Real-world data on EGFR/ALK gene status and first-line targeted therapy rate in newly diagnosed advanced non-small cell lung cancer patients in Northern China: A prospective observational study

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Keywords
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Abstract

Background: Tyrosine kinase inhibitors (TKIs) can significantly prolong overall survival for patients with advanced non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor (EGFR)-mutation or anaplastic lymphoma kinase (ALK)-rearrangement. However, the real-world evaluation status of ALK/EGFR in China remains unclear.

Methods: We conducted a prospective study including 1134 patients with cytologically or histologically confirmed advanced NSCLC (stage IIIb–IV) at 12 Chinese hospitals. The most common evaluation methods were amplification-refractory mutation system for EGFR status and immunohistochemistry targeting D5F3 for ALK status. Among patients with non-squamous, the EGFR mutation rate was 44.1% and the ALK rearrangement rate was 10.0%. Among patients with squamous cell carcinoma, the EGFR mutation rate was 8.3% and the ALK rearrangement rate was 3.7%. Among all patients, gender (HR = 1.7, 95% CI = 1.2–2.4, P = 0.006), smoking history (HR = 1.8, 95%CI = 1.3–2.7, P = 0.001), and brain metastases (HR = 5.0, 95%CI = 2.4–10.1, P < 0.001) were independent predictors of EGFR or ALK status confirmation was 7 and 5 days, respectively. Targeted therapy rate was 73.8% in EGFR-positive patients and 51.4% in ALK-positive patients. There was a negative correlation between the first-line targeted therapy rate and the EGFR mutation detection period (r = −0.152, P = 0.02), while no significant correlation among patients with ALK rearrangement (r = −0.179, P = 0.076).
Introduction

Lung cancer has become one of the most common cancers worldwide, with high morbidity and mortality rates as most patients are not eligible for radical surgery at the time of diagnosis. Furthermore, traditional radiotherapy and chemotherapy have limited effects in cases of non-small cell lung cancer (NSCLC). Recent research regarding targeted therapy such as epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) and anaplastic lymphoma kinase inhibitors (ALK-TKIs) has revealed significant improvements in overall survival rates of NSCLC patients harboring EGFR mutation or ALK rearrangement. As a result, targeted therapy has been approved as a first-line treatment for these patients.1–12

Several studies have been performed to determine the current status of EGFR-mutation/ALK-rearrangement in Asia, including China. For example, Pan et al.13 analyzed 176 NSCLC patients treated at the First Affiliated Hospital of Wenzhou Medical College, and observed that the total mutation rate of the EGFR gene in exons 19, 20, and 21 was 48.3% (85/176). They further identified several factors, including female gender, adenocarcinoma, distant metastasis, and the chemotherapy, that may increase the probability of EGFR gene mutations. Shi et al.14 analyzed 747 patients with advanced NSCLC among a subset of patients from mainland China with an adenocarcinoma history as part of the PIONEER study, and found that the overall EGFR mutation rate was 50.2% among the 741 patients that were successfully genotyped, while the activating EGFR mutation rate was 48.0% (with 1.3% of patients showing combined activating and resistance mutations). Smoking history and regional lymph nodes involvement were identified as independent predictors of EGFR mutation in multivariate analysis. Zhou et al.15 analyzed EGFR mutations of 261 patients with pathologically confirmed NSCLC from West China Hospital, and observed that the EGFR mutation rate was 48.7%, with smoking status and pathological types as independent predictors. Fu et al.16 recruited 487 lung cancer patients who underwent testing for ALK rearrangement at Sun Yat-sen University Cancer Center, and found that the ALK rearrangement rate was 9.0% (44/487), and that ALK-rearranged NSCLC tended to occur in younger individuals who were either non-smokers or light smokers with adenocarcinoma.

However, the current evaluation methods and periods of EGFR mutation and/or ALK rearrangement, as well as the first-line targeted therapy rate in patients with NSCLC harboring EGFR mutations or ALK rearrangement in China remain unclear. Moreover, previous studies have been limited by their retrospective design. Therefore, we conducted a prospective multicenter study with the goal of determining the detection methods and detection periods of EGFR mutation and ALK rearrangement, the EGFR mutation rate, ALK rearrangement rate, and first-line targeted therapy rate in patients with NSCLC harboring EGFR mutations or ALK rearrangement in northern China.

Conclusion: Squamous NSCLC patients should also be routinely tested to determine their EGFR/ALK statuses. The first-line targeted therapy rate remains low in Chinese patients with NSCLC.

Methods

Study design and patients

We conducted a prospective, epidemiological, multicenter, pan-label, and non-comparative study of EGFR mutation and ALK rearrangement evaluation status, and first-line targeted therapy rate of patients with newly diagnosed advanced (stage IIIb–IV) NSCLC. This study only involved an observational protocol, and did not affect the patients’ diagnosis and treatment. The study protocol was approved by the participating institutions’ ethics committees. Patients who were eligible for enrollment provided written informed consent for participation in the study.

Patients with locally advanced or metastatic NSCLC (stage IIIb–IV) were enrolled at 12 hospitals in northern China between March 2015 and April 2017. The inclusion criteria were: (i) age ≥ 18 years, (ii) new diagnosis of NSCLC confirmed using histology or cytology, (iii) locally advanced or metastatic NSCLC (stage IIIb–IV or recurrent cases that were not eligible for surgery or radical chemoradiotherapy), (iv) simultaneous results for EGFR mutation and ALK rearrangement testing, and (v) no previous systemic treatment (except adjuvant chemotherapy). The exclusion criteria were: (i) previous non-adjuvant systemic treatment, (ii) only sputum pathology specimens available, (iv) genetic results from sputum or blood samples, and (v) gene testing methods that did not fulfill the inclusion criteria.

