Erlotinib Treatment in Patients with Advanced Lung Adenocarcinoma with CISH-positive and CISH-negative EGFR Gene Alterations

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Abstract. Background: Epidermal growth factor receptor (EGFR) positivity as assessed by chromogenic in situ hybridization (CISH) has been demonstrated to be associated with EGFR mutation status. This study was conducted to compare the responsiveness of CISH-positive and CISH-negative lung adenocarcinomas to erlotinib. Patients and Methods: Patients received erlotinib (150 mg/day) alone until disease progression or intolerable toxicity. EGFR gene status was examined by CISH. The response rate (RR), progression-free survival (PFS), overall survival (OS) and toxicity profiles were assessed. Results: Thirty-one patients underwent response evaluations and CISH analyses, 12 of whom harboured CISH-positive adenocarcinomas. The overall RR (p=0.035), median PFS (p=0.091) and median OS (p=0.408) were higher in the CISH-positive group. No difference in toxicity profiles was observed between these two groups. Conclusion: EGFR status as assessed by CISH can predict the response to erlotinib in patients with advanced lung adenocarcinoma.

Erlotinib (Tarceva®, Roche Products Ltd., UK) is the standard of care for non-small cell lung cancer (NSCLC) that is unresponsive to cisplatin-based regimens. Epidermal growth factor receptor tyrosine kinase (EGFR-TK) domain mutation has been reported to be a key site of action. Erlotinib is a small molecule that binds to EGFR-TK and inhibits the signal transduction activity of EGFR.

Overexpression of EGFR as identified by immunohistochemical staining did not predict the clinical response of NSCLC to gefitinib (1). However, Tsao et al. reported a better response in tumors with EGFR overexpression following erlotinib treatment (2). Furthermore, better survival was observed in patients with fluorescence in situ hybridization (FISH)-confirmed EGFR overexpression (3).

Increased EGFR copy number was reported to be associated with the sensitivity of tumors to erlotinib. Most samples of such EGFR amplification were determined by either FISH or quantitative polymerase chain reaction (Q-PCR) (4-6). Recently, we reported on a novel method, chromogenic in situ hybridization (CISH), for detection of the amplification of EGFR.
EGFR genes in NSCLC (7). This retrospective analysis demonstrated a correlation between EGFR mutation status and response to gefitinib of adenocarcinoma. The present study was designed to validate the predictive value of CISH in advanced lung adenocarcinoma (ALA) following erlotinib treatment and to compare the efficacy of erlotinib in treating CISH-positive and CISH-negative ALA.

Patients and Methods

Patient selection. Patients with stage IIIB to IV or postoperative recurrent adenocarcinoma which failed or relapsed after the frontline chemotherapy and whose tumors harboured EGFR gene mutations were eligible for this study. Patients were eligible only if they refused first-line chemotherapy and would utilize EGFR-TK inhibitors (TKIs) as a first-line treatment at their own expense. Paraffin-embedded tissue blocks or fresh biopsy samples for each patient were made available at the Chang Gung Memorial Hospital. Other eligibility criteria included an Eastern Cooperative Oncology Group performance status of 0 to 3 and an estimated life expectancy of more than 12 weeks. Laboratory requirements included haemoglobin ≥10 g/dl; neutrophil count ≥2,000/μl; platelet count ≥100,000/μl; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤5.0×; alkaline phosphatase ≤6×; total bilirubin ≤1.25×; and creatinine ≤1.5× the institutional upper limit of normal; and histological or cytological proof of metastatic adenocarcinoma. Additional eligibility criteria were complete recovery from the toxic effects of previous antitumor therapy and no chemotherapy within one month of enrolment. The exclusion criteria were as follows: symptomatic brain metastasis or severe co-morbidity, such as symptomatic cardiovascular disease (e.g., uncontrolled hypertension, congestive heart failure, previous myocardial infarction within six months prior to treatment), uncontrolled diabetes, active gastric ulcer or liver cirrhosis; pregnancy or lactation; undergoing irradiation treatment, the presence of malignant pleural effusion without other measurable lesions; and active infection. Patients with a history of significant neurological or psychiatric disorders, including dementia, which would prohibit the understanding and giving of informed consent, and those receiving other concurrent experimental agents were excluded. The Institutional Review Board of our hospital approved the analyses of the EGFR gene status of the tumors and this study, and written informed consent was obtained from all enrolled patients.

