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ORIGINAL ARTICLE

Nimotuzumab combined with chemotherapy as first-line treatment for advanced lung squamous cell carcinoma

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Abstract

Background: This study was conducted to evaluate the efficacy and safety of nimotuzumab combined with chemotherapy as first-line therapy in advanced lung squamous cell carcinoma (LSCC), and to explore predictive biomarkers of the efficacy of nimotuzumab.

Methods: A retrospective study was conducted of patients with advanced LSCC administered nimotuzumab combined with chemotherapy as first-line therapy from June 2012 to December 2016 at the Department of Respiratory Medicine, Peking Union Medical College Hospital. The associations between *EGFR* expression, *EGFR* gene copy numbers, and clinical efficacy were detected by immunohistochemistry and fluorescence in situ hybridization (FISH).

Results: Twenty-six patients were enrolled, including 22 men and 4 women. The objective response rate was 50% and the disease control rate was 100%. The median progression-free survival (PFS) and overall survival were 6.7 and 16.3 months, respectively. Patients whose samples were tested via FISH and showed positive *EGFR* expression had a trend of longer median PFS (10.0 months; $P = 0.10$). Adverse effects included 15 cases (57.7%) of bone marrow suppression, 15 (57.7%) of sensory neuropathy, 14 (53.8%) of alopecia, nine (34.6%) of nausea/vomiting and one case (3.8%) of elevated creatinine level. All adverse effects were attributed to chemotherapy.

Conclusion: Nimotuzumab combined with chemotherapy might be a possible option as first-line therapy in patients with advanced LSCC. *EGFR* gene copy number examined by FISH might be a possible predictive biomarker.

Introduction

Non-small cell lung cancer (NSCLC), which accounts for approximately 85% of lung cancer cases, is a malignancy with high mortality. NSCLC targeted therapies are evolving rapidly, and include antibodies against *EGFR* overexpressed in NSCLC.[1] Nimotuzumab, a humanized anti-EGFR monoclonal antibody, shows antitumor, antiangiogenic, and proapoptotic activity in A431 squamous cell carcinoma.[2] Experimental observations have demonstrated that the intrinsic properties of nimotuzumab require bivalent binding for stable attachment to the cellular surface, meaning that nimozutumab has a greater clinical benefit and fewer dermatological adverse events than cetuximab.[3,4]

Babu *et al.* showed that nimotuzumab plus chemotherapy significantly improves the objective response rate (ORR) compared to chemotherapy alone, and is well tolerated in patients with stage IIIb/IV NSCLC.[5] Qi *et al.* demonstrated that nimotuzumab combined with docetaxel and carboplatin extended the duration of progression-free survival (PFS) and was generally well tolerated.[6] Zhao *et al.* reported that nimotuzumab and docetaxel combination therapy was well tolerated and efficacious in chemotherapy-refractory/resistant patients with advanced NSCLC.[7] A retrospective study showed that nimotuzumab combined with chemotherapy as second-line or further therapy for advanced lung squamous cell carcinoma (LSCC) was active

and well tolerated, especially in *EGFR*-positive patients.[8] However, few studies have evaluated nimotuzumab combined with chemotherapy as first-line treatment for advanced LSCC, and no biomarkers have been explored to predict nimotuzumab response.

In this study, we retrospectively analyzed the efficacy and safety of nimozutumab combined with chemotherapy as first-line treatment for advanced LSCC and elucidated the potential predictive biomarkers of nimotuzumab response.

Methods

Patients

This retrospective study was conducted through review of medical records of patients with advanced LSCC who received nimotuzumab combined with chemotherapy at the Department of Respiratory Medicine, Peking Union Medical College Hospital (PUMCH) from June 2012 to December 2016. Eligibility criteria included: (i) histological or cytological diagnosis, (ii) stage IIIb or IV, (iii) the presence of at least one measurable tumor lesion, and (iv) the choice of nimotuzumab combined with chemotherapy as first-line treatment. All eligible patients were included to reduce selection bias.

The PUMCH institutional review board determined that this study is exempt from full review.

Treatment

Nimotuzumab was administered on days 1 and 8 at a dose of 400 mg with chemotherapy every three weeks, and was continued after the end of chemotherapy until disease progression, intolerance, or patient withdrawal. Chemotherapy was administered concurrently for four to six cycles until disease progression or unacceptable toxicity.