Data collection

Demographic and clinical characteristics of patients were collected, including age at diagnosis, gender, smoking status, date of first pathological diagnosis, method of
EGFR-mutation and ALK-rearrangement analysis

Tumor samples were obtained from primary or metastatic lesions, handled and stored following the respective laboratories’ quality control requirements. The EGFR-mutation was analyzed by amplification refractory mutation system (ARMS) or next-generation sequencing (NGS), whereas the ALK-rearrangement was analyzed by fluorescence in situ hybridization (FISH), NGS, or Ventana immunohistochemistry (IHC) targeting D5F3.

Statistical analyses

All statistical analyses were performed using IBM SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). Continuous variables are expressed as the means ± standard deviation. Associations between mutations and demographic and clinical characteristics were analyzed by Fisher’s exact tests. Characteristics significantly (P < 0.05) associated with mutations were then included in a multivariate logistic model. The hazard ratio (HR) and 95%CI were calculated for all variables in the regression model. The correlation between first-line targeted therapy and the EGFR/ALK detection period were analyzed by Spearman’s correlation. All tests were two-sided, and statistical significance was set at P < 0.05. It is noteworthy that some patients underwent radical treatment and subsequently underwent EGFR/ELK gene testing several years after the diagnosis, which would not accurately reflect the EGFR/ALK detection period. Thus, the data from these cases were omitted from the related analyses of EGFR/ALK detection period.

Results

Patient characteristics

Between March 2015 and April 2017, a total of 1134 patients with cytologically or histologically confirmed advanced NSCLC (stage IIIb–IV) were enrolled in the study at 12 Chinese hospitals. Among these patients, the most common pathological type was adenocarcinoma (973 cases), followed by squamous cell carcinoma (109 cases), unclassified carcinoma (36 cases), adenosquamous carcinoma (11 cases), sarcomatoid carcinoma (3 cases), and large cell carcinoma (2 cases). The specimens were evaluated using histology (976 cases) and cytology (158 cases). The cases involved primary lesions (757 cases) or metastatic lesions (377 cases), including 172 cases of distal/local lymph node metastases, 126 cases of pleural effusion, 25 cases of pleural metastasis, 21 cases of bone metastasis, 10 cases of liver metastasis, eight cases of brain metastasis, seven cases of subcutaneous nodule metastasis, and eight cases of other metastatic sites. The most common biopsy methods were bronchoscopy (395 cases) and computed tomography-guided lung puncture (365 cases). The other cases involved ultrasound-guided puncture (191 cases), bone biopsy (16 cases), surgical biopsy (133 cases), radical resection (32 cases), and cerebrospinal fluid collection (2 cases). The most common biopsy site was the lung (66.8%), the most common biopsy method was bronchoscopy (34.6%), and the most common metastatic site was the lung (22.6%). The cases of squamous NSCLC patients frequently involved male patients (92/109) who were >60 years old (70/109), and patients with a smoking history (86/109). The cases of non-squamous NSCLC patients involved male patients (550/1025) with a smoking history (430/1025), and patients who were >60 years old (516/1025). Table 1 summarizes the clinicopathological features of 1134 NSCLC patients, 1025 non-squamous NSCLC patients, and 109 squamous NSCLC patients.

EGFR/ALK evaluation status

Among all patients, the most commonly used methods of detection for EGFR mutation and ALK rearrangement were ARMS (1029/1134, 90.7%) and IHC targeting D5F3 (692/1134, 61.0%), respectively. Among patients with non-squamous NSCLC, the most commonly used methods of detection for EGFR mutation and ALK rearrangement were ARMS (933/1025, 91.0%) and IHC targeting D5F3 (637/1025, 62.1%), respectively. Six cases were evaluated for ALK rearrangement using both NGS and IHC (2 cases) or both ARMS and IHC (4 cases), which revealed consistent findings. Twenty-two cases were evaluated for ALK rearrangement using both FISH and IHC, which revealed consistent findings in 21 cases and inconsistent findings in one case (positive IHC results and negative FISH results). Among patients with squamous NSCLC, the most commonly used methods of detection for EGFR mutation and ALK rearrangement were ARMS (96/109 88.1%) and IHC targeting D5F3 (55/109, 50.1%), respectively. One case was evaluated for ALK rearrangement using both ARMS and IHC, which revealed consistent findings. Two cases were evaluated for ALK rearrangement using both FISH and IHC, which revealed inconsistent findings (positive IHC results and negative FISH results).
The median time from the biopsy to tumor diagnosis was three days (range: 0–54 days). Sixty patients underwent genetic testing several years after undergoing radical therapy, which would not accurately reflect the EGFR/ALK detection period; thus, these cases were omitted from the following analyses. Among the remaining cases, the median time from tumor diagnosis to EGFR status confirmation was seven days (range: 0–84 days), with median times of seven days (range: 0–84 days) for ARMS and nine days (range: 0–42 days) for NGS. The median time from tumor diagnosis to ALK status confirmation was five days (range: 0–81 days), with median times of seven days (range: 0–81 days) for NGS, seven days (range: 0–55 days) for FISH, and four days (range: 0–42 days) for IHC.

EGFR/ALK evaluation results

Among all patients, the EGFR mutation rate was 40.7% (461/1134), which included 100 adenocarcinomas, four squamous cell carcinomas, two adenosquamous carcinomas, and one NSCLC. Among patients with non-squamous, the EGFR mutation rate was 44.1% (452/1025) and the ALK rearrangement rate was 10.0% (103/1025). Among patients with squamous cell carcinoma, the EGFR mutation rate was 8.3% (9/109) and the ALK rearrangement rate was 3.7% (4/109).

