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Original Article

# Therapeutic efficacy of gefitinib and erlotinib in patients with advanced lung adenosquamous carcinoma

Zhengbo Song, Baochai Lin, Lan Shao, Yiping Zhang*

*Department of Chemotherapy, Zhejiang Cancer Hospital and Key Laboratory Diagnosis and Treatment Technology on Thoracic Oncology, Zhejiang Province, Hangzhou, China*

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**Abstract**

*Background*: Adenosquamous carcinoma (ASC) of the lung is a rare subtype of nonsmall-cell lung cancer (NSCLC). To date, the efficacious targeted therapy for advanced ASC remains unclear and the epidermal growth factor receptor (EGFR) mutation rate is not well known.
*Methods*: We retrospectively reviewed clinical information of patients with ASC who were treated with gefitinib or erlotinib at Zhejiang Cancer Hospital between January 2007 and December 2011. Survival analysis was evaluated by the Kaplan-Meier method. EGFR mutations were assessed in part using direct sequencing methods.
*Results*: In total, 49 patients with a median age of 57 years were used in this study. Thirteen patients achieved a partial response and 19 had disease stabilization. The objective response rate was 26.5%, and the disease control rate was 65.3%. The median progression-free survival and overall survival were 4.3 and 17.6 months, respectively. In 21 patients with adequate specimens for molecular analysis, 7 (33.3%) had EGFR mutations (4 with deletions within exon 19 and 3 with L858R messenger mutation in exon 21). EGFR mutations were significantly more frequent in women (4/9, 44.4%) than men (3/12, 25%), never-smokers (6/15, 40%), and smokers (1/6, 16.7%).
*Conclusion*: EGFR-tyrosine kinase inhibitor (TKI) is an effective treatment for ASC. The frequency of EGFR mutation and clinical characteristics of the EGFR mutants in ASC are similar to those of Asian patients with adenocarcinoma.


*Keywords:* adenosquamous carcinoma; efficacy; EGFR-TKI; nonsmall-cell lung cancer

## 1. Introduction

Adenocarcinoma and squamous cell carcinoma (SCC) are two major subtypes of NSCLC. Adenosquamous carcinoma (ASC) is a rare subtype of NSCLC, comprising 0.4–4% of pulmonary carcinomas.[1–4] ASC is a mixed histologic tumor; according to the last WHO lung tumor classification criteria, it is defined as "a carcinoma showing components of both squamous cell carcinoma and adenocarcinoma, with each comprising to at least 10% of the tumor".[5] A clinicopathological analysis has demonstrated that ASC is more aggressive than adenocarcinoma and SCC, indicating that its biological features are different from these types of NSCLC.[6–8] The epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) including gefitinib and erlotinib are the primary drugs of choice for the treatment of NSCLC patients, especially for the patients harboring EGFR mutations.[9,10] However, no clinical study to evaluate efficacy of EGFR-TKIs and mutation frequency for ASC has been conducted thus far.

We conducted a focused analysis targeting Chinese populations in order to track the EGFR mutation status of ASC, as well as to check the feasibility of EGFR-TKIs treatment in ASC.

## 2. Methods

### 2.1. Patients

A retrospective review of patients from the Zhejiang Cancer Hospital between January 2007 and December 2011

* Corresponding author. Dr. Yiping Zhang, Department of Chemotherapy, Zhejiang Cancer Hospital, 38, Guangji Road, Hangzhou 310022, China.
*E-mail address:* yipingzhang@yahoo.cn (Y.-P. Zhang).

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was conducted. The Ethics Committee at Zhejiang Cancer Hospital approved the study. NSCLC staging was performed according to the 7th TNM classification. The inclusion criteria were as follows: (1) pathologically proven primary ASC, (2) all patients were supplied with TKI during the treatment course, (3) disease recurrence was confirmed using chest computed tomography (CT), brain MRI and bone scan as well as ultrasound examination and/or CT of the abdomen, (4) without any local treatment such as radiotherapy or interventional therapy during the period of TKI therapy, and (5) at least one measurable lesion and an Eastern Cooperative Oncology Group performance status of 0 to 3.