EGFR gene analysis. CISH was performed using 4-μm-thick formalin-fixed paraffin-embedded tissue sections on coated slides. The EGFR probe was digoxigenin-labelled (Zymed Inc., South San Francisco, CA, USA). Briefly, the unstained tissue sections were pre-treated with 100 mM Tris and 50 mM EDTA (pH 7.0), heated to 92°C for 15 min and then washed with phosphate-buffered saline (PBS), followed by enzymatic digestion with 300 μl of Digest-all (Zymed) at 37°C for 10 min. The digestion was stopped by the addition of 10% phosphate buffered-formalin at room temperature for 1 min. Ten microliters of the EGFR probe (Zymed) were applied to each dehydrated and air-dried section, which was then denatured at 94°C for 3 min. The hybridization was performed overnight at 37°C in a humidified chamber. Post-hybridization washes were performed using 0.5x standard saline citrate at 72°C for 5 min, and samples were then rinsed in PBS containing 0.25% Tween 20 (Sigma-Aldrich Co., St. Louis, MO, USA). Detection was performed with a CISH Detection Kit (Zymed) according to the manufacturer’s recommended protocol. Non-specific staining was blocked by Cas-Block (Zymed) incubation for 10 min, after which samples were incubated with mouse anti-digoxigenin antibody for 1 h and then incubated with horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody (Zymed) for 30 min. Diaminobenzidine (DAS; Sigma-Aldrich Co.) was used as a chromogen and each sample was incubated in DAB for 20 min. Finally, the tissue sections were counterstained with hematoxylin for 30 s. The CISH study results were evaluated using a regular light microscope (Nikon E600; Nikon Corporation, Tokyo, Japan) and counting was performed by a pathologist (SF Huang). At least 100 non-overlapping and intact tumor nuclei were evaluated. A tumor was considered CISH-positive (exhibiting significant genomic gain) if the copy number was ≥5 signals per nucleus in more than 40% of the tumor cells, which was modified from the criteria set by Hirsch et al. (5). A tumor was considered CISH-negative (exhibiting no significant genomic gain) if the copy number of the EGFR gene was ≤4 signals per nucleus in more than 60% of the tumor cells (7).

Drug administration. Erlotinib (150 mg/day) was orally administered once daily. The patients continued treatment until disease progression, intolerable toxicity or withdrawal of consent. Second-line chemotherapy or other treatments after erlotinib were not prohibited by the protocol.

Treatment assessment. This study is a part of a two-arm, open label study. The total number of evaluable patients in each arm was intended to be 37, and thus, a total of 74 patients were to be enrolled. The study enrolment period is 2 years. All patients underwent the following procedure before this study: complete blood cell counts, chemistry profile, chest x-ray and computer tomographic evaluation of the chest and abdomen. Treatment was repeated every four weeks until disease progression or unbearable toxicity. Tumor assessment was performed after every two cycles of treatment. Tumor responses were evaluated as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD) in accordance with WHO criteria (Response Evaluation Criteria in Solid Tumors, version 1.0). The response rate (RR) was defined as the proportion of patients whose best response was CR or PR among all per-protocol patients. The disease control rate was defined as the best tumor response of CR, PR or SD that was confirmed and sustained for at least four weeks. Baseline assessments were performed within 14 days before treatment. During the treatment, assessments were performed every eight weeks until disease progression was observed. All adverse events (AEs) were reported, and their severity was graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0) grading system. Data were collected when erlotinib treatment was interrupted or withdrawn because of AEs. Routine clinical and laboratory assessments were performed at least every four weeks.

Statistical analysis. Group sample sizes of 37 achieve 81% power to detect a difference of 30% between the null hypothesis that the RRs of both the CISH-positive and CISH-negative groups are 50% and the alternative hypothesis that the RR in the CISH-negative
group is 20% by using a one-sided chi-square test with continuity correction and a significance level of 0.05. The primary endpoint was the difference in the RR of ALA to erlotinib between patients with CISH-positive and those with CISH-negative tumors. The secondary objectives were to analyze the median progression-free survival (PFS) and overall survival (OS) between patients with CISH-positive and CISH-negative ALA. The survival distribution was estimated by the Kaplan-Meier analysis.