Among all patients, univariate analyses showed that the EGFR mutation rate was significantly higher in females ($P < 0.001$), without a smoking history ($P < 0.001$), non-squamous ($P < 0.001$), stage IV tumor ($P < 0.001$), bone metastases ($P = 0.014$), brain metastases ($P = 0.002$), pleural effusion ($P = 0.016$) and pleural nodules ($P = 0.014$) (Table 3). Multivariate analysis further identified gender (HR = 1.7, 95%CI = 1.2–2.4, $P = 0.006$), smoking history (HR = 1.8, 95%CI = 1.3–2.7, $P = 0.001$), histology (HR = 5.0, 95%CI = 2.4–10.1, $P < 0.001$), and brain metastases (HR = 1.5, 95%CI = 1.1–2.2, $P = 0.017$) as independent predictors of EGFR mutation. Among patients with non-squamous, univariate analyses showed that the EGFR mutation rate was significantly higher in females ($P < 0.001$), without a smoking history ($P < 0.001$), stage IV tumor ($P = 0.002$), brain metastases ($P = 0.027$), and pleural nodules metastases ($P = 0.034$) (Table 4).

### Table 1 Clinical and pathological features of 1134 NSCLC patients

| Clinicopathology | All patients | Non-squamous | Squamous |
|------------------|-------------|--------------|----------|
| **No. (%)**      | **No. (%)** | **No. (%)**  | **No. (%)** |
| **Age**          |             |              |           |
| Medium (Range)   | 60 (21–88)  | 60 (21–88)   | 63 (42–87) |
| <60 years old    | 548 (48.4%) | 509 (49.7%)  | 39 (35.8%) |
| >60 years old    | 586 (51.6%) | 516 (50.3%)  | 70 (64.2%) |
| **Gender**       |             |              |           |
| Male             | 642 (56.6%) | 550 (53.7%)  | 92 (84.4%) |
| Female           | 492 (43.4%) | 475 (46.3%)  | 17 (15.6%) |
| **Smoking history** |          |              |           |
| No               | 588 (51.9%) | 565 (55.1%)  | 23 (21.1%) |
| Yes              | 515 (45.4%) | 430 (42.0%)  | 85 (78.0%) |
| Unknown          | 31 (2.7%)   | 30 (2.9%)    | 1 (0.9%)  |
| **Stage**        |             |              |           |
| IIIb             | 165 (14.6%) | 120 (11.7%)  | 45 (41.3%) |
| IV               | 969 (85.4%) | 905 (88.3%)  | 64 (58.7%) |
| **Pathology**    |             |              |           |
| Non-squamous     | 1025 (90.4%)| 1025 (100.0%)| 0 (0.0%)  |
| Squamous         | 109 (9.6%)  | 0 (0.0%)     | 109 (100.0%)|
| **Diagnostic methods** |       |              |           |
| Histology        | 976 (86.1%) | 867 (84.6%)  | 109 (100.0%)|
| Cytology         | 158 (13.9%) | 158 (15.4%)  | 0 (0.0%)  |
| **EGFR mutation** |            |              |           |
| Mutant type      | 461 (40.7%) | 452 (44.1%)  | 9 (8.3%)  |
| Wild type        | 673 (59.3%) | 573 (55.9%)  | 100 (91.7%)|
| **ALK rearrangement** |       |              |           |
| Mutant type      | 107 (9.4%)  | 103 (10.0%)  | 4 (3.7%)  |
| Wild type        | 1027 (90.6%)| 922 (90.0%)  | 105 (96.3%)|

EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase.
Among four patients with squamous harboring ALK rearrangement, two showed inconsistent test results (positive for IHC and negative for FISH). Thus, IHC and FISH testing appear to provide inconsistent results regarding the squamous NSCLC patient’s ALK status. Nevertheless, further validation of this result is needed, given the small sample size of this subgroup in the present study. A previous report regarding ALK rearrangement in NSCLC indicated that FISH provides higher sensitivity and specificity than IHC regarding the effects of targeted therapy, and

**Table 2** Mutation patterns of 19 cases with EGFR double mutations

| Pts. | 19del | L858R | L861Q | G719X | S768I | T790M | 20ins | G | Age | SH | P | TNM stage |
|------|-------|-------|-------|-------|-------|-------|-------|---|-----|---|---|------------|
| 1    | −     | −     | −     | −     | −     | −     | −     | − | M   | 76 | N | AS | T2N3M0     |
| 2    | −     | −     | −     | −     | −     | −     | −     | − | F   | 54 | N | A | T1N0M1     |
| 3    | −     | −     | −     | +     | +     | −     | −     | − | M   | 60 | Y | A | T4N2M1     |
| 4    | −     | −     | −     | +     | +     | −     | −     | − | M   | 49 | Y | A | T2N3M1     |
| 5    | −     | −     | −     | +     | +     | −     | −     | − | F   | 51 | N | A | T2N3M1     |
| 6    | +     | −     | −     | −     | −     | −     | +     | − | F   | 68 | Y | AS | T4N3M0     |
| 7    | +     | −     | −     | −     | −     | −     | −     | − | F   | 80 | Y | A | T2N2M1     |
| 8    | +     | −     | −     | −     | −     | −     | −     | − | M   | 65 | Y | A | T2N0M1     |
| 9    | +     | −     | −     | −     | −     | −     | −     | − | M   | 71 | Y | A | T1N3M0     |
| 10   | +     | −     | −     | −     | −     | −     | −     | − | M   | 59 | Y | A | T1N3M1     |
| 11   | +     | −     | −     | −     | −     | −     | +     | − | M   | 64 | Y | A | T4N3M1     |
| 12   | +     | −     | −     | −     | −     | −     | −     | − | M   | 66 | Y | S | T4N2M1     |
| 13   | +     | −     | −     | −     | −     | −     | +     | − | F   | 60 | N | A | T1N1M1     |
| 14   | +     | −     | −     | −     | −     | −     | −     | − | F   | 76 | N | A | T4N3M1     |
| 15   | +     | −     | −     | −     | −     | −     | +     | − | M   | 46 | Y | A | T2N2M1     |
| 16   | +     | −     | −     | −     | −     | −     | −     | − | F   | 76 | N | A | T4N0M1     |
| 17   | +     | −     | −     | −     | −     | −     | −     | − | M   | 66 | N | A | T2N0M1     |

