins were recognized as independent factors for OS (Table 2).

On analysis of RFS, pathologic staging, pleural invasion, and positive surgical margins were significantly related to RFS (log-rank test, $P = .04$, $P = .032$, $P = .016$, respectively), although these factors were not recognized as independent factors on multivariate analysis. Neither *EGFR* amplification nor protein expression was associated with RFS (Figure 1; Supplemental Figure 1; available in the online version).

Discussion

Our study, which explored whether *EGFR* amplification is associated with clinicopathologic factors and its influence on survival revealed that an increased *EGFR* gene copy number was a poor prognostic factor for patients with operable NA-NSCLC. The frequency of *EGFR* gene amplification (7.9%) and high polysomy (31.6%) in NA-NSCLC patients in our study was comparable to the assessments of nonadenocarcinoma described in other populations. For Western patients, Hirsch et al[4] detected a high *EGFR* gene copy number in 29% of squamous cell carcinomas and 13% of

Figure 1 (A) Overall Survival (OS) and (B) Relapse-Free Survival (RFS) for Patients With Epidermal Growth Factor Receptor (*EGFR*) Fluorescence In Situ Hybridization (FISH)- Negative (Solid Line) and *EGFR* FISH-Positive (Dashed Line) Status



nonsquamous cell carcinomas by FISH analysis. For Asian patients, Jeon et al[8] showed a high *EGFR* gene copy number in 30% of NSCLC cases (26.7% of squamous cell carcinoma and 33% of adenocarcinoma cases) using a FISH method. Lee et al[14] reported an increased *EGFR* gene copy number in 26.7% of patients with advanced squamous cell carcinoma of the lung using FISH evaluation. Liu et al[10] reported *EGFR* polysomy in 30% to 46% of NSCLC cases by chromogenic in situ hybridization. In a different FISH analysis, 62% of NSCLC patients were positive for *EGFR*. Using next-generation sequencing methods, amplification of *EGFR* was found in 7% cases of squamous cell carcinoma of the lung in The Cancer Genome Atlas data set.[1] Whole exome sequencing of Korean patients with squamous cell carcinoma showed that the frequency of somatic copy number variation of *EGFR* with low-level gain or focal high-level gain was 18%.[15] To use *EGFR* FISH for analysis of the prognosis in patients with operable NA-NSCLC patients, it is necessary to define the optimal assay for clinical testing of *EGFR* amplification.

Some previous studies have reported that a high *EGFR* gene copy number (high polysomy and amplification) is accompanied by inferior prognosis in patients with advanced NSCLC; however, similar research studies for early-stage surgically resected NA-NSCLC patients are very limited.[16,17] Hirsch et al[4] showed that a high *EGFR* gene copy number was related to a tendency toward shorter survival in patients with stage I to III resected NSCLC ($P = .13$). Our study revealed that patients with *EGFR* FISH$^+$ NA-NSCLC had poorer survival than FISH$^-$ patients. Jeon et al[8] revealed that a high *EGFR* gene copy number was associated with significantly inferior survival for patients with stage I squamous cell carcinoma of the lung ($P = .04$); however, the *EGFR* gene copy status did not meaningfully affect the OS of adenocarcinoma patients. These data suggest that the association of *EGFR* gene copy number with prognosis in NSCLC might be influenced by the histologic tumor type.

In addition, this finding could suggest that an amplified wild-type *EGFR* tumor is dependent on the EGFR signaling pathway for tumor growth, as proposed by preclinical and clinical studies of advanced NA-NSCLC in which 75% (3 of 4 human cancer samples) of *EGFR* wild-type lung cancer stem cells with amplified *EGFR* were sensitive to erlotinib.[5] Lee et al[14] reported that *EGFR* FISH$^+$ patients with squamous cell carcinoma of the lung showed a significantly better response rate to EGFR-tyrosine kinase inhibitors compared with *EGFR* FISH$^-$ patients. Moreover, patients with *EGFR* FISH$^+$ tumors had a lower probability of progression during EGFR tyrosine kinase inhibitor treatment compared with those with *EGFR* FISH$^-$ tumors.[14]

This hypothesis requires validation in further NA-NSCLC studies because the reports of the influence of *EGFR* gene copy number on outcomes have been inconsistent. Liu et al[10] did not observe any influence of high polysomy ($\geq$ 5 copies), determined using chromogenic in situ hybridization, in surgically treated patients from Taiwan. Tsao et al[7] reported that the tumor samples from the NSCLC CTG JBR.19 study, a comparison of adjuvant chemotherapy with vinorelbine/cisplatin against observation, showed no differences in OS between patients with high- and low-copy *EGFR*.

It is known that the *EGFR* gene copy number and protein expression are well correlated in NSCLC.[8] In our study, we detected EGFR expression in 61% of NA-NSCLC cases and found a strong association between EGFR protein expression and *EGFR* gene copy status. However, EGFR protein expression did not affect the overall prognosis. Thus, survival was more highly related to gene copy number alterations than to the protein level of EGFR. Processes other than amplified gene copy number, such as increased

transcription or post-translational modification, could also be involved in high EGFR expression in NA-NSCLC.[8]

Molecular studies have demonstrated that *SOX2* and *FGFR1* amplifications are independent prognostic factors in surgically resected squamous cell carcinoma.[18,19] Therefore, comprehensive investigation of *EGFR*, *FGFR1,* and *SOX2* amplification will be required to accurately predict the prognosis of patients with NA-NSCLC.