Results

Patients’ characteristics. From April 2010 to March 2011, 43 patients were enrolled, and the EGFR status of their tumors was assessed by CISH. Thirty-nine patients were assessed for EGFR mutations by CISH, and all patients received erlotinib as treatment. Thirty-one patients were fully assessable for efficacy, but eight patients were not assessable due to early termination of erlotinib treatment because of rapid disease progression or loss to follow-up (Figure 1). Among the 31 patients, EGFR amplification as assessed by CISH (i.e. CISH-positive) was detected in 12 patients (38.7%), whereas 19 patients (61.3%) had no EGFR amplification identified by CISH (i.e. CISH-negative). The relationships between the patient characteristics and the EGFR mutations are shown in Table I. There were no significant differences between the CISH-positive and CISH-negative groups regarding gender, age, performance status or cancer staging.

Response and survival. The objective tumor responses are listed in Table II. The overall RRs were 58.3 and 21.1% ($p=0.035$) for the CISH-positive and CISH-negative groups, respectively. The disease control rate was 75% in the CISH-positive group, compared with 63.2% in the CISH-negative group. The median PFS was 9.8 (95% confidence interval (CI)=4.6 to 15 months) and 6.1 months (95% CI=2.7 to 9.5 months) in the CISH-positive and CISH-negative groups ($p=0.091$), respectively (Figure 2A). The median OS was 11.1 (95% CI=8.2 to 14.1 months) and 14.7 months (95% CI=12.9 to 16.5 months) in the CISH-positive and CISH-negative groups ($p=0.408$), respectively (Figure 2B). No life-threatening toxicity was observed in any patient. The most frequent AE was skin rash of any grade (83%), although 33% of skin rashes were grade 3/4 in the CISH-positive group (Table III).

Discussion

Many reports suggest that patients with advanced NSCLC should be evaluated for EGFR mutation status because patients with active EGFR mutations exhibit high RRs and long PFS (4, 8-13). There have been more than ten published reports on EGFR gene copy number changes, which were related to EGFR mutations and TKI sensitivity in patients with NSCLC (2, 4-6, 14-22). According to these reports, the role of CISH in predicting the response to EGFR-TKI is controversial. Some studies suggest that CISH is a good method for detecting EGFR mutations, but other studies asserted an opposing opinion.
In our study series, increased \textit{EGFR} copy number (≥5 copies per nucleus) was significantly correlated with \textit{EGFR} mutation status in adenocarcinoma, although it was less correlated with TKI responsiveness and OS than \textit{EGFR} mutation status (7). CISH can predict a lack of response in CISH-negative patients (23). We also supposed that CISH might be a good alternative molecular predictor of EGFR-TKI responsiveness, and thus, we conducted this study to test this hypothesis. In the results of this study, the RR was significantly different better in the CISH-positive than in the CISH-negative group. A similar trend for a higher PFS in the CISH-positive group was observed. The disease control rates

Figure 1. The scheme of patient selection. \textit{EGFR}, Epidermal growth factor receptor; \textit{CISH}, Chromogenic in situ hybridization.

Figure 2. Kaplan-Meier plot of progression-free survival (A) and overall survival (B) for patients with chromogenic in situ hybridization (CISH)-positive versus CISH-negative tumors. The median PFS was 9.8 (95% CI=4.595-15.005) and 6.1 (95% CI=2.687-9.513) months, respectively. The median OS was 11.1 (95% CI=8.17-14.097) and 14.7 (95% CI=12.852-16.548) months, respectively.
(CR, PR and SD) for the CISH-positive and CISH-negative groups were 75% and 63.2% (p=0.492), respectively. The low number of enrolled patients or inconsistent CISH performance might explain this finding. Several samples could not be assessed for CISH status because of there being too few tumor cells in the specimen. Although CISH status is less correlated with TKI responsiveness than EGFR mutation status, CISH can be performed using paraffin sections and can be analyzed much faster than DNA sequencing. Time and money can be saved by using CISH to evaluate EGFR mutation status, and this technique appears to predict a higher RR to erlotinib in patients with CISH-positive ALA.

References


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