A, adenocarcinoma; AS, adenosquamous carcinoma; F, female; G, gender; M, male; N, no, never smoker; P, pathology; S, squamous carcinoma; SH, smoking history; Y, yes, former or current smoker.

**Discussion**

The present study revealed that although adenocarcinoma was the most common pathological type to be submitted for EGFR/ALK evaluation, patients with squamous carcinoma had an EGFR mutation rate of 8.3% and an ALK rearrangement rate of 3.7%. Because of the relatively high mutation rate, patients with squamous cell carcinoma should also be routinely tested to determine their EGFR and ALK statuses.

Among four patients with squamous harboring ALK rearrangement, two showed inconsistent test results (positive for IHC and negative for FISH). Thus, IHC and FISH testing appear to provide inconsistent results regarding the squamous NSCLC patient’s ALK status. Nevertheless, further validation of this result is needed, given the small sample size of this subgroup in the present study. A previous report regarding ALK rearrangement in NSCLC indicated that FISH provides higher sensitivity and specificity than IHC regarding the effects of targeted therapy, and
that the interpretation of FISH results is more objective than that of IHC results.\textsuperscript{17} However, that study included a much smaller number of squamous cell carcinoma cases than adenocarcinoma cases (303 vs. 25,596). Therefore, further studies are needed to examine whether IHC and/or FISH are the most appropriate techniques for determining the \textsuperscript{ALK} status, especially for patients with squamous cell carcinoma.

### Table 3: Fisher exact probability method analysis of EGFR mutation and clinical characteristics in 1134 NSCLC patients

| Characteristics                        | No. (%) | Wild type (%) | Mutant type (%) | \(P\)-value |
|----------------------------------------|---------|---------------|-----------------|-------------|
| **Age**                                |         |               |                 |             |
| <60 years old                          | 548 (48.3%) | 317 (57.8%)  | 231 (42.2%)    | 0.333       |
| ≥60 years old                          | 586 (51.7%) | 356 (60.8%)  | 230 (39.2%)    |             |
| **Gender**                             |         |               |                 |             |
| Male                                   | 642 (56.6%) | 450 (70.1%)  | 192 (29.9%)    | <0.001      |
| Female                                 | 492 (43.4%) | 223 (45.3%)  | 269 (54.7%)    |             |
| **Smoking history**                    |         |               |                 |             |
| No                                     | 588 (53.3%) | 282 (48.0%)  | 30 (52.0%)     | <0.001      |
| Yes                                    | 515 (46.7%) | 380 (73.8%)  | 135 (26.2%)    |             |
| **Pathology**                          |         |               |                 |             |
| Non-squamous                           | 1025 (90.4%) | 573 (55.9%)  | 452 (44.1%)    | <0.001      |
| Squamous                               | 109 (9.6%)    | 100 (91.7%)  | 9 (8.3%)       |             |
| **T stage**                            |         |               |                 |             |
| 1                                      | 153 (13.5%) | 83 (54.2%)   | 70 (45.8%)     | 0.093       |
| 2                                      | 268 (23.6%) | 154 (57.5%)  | 114 (42.5%)    |             |
| 3                                      | 107 (9.4%)   | 72 (67.3%)   | 35 (32.7%)     |             |
| 4                                      | 526 (46.4%) | 323 (61.4%)  | 203 (38.6%)    |             |
| x                                      | 80 (7.1%)    | 41 (51.3)    | 39 (48.8)      |             |
| **N stage**                            |         |               |                 |             |
| 0                                      | 161 (14.2%) | 82 (50.9%)   | 79 (49.1%)     | 0.058       |
| 1                                      | 48 (4.2%)   | 26 (54.2%)   | 22 (45.8%)     |             |
| 2                                      | 298 (26.3%) | 174 (58.4%)  | 124 (41.6%)    |             |
| 3                                      | 593 (52.3%) | 373 (62.9%)  | 220 (37.1%)    |             |
| x                                      | 34 (3.0%)   | 18 (52.9%)   | 16 (47.1%)     |             |
| **M stage**                            |         |               |                 |             |
| 0                                      | 166 (14.6%) | 126 (75.9%)  | 40 (24.1%)     | <0.001      |
| 1                                      | 968 (85.4%) | 547 (56.5%)  | 421 (43.5%)    |             |
| **Lung metastases**                    |         |               |                 |             |
| No                                     | 724 (63.8%) | 442 (61.0%)  | 282 (39.0%)    | 0.131       |
| Yes                                    | 410 (36.2%) | 231 (56.3%)  | 179 (43.7%)    |             |
| **Bone metastases**                    |         |               |                 |             |
| No                                     | 733 (64.6%) | 455 (62.1%)  | 278 (37.9%)    | 0.014       |
| Yes                                    | 401 (35.4%) | 218 (54.4%)  | 183 (45.6%)    |             |
| **Brain metastases**                   |         |               |                 |             |
| No                                     | 948 (16.4%) | 582 (61.4%)  | 366 (38.6%)    | 0.002       |
| Yes                                    | 186 (83.6%) | 91 (48.9%)   | 95 (51.1%)     |             |
| **Adrenal metastases**                 |         |               |                 |             |
| No                                     | 1046 (92.2%) | 613 (58.6%)  | 433 (41.4%)    | 0.090       |
| Yes                                    | 88 (7.8%)   | 60 (68.2%)   | 28 (31.8%)     |             |
| **Liver metastases**                   |         |               |                 |             |
| No                                     | 1040 (91.7%) | 610 (58.7%)  | 430 (41.3%)    | 0.125       |
| Yes                                    | 94 (8.3%)   | 63 (67.0%)   | 31 (33.0%)     |             |
| **Pleural effusion**                   |         |               |                 |             |
| No                                     | 813 (71.7%) | 501 (61.6%)  | 312 (38.4%)    | 0.016       |
| Yes                                    | 321 (28.3%) | 172 (53.6%)  | 149 (46.4%)    |             |
| **Pleural nodules**                    |         |               |                 |             |
| No                                     | 1002 (88.4%) | 608 (60.7%)  | 394 (39.3%)    | 0.014       |
| Yes                                    | 132 (11.6%) | 65 (49.2%)   | 67 (50.8%)     |             |