### 2.2. Pathology and EGFR mutation examination method

To confirm the histology of ASC, each of the slides previously identified was examined independently by two specialists according to the World Health Organization criteria (2004 version). The molecular analysis of EGFR was performed using direct sequencing methods with formalin-fixed paraffin embedded archival tissue blocks. Additionally, EGFR mutation analysis using direct sequencing occurred as previously described.[11] Briefly, DNA was extracted from the tumors using a QIAmp DNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocols. The tyrosine kinase domain of the EGFR coding sequences were analyzed by Sequencer 3.1.1. software (Applied Biosystems) to compare variations. Exons 18-21 of EGFR were then examined.

### 2.3. Responses and toxicity

Tumor responses were assessed with computed tomography (CT) at 4−8-week intervals until the lesions were evaluated as the progressive disease. The tumor response was evaluated based on the Response Evaluation Criteria in Solid Tumor (RECIST) version 1.1. Objective tumor responses include complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Objective response included the CR and PR. Disease control rate (DCR) was defined as the addition of objective response and stabilization rates (CR + PR + SD).

The toxicity of TKI treatment was evaluated in accordance with the National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.0.

### 2.4. Follow-up

All patients that were evaluated for the TKI tumor response had a progression-free survival (PFS), and none of the patients were lost to follow-up. The median follow-up period was 20.2 months (3.9-52); the last follow-up time was January 30, 2012.

### 2.5. Statistical analysis

Categorical variables were compared using the $\chi^2$ test and continuous variables by the Mann-Whitney nonparametric test. Survival was recorded from the first-line of treatment to the date of death or that of the last follow-up visit. The PFS encompassed the time from the TKI therapy to documented progression or death from any cause. The survival curves were calculated according to the method of Kaplan-Meier. The Cox proportional model was used to evaluate various prognostic factors. Values of $p < 0.05$ were considered significant. Analyses were conducted using the computer software SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Patient characteristics

Overall, 1679 patients were diagnosed with primary NSCLC between January 2007 and December 2011. Of these, 975 had advanced disease or recurrence after surgery. TKI therapy was administered to these 975 patients (551 with adenocarcinoma, 102 with squamous cell carcinoma, 49 with ASC, 67 with other histologies).

Of the 49 ASC patients enrolled in the clinical study of TKI treatment, there were 26 males and 23 females. The performance status (PS) was 0−1 in 40 patients (81.6%) and PS 2-3 accounted for 18.4%. The median age of the patients was 56 years (range 40−76 years). Twenty-one of them were in advanced stage on presentation and 28 presented with a disease recurrence. Thirteen patients had a history of smoking. Thirty-eight patients received TKI treatment in the second-line and 11 in the third-line or further-line treatment. Thirty-one patients received gefitinib and 18 received erlotinib treatment. The patients' baseline characteristics are listed in Table 1.

### 3.2. EGFR mutation analysis

Twenty-one patients provided tumor samples for EGFR mutation analysis out of a total of 49 ASC patients (9 female and 12 male). EGFR mutations were identified in seven (30%) patients (4 with deletion in exon 19 and 3 with L858R in exon 21). EGFR mutations occurred significantly more frequently in women (4/9, 44.4%) than men (3/12, 25%) ($p = 0.64$). Patients who had never smoked (6/15, 40%) had EGFR mutations more commonly than smokers (1/6, 16.7%) ($p = 0.61$). One hundred and ninety-two patients provided tumor samples for EGFR mutation analysis in adenocarcinoma. EGFR mutations were identified in 58 (30.2%) patients. The EGFR mutations were identified in four patients among 74 SCC tumor samples (4/74, 5.41% ). There was a significant difference among the ASC, adenocarcinoma, and SCC patients in EGFR mutation frequency ($p < 0.001$).