Because our study was a retrospective, single-institution study with relatively small patient numbers, we have some limitations. The effect of patient comorbidity and subsequent treatment could have affected survival in our study. Because the *EGFR* gene copy number predicted for OS but not RFS in our study, it could be related to postprogression survival (data not shown). A prospective study is intended to confirm the ability of *EGFR* FISH evaluation of surgical tumor tissue to predict the prognosis of NA-NSCLC patients.

Conclusion

We have demonstrated that the *EGFR* gene copy number is associated with a worse prognosis in patients with surgically resected NA-NSCLC.

Clinical Practice Points

- A high *EGFR* gene copy number (high polysomy and amplification) is known to be accompanied by an inferior prognosis in patients with advanced NSCLC; however, similar research studies for early-stage NA-NSCLC patients are very limited.
- For patients who underwent curative intent surgical resection, *EGFR* gene amplification and high polysomy were significantly associated with shorter OS.
- *EGFR* FISH evaluation, in addition to clinicopathologic factors, might better predict the prognosis of early-stage or locally advanced NA-NSCLC patients.

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Disclosure

The authors declare that they have no competing interests.

Supplemental Data

The supplemental figure accompanying this article can be found in the online version at https://doi.org/10.1016/j.cllc.2018.06.003.

References

1. Cancer Genome Atlas Research Network. Comprehensive genomic characterization of squamous cell lung cancers. *Nature* 2012; 489:519-25.
2. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; 304:1497-500.
3. Soda M, Choi YL, Enomoto M, et al. Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 2007; 448:561-6.
4. Hirsch FR, Varella-Garcia M, Bunn PA Jr, et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol* 2003; 21:3798-807.
5. Sette G, Salvati V, Mottolese M, et al. Tyr1068-phosphorylated epidermal growth factor receptor (EGFR) predicts cancer stem cell targeting by erlotinib in preclinical models of wild-type EGFR lung cancer. *Cell Death Dis* 2015; 6:e1850.
6. Chang H, Zhang X, Cho BC, et al. Tumor MET expression profile predicts the outcome of non-small cell lung cancer patients receiving epidermal growth factor receptor tyrosine kinase inhibitors. *Thorac Cancer* 2014; 5:517-24.
7. Tsao MS, Sakurada A, Ding K, et al. Prognostic and predictive value of epidermal growth factor receptor tyrosine kinase domain mutation status and gene copy number for adjuvant chemotherapy in non-small cell lung cancer. *J Thorac Oncol* 2011; 6:139-47.
8. Jeon YK, Sung SW, Chung JH, et al. Clinicopathologic features and prognostic implications of epidermal growth factor receptor (EGFR) gene copy number and protein expression in non-small cell lung cancer. *Lung Cancer* 2006; 54:387-98.
9. Chang H, Oh J, Zhang X, et al. EGFR protein expression using a specific intracellular domain antibody and PTEN and clinical outcomes in squamous cell lung cancer patients with EGFR-tyrosine kinase inhibitor therapy. *Onco Targets Ther* 2016; 9:5153-62.
10. Liu HP, Isaac Wu HD, Chang JW, et al. Prognostic implications of epidermal growth factor receptor and KRAS gene mutations and epidermal growth factor receptor gene copy numbers in patients with surgically resectable non-small cell lung cancer in Taiwan. *J Thorac Oncol* 2010; 5:1175-84.
11. Beasley MB, Brambilla E, Travis WD. The 2004 World Health Organization classification of lung tumors. *Semin Roentgenol* 2005; 40:90-7.
12. Goldstraw P, Crowley J, Chansky K, et al. The IASLC lung cancer staging project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours. *J Thorac Oncol* 2007; 2:706-14.
13. Lee HJ, Xu X, Choe G, et al. Protein overexpression and gene amplification of epidermal growth factor receptor in nonsmall cell lung carcinomas: comparison of four commercially available antibodies by immunohistochemistry and fluorescence in situ hybridization study. *Lung Cancer* 2010; 68:375-82.
14. Lee Y, Shim HS, Park MS, et al. High EGFR gene copy number and skin rash as predictive markers for EGFR tyrosine kinase inhibitors in patients with advanced squamous cell lung carcinoma. *Clin Cancer Res* 2012; 18:1760-8.
15. Kim Y, Hammerman PS, Kim J, et al. Integrative and comparative genomic analysis of lung squamous cell carcinomas in East Asian patients. *J Clin Oncol* 2014; 32:121-8.
16. Zhu CQ, da Cunha Santos G, Ding K, et al. Role of KRAS and EGFR as biomarkers of response to erlotinib in National Cancer Institute of Canada Clinical Trials Group Study BR.21. *J Clin Oncol* 2008; 26:4268-75.
17. Douillard JY, Shepherd FA, Hirsh V, et al. Molecular predictors of outcome with gefitinib and docetaxel in previously treated non-small-cell lung cancer: data from the randomized phase III INTEREST trial. *J Clin Oncol* 2010; 28:744-52.
18. Wilbertz T, Wagner P, Petersen K, et al. SOX2 gene amplification and protein overexpression are associated with better outcome in squamous cell lung cancer. *Mod Pathol* 2011; 24:944-53.
19. Kim HR, Kim DJ, Kang DR, et al. Fibroblast growth factor receptor 1 gene amplification is associated with poor survival and cigarette smoking dosage in patients with resected squamous cell lung cancer. *J Clin Oncol* 2013; 31:731-7.

Supplemental Figure 1 (A) Overall Survival (OS) and (B) Relapse-Free Survival for Patients With Epidermal Growth Factor Receptor (EGFR) Immunohistochemistry-Negative (IHC^-) (Solid Line) and EGFR IHC^+ (Dashed Line) Status. *P* Value for Log-Rank Test Was 0.135 for OS and 0.207 for RFS, respectively