EGFR, epidermal growth factor receptor.
The present study revealed that the overall EGFR mutation rate was 40.7%, and gender, smoking history, and histology were independent predictors of EGFR mutation. These findings are consistent with the results of previous studies. Furthermore, we found that patients with EGFR mutations were more likely to have baseline brain metastases, which may be related to the downstream effects of EGFR on brain metastases. It is reported that EGFR inhibition decreased the rate of brain metastases in human DMA-MB-231 breast cancer cell lines. Although the patients with NSCLC were not evaluated, results suggested that EGFR may affect the phosphoinositide 3 kinase/protein kinase B/phospholipase Cγ pathway and subsequently lead to brain metastasis. 

Table 4  Fisher exact probability method analysis of EGFR mutation and clinical characteristics in 1025 non-squamous NSCLC patients

| characteristics | No. | Wild type | Mutant type | P-value |
|-----------------|-----|-----------|-------------|---------|
| Age (N,%)       |     |           |             |         |
| <60 years old   | 509 (49.7%) | 285 (56.0%) | 224 (44.0%) | 1.000   |
| ≥60 years old   | 516 (50.8%) | 288 (55.8%) | 228 (44.2%) |         |
| Gender (N,%)    |     |           |             |         |
| Male            | 550 (53.7%) | 362 (65.8%) | 188 (34.2%) | <0.001  |
| Female          | 475 (46.3%) | 211 (44.4%) | 264 (55.6%) |         |
| Smoking history (N,%) |     |           |             |         |
| No              | 565 (55.1%) | 264 (46.7%) | 301 (53.3%) | <0.001  |
| Yes             | 430 (42.0%) | 299 (69.5%) | 131 (30.5%) |         |
| Unknown         | 30 (2.9%) | 10 (33.3%) | 20 (66.7%) |         |
| T stage (N,%)   |     |           |             |         |
| 1               | 148 (14.4%) | 78 (52.7%) | 70 (47.3%) | 0.415   |
| 2               | 247 (24.1%) | 136 (55.1%) | 111 (44.9%) |         |
| 3               | 96 (9.4%) | 61 (63.5%) | 35 (36.5%) |         |
| 4               | 457 (44.6%) | 259 (56.7%) | 198 (43.3%) |         |
| X               | 77 (7.5%) | 39 (50.6%) | 38 (49.4%) |         |
| N stage (N,%)   |     |           |             |         |
| 0               | 154 (15.0%) | 75 (48.7%) | 79 (51.3%) | 0.107   |
| 1               | 42 (4.1%) | 21 (50.0%) | 21 (50.0%) |         |
| 2               | 263 (25.7%) | 142 (54.0%) | 121 (46.0%) |         |
| 3               | 533 (52.0%) | 318 (59.7%) | 215 (40.3%) |         |
| X               | 33 (3.2%) | 17 (51.5%) | 16 (48.5%) |         |
| M stage (N,%)   |     |           |             |         |
| 0               | 121 (14.6%) | 84 (69.4%) | 37 (30.6%) | 0.002   |
| 1               | 904 (85.4%) | 489 (54.1%) | 415 (45.9%) |         |
| Lung metastases |     |           |             |         |
| No              | 645 (62.9%) | 370 (57.4%) | 275 (42.6%) | 0.241   |
| Yes             | 380 (37.1%) | 203 (53.4%) | 177 (46.6%) |         |
| Bone metastases |     |           |             |         |
| No              | 650 (63.4%) | 378 (58.2%) | 272 (41.8%) | 0.058   |
| Yes             | 375 (36.6%) | 195 (52.0%) | 180 (48.0%) |         |
| Brain metastases |     |           |             |         |
| No              | 841 (82.0%) | 484 (57.6%) | 357 (42.4%) | 0.027   |
| Yes             | 184 (18.0%) | 89 (48.4%) | 95 (51.6%) |         |
| Adrenal metastases |     |           |             |         |
| No              | 942 (91.9%) | 518 (55.0%) | 424 (45.0%) | 0.050   |
| Yes             | 83 (8.1%) | 55 (66.3%) | 28 (33.7%) |         |
| Liver metastases |     |           |             |         |
| No              | 938 (91.5%) | 516 (55.0%) | 422 (45.0%) | 0.071   |
| Yes             | 87 (8.5%) | 57 (65.5%) | 30 (34.5%) |         |
| Pleural effusion |     |           |             |         |
| No              | 721 (70.3%) | 414 (57.4%) | 307 (42.6%) | 0.148   |
| Yes             | 304 (29.7%) | 159 (52.3%) | 145 (47.7%) |         |
| Pleural nodules |     |           |             |         |
| No              | 901 (87.9%) | 515 (57.2%) | 386 (42.8%) | 0.034   |
| Yes             | 124 (12.1%) | 58 (46.8%) | 66 (53.2%) |         |