### 3.3. Efficacy and comparison among adenocarcinoma, SCC, and ASC

Among the 49 ASC patients, 13 had PR and 19 patients had SD in the gefitinib or erlotinib treatment, accounting for a disease control rate of 65.3% (32/49). The median PFS during

Table 1
Comparison of baseline characteristics and efficacy between the ASC, adenocarcinoma, and SCC treatments.

| Variables | ASC ($n = 49$) | Adenocarcinoma ($n = 512$) | SCC ($n = 102$) | $p$ |
|---|---|---|---|---|
| Gender | | | | |
| Male | 26 | 262 | 74 | <0.001 |
| Female | 23 | 250 | 28 | |
| PS | | | | |
| 0–1 | 40 | 394 | 83 | 0.502 |
| 2–3 | 9 | 118 | 19 | |
| Age (y) | | | | |
| <65 | 39 | 410 | 80 | 0.930 |
| ≥65 | 10 | 102 | 22 | |
| Smoking | | | | |
| Yes | 36 | 352 | 83 | 0.034 |
| No | 13 | 160 | 19 | |
| Treatment | | | | |
| Gefitinib | 31 | 291 | 37 | <0.001 |
| Erlotinib | 18 | 221 | 65 | |
| Prior chemotherapy | | | | |
| 1 | 38 | 334 | 41 | <0.001 |
| ≥2 | 11 | 178 | 61 | |
| ORR | 26.50% | 19.50% | 8.80% | 0.012 |
| DCR | 65.30% | 59.60% | 36.30% | <0.001 |
| Median PFS (mo) | 4.3 | 4.2 | 1.93 | 0.047 |
| Median OS (mo) | 17.6 | 18.7 | 12.2 | <0.001 |

ASC = adenosquamous carcinoma; DCR = disease control rate; ORR = objective response rate; OS = overall survival; PFS = progression-free survival; PS = performance status; SCC = squamous cell carcinoma.

gefitinib and erlotinib treatment was 4.3 months (95% CI: 2.5-6.1). The median survival time for all patients was 17.6 months (95% CI: 12.1-20.5).

Among the 551 patients with adenocarcinoma who received gefitinib or erlotinib treatment, 39 received the drug as part of the first-line treatment and 512 as part of the second-line or further-line treatment. One hundred and two SCC patients received TKI treatment. There were significant difference in PFS among ASC, adenocarcinoma, and SCC treatment (4.3 months vs. 4.2 months vs.1.93 months, $p = 0.047$) (Table 1 and Fig. 1).

### 3.4. The relationship between EGFR mutation and efficacy

Among the 49 ASC patients, there were 21 patients with adequate specimens for EGFR mutation analysis, among the 21 patients, 7 with EGFR mutation and 14 with EGFR wild-type. Five patients had PR, one had SD, and one had PD in the patients harboring EGFR mutation. In contrast, the objective response rate (ORR) was 7.1% (1/14) and DCR was 42.9% (5/14) in the wild-type group. In the 21 ASC patients, the PFS was 2.1 months and 8.7 months in the wild-type and mutation groups, respectively ($p = 0.013$) (Fig. 2). In 58 adenocarcinoma patients with EGFR mutation, the ORR was 65.5% (38/58) and DCR was 84.5% (49/58). Among the 134 patients with wild-type, the ORR 6.72% (9/134) and DCR was 36.57% (49/134). The PFS was 2.4 months and 9.4 months in wild-type and mutation group, respectively

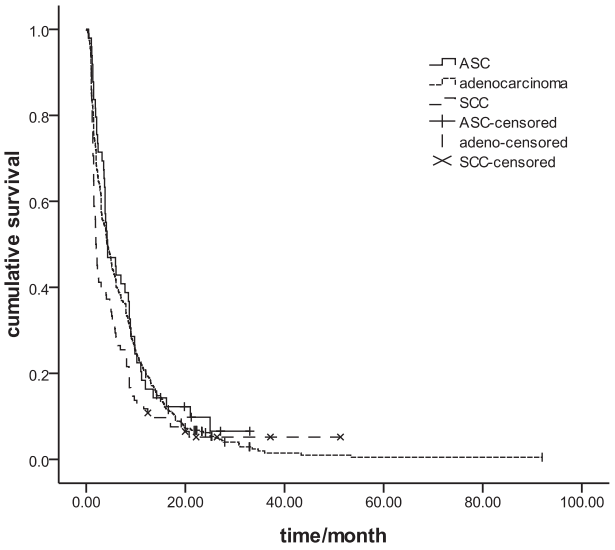


Fig. 1. Progression-free survival in ASC, adenocarcinoma and SCC patients ($p = 0.047$).