Assessment

Tumor response was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 at intervals of six weeks until disease progression. Both doctors and radiologists assessed efficacy to reduce bias. Efficacy was divided into four categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). The ORR was calculated as (CR + PR)/(CR + PR + SD + PD), and the disease control rate (DCR) as (CR + PR + SD)/(CR + PR + SD + PD). PFS was calculated from the time of first infusion of nimotuzumab to the date of disease progression.

Complete blood counts and serum chemistry were tested at baseline and every week during the treatment. Clinical adverse events (AEs) and changes in the laboratory parameters were assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. Performance status (PS) was assessed according to Eastern Cooperative Oncology Group (ECOG) scores at baseline.

EGFR expression detection by immunohistochemistry

Immunohistochemical analysis was performed on 4 μm thick formalin-fixed, paraffin-embedded sections with anti-EGFR antibody (clone 5B7) using standard autostaining protocol on a Ventana BenchMark XT autostainer (Ventana Medical Systems Inc., Tucson, AZ, USA). The algorithm for *EGFR* evaluation was adopted from that used for *HER-2* evaluation in breast cancer: score 0: no staining; score 1+: weak and incomplete membrane staining in > 10% of the invasive tumor cells; score 2+: weak complete staining of the membrane in > 10% of invasive cancer cells; and score 3+: strong complete homogenous membrane staining in > 30% of the invasive tumor cells.[9] Score 3 was considered positive *EGFR* expression and all other scores were considered negative.

EGFR gene copy number (GCN) detection by fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) was performed on 4 um thick formalin-fixed, paraffin-embedded slices using the Vysis LSI EGFR SO/CEP 7 SG Probe (Vysis LSI EGFR Dual Color Probe-Hyb Set FISH probes; Abbott Molecular, Abbott, Park, IL, USA) for *EGFR* gene copy number (GCN) using ThermoBrite Elite (Leica, Richmond, CA, USA) according to the manufacturer's instructions. FISH study results were analyzed using DM6000B (Leica) with orange, green, and 4,6-diamid-ino-2-phenylindole filters. FISH results were defined by the Colorado scoring system: FISH positive was defined as ≥ 40% of cells displaying ≥ 4 *EGFR* counts; or the presence of gene amplification as defined by either mean *EGFR*/CEN-7 ratio ≥ 2, > 10% of cells displaying >15 *EGFR* counts, > 10% of the cells displaying the presence of loose or tight *EGFR* signal cluster, or atypically large *EGFR* signals (*EGFR* cluster scored).[10] Images were captured using Ariol SL200 (Leica).

Statistical analysis

Descriptive statistics, including median, frequency, and proportion were used to summarize the demographics and AEs. A Fisher's exact test was used to compare ORR, DCR, and demographic data between the subgroups. A Student's *t*-test was used to compare means. Kaplan–Meier and log-rank tests were used to estimate PFS. All statistical tests

were calculated using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) and GraphPad prism 5 (GraphPad, La Jolla, CA, USA). A two-sided P value of < 0.05 was considered statistically significant.

Results

In total, 26 patients were enrolled in the study out of 216 advanced LSCC patients treated from June 2012 to December 2016 at the Department of Respiratory Medicine, PUMCH. Table 1 shows the baseline characteristics of patients and chemotherapy regimens. Sixteen patients discontinued nimotuzumab because of the inconvenience of treatment. Patients received a median of 4 (range 2–8) cycles of nimotuzumab. No patients were lost follow-up.

Efficacy analysis

The median follow-up duration was 14.6 (range: 3.6–38.1) months. At the date of analysis, seven patients were alive, and disease had not progressed in three patients. The ORR, DCR, median PFS, and median overall survival (OS) were 50%, 100%, 6.7 months, and 16.3 months, respectively.

EGFR expression and efficacy

Thirteen biopsy samples were available for *EGFR* immunohistochemistry (IHC). *EGFR* expression evaluated by IHC staining was not associated with efficacy (Table 2).

EGFR GCN and efficacy

Gene copy numbers were analyzed in 13 biopsy samples using FISH. The stage and ECOG PS were well balanced between FISH *EGFR* negative and positive groups. Table 2 summarizes the efficacy stratified with GCNs, which showed that patients with positive *EGFR* via FISH had a trend of longer median PFS (10.0 vs. 7.0 months; $P = 0.10$) compared to patients with negative *EGFR* via FISH (Fig 1).

Chemotherapy regimens and efficacy

Paclitaxel/carboplatin (PC) and gemcitabine/cisplatin (GC) regimens were administered to most patients with advanced LSCC. Patients in the GC group were younger. There were no significant differences in median PFS or OS between the two groups.