EGFR, epidermal growth factor receptor.
TKI therapy induced MET expression and phosphorylation, which may be associated with subsequent brain metastases in patients with NSCLC.\(^{22}\) While this relationship can only explain the increase in brain metastases after EGFR-TKI treatment, it does not explain the relationship between EGFR mutations and baseline brain metastasis. Therefore, further studies are needed to better understand the relationship between EGFR mutations and baseline brain metastasis, and clinicians should be aware of this relationship when they encounter cases of EGFR-mutated NSCLC, or cases with brain metastasis. This study observed the ALK-rearrangement rate was 9.4%, which is much higher than that reported among Asian patients with NSCLC of 4.1–5%.\(^{23}\) This difference

| Table 5 Fisher exact probability method analysis of EGFR mutation and clinical characteristics in 109 squamous NSCLC patients |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Characteristics             | No.                         | Wild type                   | Mutant type                 |
| Age (N,%), <60 years old    | 39 (35.8%)                  | 34 (87.2%)                  | 5 (12.8%)                   |
| >60 years old               | 70 (64.2%)                  | 66 (94.3%)                  | 4 (5.7%)                    |
| Gender, Male                | 92 (84.4%)                  | 88 (95.7%)                  | 4 (4.3%)                    |
| Female                      | 17 (15.6%)                  | 12 (70.6%)                  | 5 (29.4%)                   |
| Smoking history, No         | 23 (21.1%)                  | 18 (78.3%)                  | 5 (21.7%)                   |
| Yes                         | 85 (78.0%)                  | 81 (95.3%)                  | 4 (4.7%)                    |
| Unknown                     | 1 (0.9%)                    | 1 (100.0%)                  | 0 (0.0%)                    |
| T stage, 1                  | 5 (4.6%)                    | 5 (100.0%)                  | 0 (0.0%)                    |
| 2                           | 21 (19.3%)                  | 18 (85.7%)                  | 3 (14.3%)                   |
| 3                           | 11 (10.1%)                  | 11 (100.0%)                 | 0 (0.0%)                    |
| 4                           | 69 (63.3%)                  | 64 (92.8%)                  | 5 (7.2%)                    |
| x                           | 3 (2.8%)                    | 2 (66.7%)                   | 1 (33.3%)                   |
| N stage, 0                  | 7 (6.4%)                    | 7 (100.0%)                  | 0 (0.0%)                    |
| 1                           | 6 (5.5%)                    | 5 (83.3%)                   | 1 (16.7%)                   |
| 2                           | 35 (32.1%)                  | 32 (91.4%)                  | 3 (8.6%)                    |
| 3                           | 60 (55.0%)                  | 55 (91.7%)                  | 5 (8.3%)                    |
| x                           | 1 (0.9%)                    | 1 (100.0%)                  | 0 (0.0%)                    |
| M stage, 0                  | 45 (41.3%)                  | 42 (93.3%)                  | 3 (6.7%)                    |
| 1                           | 64 (58.7%)                  | 58 (90.6%)                  | 6 (9.4%)                    |
| Lung metastases, No         | 79 (72.5%)                  | 72 (91.1%)                  | 7 (8.9%)                    |
| Yes                         | 30 (27.5%)                  | 28 (93.3%)                  | 2 (6.7%)                    |
| Bone metastases, No         | 83 (76.1%)                  | 77 (92.8%)                  | 6 (7.2%)                    |
| Yes                         | 26 (23.9%)                  | 23 (88.5%)                  | 3 (11.5%)                   |
| Brain metastases, No        | 107 (98.2%)                 | 98 (91.6%)                  | 9 (8.4%)                    |
| Yes                         | 2 (1.8%)                    | 2 (100.0%)                  | 0 (0.0%)                    |
| Adrenal metastases, No      | 104 (95.4%)                 | 95 (91.3%)                  | 9 (8.7%)                    |
| Yes                         | 5 (4.6%)                    | 5 (100.0%)                  | 0 (0.0%)                    |
| Liver metastases, No        | 102 (93.6%)                 | 94 (92.2%)                  | 8 (7.8%)                    |
| Yes                         | 7 (6.4%)                    | 6 (85.7%)                   | 1 (14.3%)                   |
| Pleural effusion, No        | 92 (84.4%)                  | 87 (94.6%)                  | 5 (5.4%)                    |
| Yes                         | 17 (15.6%)                  | 13 (76.5%)                  | 4 (23.5%)                   |
| Pleural nodules, No         | 101 (92.7%)                 | 93 (92.1%)                  | 8 (7.9%)                    |
| Yes                         | 8 (7.3%)                    | 7 (87.5%)                   | 1 (12.5%)                   |