($p < 0.001$). There were only four cases with EGFR mutation among 74 SCC patients who could provide tumor samples for EGFR mutation analysis. The ORR was 75% in the mutation and 5.71% in wild-type patients. The PFS was 7.0 months and 1.93 months in mutation and wild-type patients, respectively ($p < 0.001$). There were no significant difference in PFS among the ASC, adenocarcinoma and SCC patients in the EGFR mutation patients ($p = 0.343$) (Fig. 3).

### 3.5. Factors affecting PFS by univariate and multivariate analysis

The performance status (PS) score was the only factor influence the PFS ($p = 0.031$). No other factors correlated

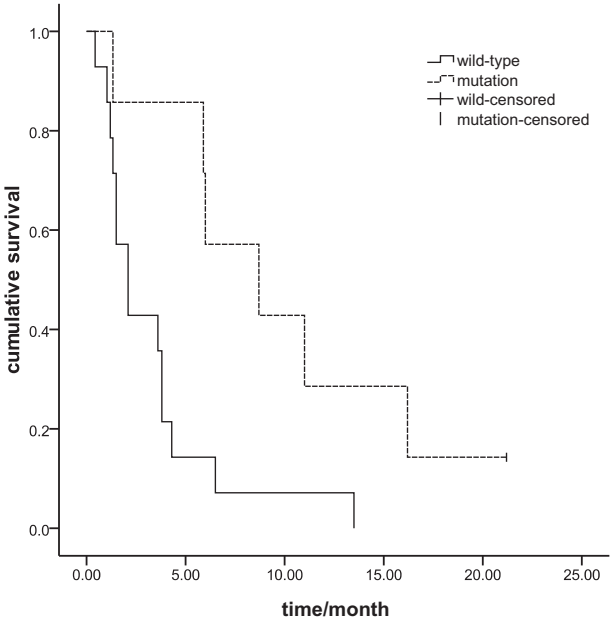


Fig. 2. Progression-free survival of ASC in EGFR mutation patients ($p = 0.013$).

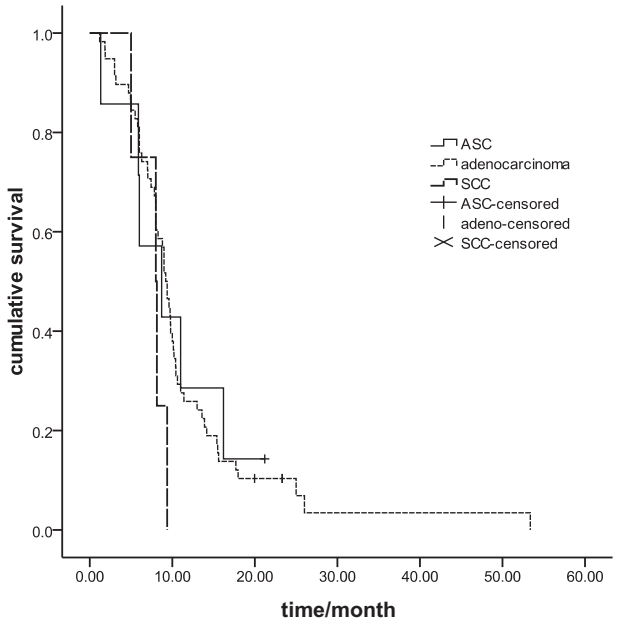


Fig. 3. Progression-free survival of ASC, adenocarcinoma and SCC in EGFR mutation patients ($p = 0.343$).

significantly with PFS, but we observed the prolong trends in female and nonsmokers ($p = 0.099$ and 0.097, respectively).