Safety profile

Table 3 summarizes the AEs of treatment, which primarily included bone marrow suppression and sensory neuropathy. Sensory neuropathy were observed in 15 patients administered paclitaxel or cisplatin, and an elevated serum creatinine level was observed in one patient administered cisplatin. All AEs were attributed to chemotherapy.

Table 1 Baseline patient characteristics

Parameters	No. of patients (n = 26)	Percentage
Age (years)	Median 64.5 (range 43–76)	
Male/female	22/4	84.6/15.4
ECOG PS		
0	15	57.7
1	8	30.8
2	3	11.5
Tumor stage		
IIIB	2	7.7
IV	24	92.3
EGFR genotype		
Wild type	8	30.8
Unknown	18	69.2
Smoking history		
Yes	22	84.6
Never	4	15.4
Chemotherapy regimen		
Paclitaxel/carboplatin	13	50.0
Gemcitabine/cisplatin	9	34.6
Gemcitabine	2	7.7
Etoposide	2	7.7

ECOG PS, Eastern Cooperative Oncology Group performance status.

Discussion

Platinum-based chemotherapy is widely used for the treatment of advanced NSCLC; however, the efficacy of such treatment is dissatisfactory, with response, one-year survival, and PFS rates of 19–30.6%, 30–36%, and approximately five months, respectively.[11,12] The efficacy of nimozutumab plus chemotherapy is superior to platinum-based regimens. In this study, the ORR was 50% and median PFS was 6.7 months. Similarly, a previous phase II study reported an ORR of 54% in a group of NSCLC patients administered nimotuzumab plus chemotherapy.[5]

The half-life durations of nimotuzumab, administered in doses of 200 mg and 400 mg have been reported as 302.95 ± 44.14 and 345.1 ± 41.23 hours, respectively.[13] Nimotuzumab is well tolerated up to 400 mg per week.[14] In previous clinical trials, nimotuzumab was administered in weekly doses of 200 mg or 400 mg.[5,15] Considering the half life of nimotuzumab, safety, and the need for fewer infusions, nimotuzumab was administered at a dose of 400 mg on days 1 and 8 for 21 days in this study.

Nimotuzumab is a highly specific humanized antibody with highly affinity and is associated with fewer AEs than cetuximab. Qi *et al.* reported only three cases (10%) of grade 1–2 skin rash.[6] Xu *et al.* reported that skin and subcutaneous tissue disorders related to nimotuzumab

Table 2 *EGFR* immunohistochemistry, *EGFR* gene copy numbers, chemotherapy regimens, and efficacy

Variables	*EGFR* negative (*n* = 6)	*EGFR* positive (*n* = 7)	*P*	*EGFR*– via FISH (*n* = 5)	*EGFR*+ via FISH (*n* = 8)	*P*	Paclitaxel/carboplatin (*n* = 13)	Gemcitabine/cisplatin (*n* = 9)	*P*
Gender M/F	4/2	6/1	0.56	3/2	7/1	0.51	12/1	7/2	0.54
Age Median (range)	68 (43–71)	62 (52–75)	0.89	65.0 (46–71)	62.5 (43–75)	0.75	67.0 (52–76)	54.0 (43–71)	0.03
Stage IIIB/IV	1/5	1/6	1.0	1/4	1/7	1.0	0/13	2/7	0.16
ECOG PS 0/1/2	5/1/0	3/4/0	0.27	3/2/0	5/3/0	1.0	6/7/0	8/1/0	0.07
PR/SD/PD	2/4/0	5/2/0	0.59	2/3/0	6/2/0	0.29	6/7/0	3/6	0.67
mPFS (months)	7.8	7.1	0.61	7.0	10.0	0.10	6.0	7.0	0.28
mOS (months)	17.8	13.8	0.44	16.3	28.9	0.51	15.4	16.3	0.59

FISH, fluorescence in situ hybridization; ECOG PS, Eastern Cooperative Oncology Group performance status; mPFS, median progression-free survival; mOS, median overall survival; PR, partial response; PD, progressive disease; SD, stable disease.

administration only accounted for 3.7% of all AEs.[16] In this study, nimotuzumab was well tolerated. AEs were associated with chemotherapy. The most common AEs related to *EGFR* inhibitors, including dermatologic toxicity and diarrhea, were not observed in this study. We infer that it this may be a result of the excellent safety profile of nimotuzumab, but may relate to the small sample size.