EGFR, epidermal growth factor receptor.
may be related to the clinicopathological characteristics of the included patients. Previous reports indicated an ALK rearrangement rate of 13.5% (19/141) among patients with NSCLC who were female, Asian, did not smoke or smoked relatively small amounts, and adenocarcinoma. In the present study, 85.8% (973/1134) of the enrolled patients had adenocarcinoma, which may explain the relatively high ALK rearrangement rate. The present study also revealed

Table 6 Fisher exact probability method analysis of ALK rearrangement and clinical characteristics in 1134 NSCLC patients

| Characteristics                     | No.     | Wild type | Mutant type | P-value |
|-------------------------------------|---------|-----------|-------------|---------|
| Age (N, %)                          |         |           |             |         |
| ≤60 years old                       | 548 (48.0%) | 473 (86.2%) | 75 (13.8%)  | <0.001  |
| >60 years old                       | 586 (52.0%) | 554 (94.6%) | 32 (5.4%)   |         |
| Gender (N, %)                       |         |           |             |         |
| Male                                | 642 (56.6%) | 597 (93.0%) | 45 (7.0%)   | 0.002   |
| Female                              | 492 (43.4%) | 430 (87.4%) | 62 (12.6%)  |         |
| Smoking history (N, %)              |         |           |             |         |
| No                                  | 588 (51.9%) | 513 (87.2%) | 75 (12.8%)  | <0.001  |
| Yes                                 | 515 (45.4%) | 484 (94.0%) | 31 (6.0%)   |         |
| Unknown                             | 31 (2.7%)  | 30 (96.8%) | 1 (3.2%)    |         |
| Pathology (N, %)                    |         |           |             |         |
| Non-squamous                        | 1025 (90.4%) | 922 (90.0%) | 103 (10.0%) | 0.025   |
| Squamous                            | 109 (9.6%)  | 105 (96.3%) | 4 (3.7%)    |         |
| T stage (N, %)                      |         |           |             |         |
| 1                                   | 153 (13.5%) | 135 (88.2%) | 18 (11.8%)  | 0.586   |
| 2                                   | 268 (23.6%) | 243 (90.7%) | 25 (9.3%)   |         |
| 3                                   | 107 (9.4%)  | 94 (87.9%)  | 13 (12.1%)  |         |
| 4                                   | 526 (46.4%) | 482 (91.6%) | 44 (8.4%)   |         |
| x                                   | 80 (7.1%)  | 73 (91.3%)  | 7 (8.7%)    |         |
| N stage (N, %)                      |         |           |             |         |
| 0                                   | 161 (14.2%) | 150 (93.2%) | 11 (6.8%)   | 0.818   |
| 1                                   | 48 (4.2%)  | 44 (91.7%)  | 4 (8.3%)    |         |
| 2                                   | 298 (26.3%) | 269 (90.3%) | 29 (9.7%)   |         |
| 3                                   | 593 (52.3%) | 533 (89.9%) | 60 (10.1%)  |         |
| x                                   | 34 (3.0%)  | 31 (91.2%)  | 3 (8.8%)    |         |
| M stage (N, %)                      |         |           |             |         |
| 0                                   | 166 (14.6%) | 150 (90.4%) | 16 (9.6%)   | 0.886   |
| 1                                   | 968 (85.4%) | 877 (90.6%) | 91 (9.4%)   |         |
| Lung metastases                     |         |           |             |         |
| No                                  | 724 (63.8%) | 652 (90.1%) | 72 (9.9%)   | 0.461   |
| Yes                                 | 410 (36.2%) | 375 (91.5%) | 35 (8.5%)   |         |
| Bone metastases                     |         |           |             |         |
| No                                  | 733 (64.6%) | 656 (89.5%) | 77 (10.5%)  | 0.111   |
| Yes                                 | 401 (35.4%) | 371 (92.5%) | 30 (7.5%)   |         |
| Brain metastases                    |         |           |             |         |
| No                                  | 948 (16.4%) | 857 (90.4%) | 91 (9.6%)   | 0.784   |
| Yes                                 | 186 (83.6%) | 170 (91.4%) | 16 (8.6%)   |         |
| Adrenal metastases                  |         |           |             |         |
| No                                  | 1046 (92.2%) | 944 (90.2%) | 102 (9.8%)  | 0.257   |
| Yes                                 | 88 (7.8%)  | 83 (94.3%)  | 5 (5.7%)    |         |
| Liver metastases                    |         |           |             |         |
| No                                  | 1040 (91.7%) | 947 (91.1%) | 93 (8.9%)   | 0.066   |
| Yes                                 | 94 (8.3%)  | 80 (85.1%)  | 14 (14.9%)  |         |
| Pleural effusion                    |         |           |             |         |
| No                                  | 813 (71.7%) | 733 (90.2%) | 80 (9.8%)   | 0.500   |
| Yes                                 | 321 (28.3%) | 294 (91.6%) | 27 (8.4%)   |         |
| Pleural nodules                     |         |           |             |         |
| No                                  | 1002 (88.4%) | 910 (90.8%) | 92 (9.2%)   | 0.428   |
| Yes                                 | 132 (11.6%) | 117 (88.6%) | 15 (11.4%)  |         |

ALK, anaplastic lymphoma kinase.
that only age was independently associated with ALK rearrangement, which is in line with previous reports.\textsuperscript{27,28}

The study suggested that first-line targeted therapy rate for patients with NSCLC with EGFR-activating mutation or ALK rearrangement were still low. This relatively low EGFR-TKI treatment rate could be explained by a long interval until the EGFR mutation is detected; thus, it may be possible to increase this rate by shortening the EGFR mutation detection period, which may be achieved by obtaining sufficient pathological specimens in a timely manner and popularizing EGFR gene detection technology. However, there was no significant correlation between the ALK rearrangement detection period and first-line targeted therapy rate. Although the ALK rearrangement detection