A multivariate Cox regression model was constructed incorporating patient age, sex, PS, smoking history, TKI type (erlotinib or gefitinib) and prior chemotherapy. PS remained as the independent prognostic factor for PFS (Table 2).

### 3.6. Toxicity of TKI treatment

Toxicity was evaluated in all the 49 patients. The most common adverse event was skin toxicity in 25 patients (51.0%), including five patients with Grade 3. Other common toxicities included diarrhea (12 cases) and fatigue (11 cases). One patient showed deteriorated hepatic function with erlotinib therapy. However, no dosage reduction occurred.

## 4. Discussion

ASC is an uncommon lung cancer. Owing to its rarity, this disease has received far less attention in its clinical diagnosis and prognosis, and few studies have been published about ASC. In this series of patients with ASC who received gefitinib or erlotinib, the objective response and disease control rates were 26.5% and 65.3%, respectively, and the overall median survival duration was 17.6 months. To the best of our knowledge, this is the first report that focused on the efficacy of EGFR-TKIs for ASC.

Table 2
Cox regression analysis about PFS of ASC patients.

| Variables | HR | 95% confidence interval | $p$ |
|---|---|---|---|
| Sex | 0.498 | 0.215−1.152 | 0.670 |
| Age | 0.913 | 0.363−2.294 | 0.846 |
| PS | 3.228 | 1.277−8.160 | 0.013 |
| TKI type | 2.230 | 0.861−5.780 | 0.199 |
| Prior chemotherapy | 0.961 | 0.499−1.852 | 0.906 |
| Smoking history | 0.641 | 0.230−1.639 | 0.331 |
| Surgical history | 1.576 | 0.769−2.572 | 0.898 |

CI = confidence interval; HR = hazard ratio; PS = performance status; TKI = tyrosine kinase inhibitor.

Owing to the low incidence, available data on EGFR mutation status in ASC was very limited. Several cases of ASC showing EGFR mutations have been reported with an EGFR mutation frequency of about 30-44%,[12−15] which indicated that the mutation incidence is similar to adenocarcinoma. The female, nonsmokers had a high frequency EGFR mutation in Kang's report with 25 Korean patients, which was confirmed by Sasaki et al and Toyooka et al.[12−14] However, the EGFR mutation was not associated with gender, age, and smoking history in Jia's report ,which included 55 Chinese ASC patients.[15] Among the 21 patients in our data, there were seven patients with EGFR mutation. The female, nonsmoking patients had a high frequency of EGFR mutation.

Owing to the rarity of this subtype carcinoma, the efficacy of ASC with TKI was not well known until now. Seven cases of ASC showing EGFR mutations have been reported.[16,17] A pooled analysis by Shukuya et al included three patients of ASC with EGFR mutation, with all three patients with L858R mutation in exon 21. Two of the patients reached SD, and one had PR with gefitinib treatment.[18]

For Asian patients with adenocarcinoma who received gefitinib or erlotinib in the second- or third-line treatment, the PFS was ranged from 3 months to 5 months.[19] However, the treatment efficacy is much lower in patients with squamous lung cancer. In the current study, the PFS of ASC was 4.3 months, which was similar to the adenocarcinoma. For the mutation patients, the PFS was about 9 months with the TKI therapy in adenocarcinoma. The median PFS in our seven patients was 8.7 months, which is also similar to the previous studies.

The major limitation of the present study is its retrospective nature. In addition, direct sequencing was used to analyze the EGFR mutations in our patients. It has a lower sensitivity than the amplification refractory mutation system (ARMS), which may increase the false-negatives in the EGFR mutation result. Less than half of our patients had EGFR mutation information, which also influenced our clinical analysis. However, with few cases even in limited clinical trials, our retrospective study can also be considered to be meaningful.

In conclusion, a significant proportion of ASC patients would derive clinical benefit from TKI treatment. A similar mutation frequency was found in ASC and in adenocarcinoma. Prospective studies with a larger cohort should be conducted to verify the efficacy of TKI in ASC patients and mutation frequency.

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