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
Characteristics & No. & Wild type & Mutant type & \textit{P}-value \\
\hline
\hline
Age (N,\%) & & & & \\
<60 years old & 509 (49.7\%) & 436 (85.7\%) & 73 (14.3\%) & <0.001 \\
\geq 60 years old & 516 (50.3\%) & 486 (94.2\%) & 30 (5.8\%) & \\
\hline
Gender (N,\%) & & & & \\
Male & 550 (53.7\%) & 508 (92.4\%) & 42 (7.6\%) & 0.007 \\
Female & 475 (46.3\%) & 414 (87.2\%) & 61 (12.8\%) & \\
\hline
Smoking history (N,\%) & & & & \\
No & 565 (55.1\%) & 491 (86.9\%) & 74 (13.1\%) & 0.001 \\
Yes & 430 (42.0\%) & 402 (93.5\%) & 28 (6.5\%) & \\
Unknown & 30 (2.9\%) & 29 (96.7\%) & 1 (3.3\%) & \\
\hline
T stage (N,\%) & & & & \\
1 & 148 (14.4\%) & 130 (87.8\%) & 18 (12.2\%) & 0.700 \\
2 & 247 (24.1\%) & 222 (89.9\%) & 25 (10.1\%) & \\
3 & 96 (9.4\%) & 84 (87.5\%) & 12 (12.5\%) & \\
4 & 457 (44.6\%) & 416 (91.0\%) & 41 (9.0\%) & \\
x & 77 (7.5\%) & 70 (90.0\%) & 7 (9.9\%) & \\
\hline
N stage (N,\%) & & & & \\
0 & 154 (15.0\%) & 143 (92.9\%) & 11 (7.1\%) & 0.736 \\
1 & 42 (4.1\%) & 38 (90.5\%) & 4 (9.5\%) & \\
2 & 263 (25.7\%) & 237 (90.1\%) & 26 (9.9\%) & \\
3 & 533 (52.0\%) & 474 (88.9\%) & 59 (11.1\%) & \\
x & 33 (3.2\%) & 30 (90.9\%) & 3 (9.1\%) & \\
\hline
M stage (N,\%) & & & & \\
0 & 121 (11.8\%) & 108 (89.3\%) & 13 (10.7\%) & 0.749 \\
1 & 904 (88.2\%) & 814 (90.0\%) & 90 (10.0\%) & \\
\hline
Lung metastases & & & & \\
No & 645 (62.9\%) & 576 (89.3\%) & 69 (10.7\%) & 0.391 \\
Yes & 380 (37.1\%) & 346 (91.1\%) & 34 (8.9\%) & \\
\hline
Bone metastases & & & & \\
No & 650 (63.4\%) & 577 (88.8\%) & 73 (11.2\%) & 0.106 \\
Yes & 375 (36.6\%) & 345 (92.0\%) & 30 (8.0\%) & \\
\hline
Brain metastases & & & & \\
No & 841 (82.0\%) & 754 (89.7\%) & 87 (10.3\%) & 0.589 \\
Yes & 184 (18.0\%) & 168 (91.3\%) & 16 (8.7\%) & \\
\hline
Adrenal metastases & & & & \\
No & 942 (91.9\%) & 844 (89.6\%) & 98 (10.4\%) & 0.254 \\
Yes & 83 (8.1\%) & 78 (94.0\%) & 5 (6.0\%) & \\
\hline
Liver metastases & & & & \\
No & 938 (91.5\%) & 849 (90.5\%) & 89 (9.5\%) & 0.061 \\
Yes & 87 (8.5\%) & 73 (83.9\%) & 14 (16.1\%) & \\
\hline
Pleural effusion & & & & \\
No & 721 (70.3\%) & 645 (89.5\%) & 76 (10.5\%) & 0.495 \\
Yes & 304 (29.7\%) & 277 (91.1\%) & 27 (8.9\%) & \\
\hline
Pleural nodules & & & & \\
No & 901 (87.9\%) & 813 (90.2\%) & 88 (9.8\%) & 0.425 \\
Yes & 124 (12.1\%) & 109 (87.9\%) & 15 (12.1\%) & \\
\hline
\end{tabular}
\caption{Fisher exact probability method analysis of ALK rearrangement and clinical characteristics in 1025 non-squamous NSCLC patients}
\end{table}

ALK, anaplastic lymphoma kinase.
period was shorter than the EGFR mutation detection period (5 days vs. 7 days), the first-line ALK-TKIs treatment rate was lower than the first-line EGFR-TKIs treatment rate (51.4% vs. 73.8%). This finding may be related to limited access to ALK inhibitors, based on their high cost.

In addition to the important discoveries revealed in the present study, there are also some limitations. First, these data are preliminary, and thus additional follow-up is needed to examine the effects of targeted therapy in cases of NSCLC harboring EGFR mutation or ALK rearrangement. Second, different centers had varying numbers of patients who were eligible for enrollment, and we were unable to perform regional subanalyses of EGFR/ALK status.

In summary, the present study demonstrated that patients with squamous cell carcinoma should also be routinely tested to determine their EGFR and ALK gene status. First-line targeted therapy for EGFR-positive patients was negative associated with the time from the pathological diagnosis to EGFR gene status confirmation. Further research is needed to identify whether IHC and/or FISH are the most appropriate techniques for determining the ALK status of patients with squamous cell carcinoma.

Disclosure

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