The FLEX study, a retrospective biomarker analysis, showed that cetuximab was beneficial to a group with high *EGFR* expression.[17,18] Another study analyzed the clinical response to nimotuzumab in esophageal squamous cell carcinoma patients with *EGFR* expression detected using IHC staining, but concluded that detection of *EGFR* expression via IHC was not a predictive biomarker.[19] In this study,

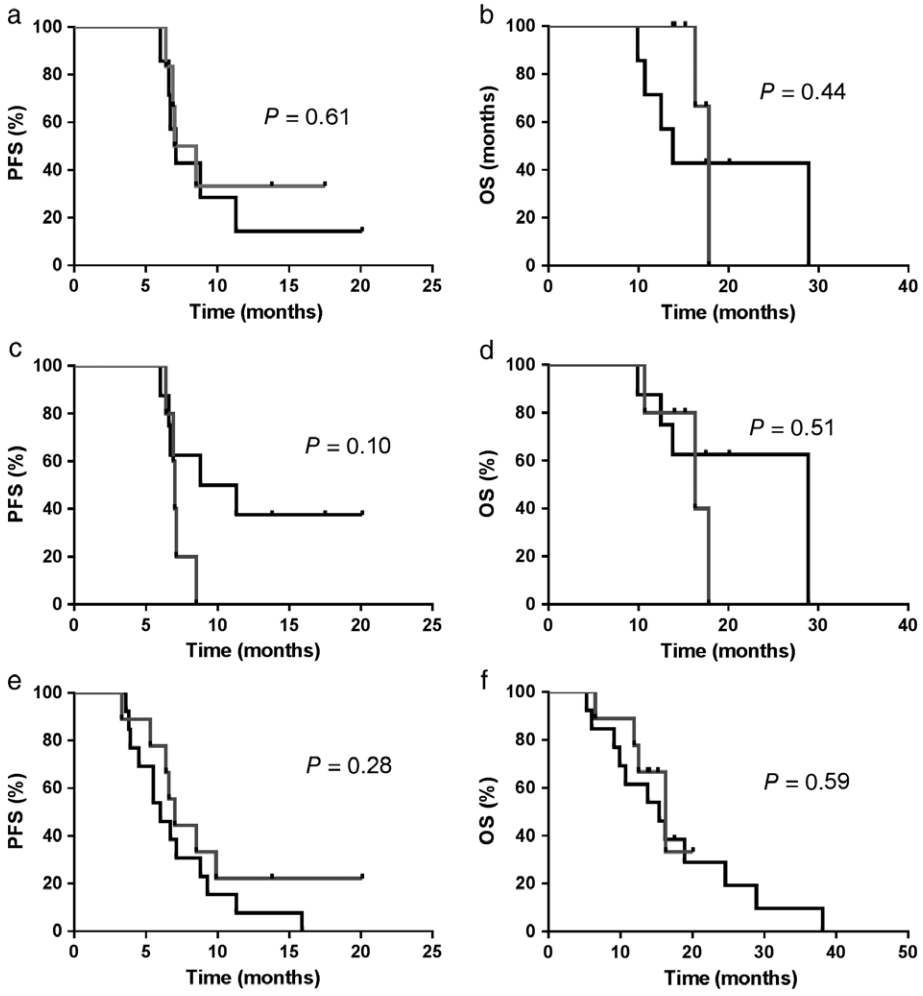

Figure 1 (**a**) Progression-free survival (PFS) and (**b**) overall survival (OS) of EGFR status (positive and negative) via immunohistochemistry (IHC). (**c**) PFS and (**d**) OS of patients with positive and negative *EGFR* gene copy number (GCN). (**e**) PFS and (**f**) OS of patients administered paclitaxel/carboplatin (PC) and gemcitabine/cisplatin (GC). (—▪—) IHC EGFR+; (—▪—) IHC EGFR–; (—▪—) EGFR+ GCN; (—▪—) EGFR– GCN; (—▪—) PC; (—▪—) GC.

Table 3 Adverse events of nimotuzumab-combined chemotherapies in patients with advanced lung squamous cell carcinoma

Adverse event	No. (%)	Grade 1–2†	Grade 3–4†
Bone marrow suppression	15 (57.7)	5	10
Sensory neuropathy	15 (57.7)	14	1
Alopecia	14 (53.8)	14	0
Nausea/vomiting	9 (34.6)	8	1
Elevated creatinine level	1 (3.8)	1	0

†According to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0.

there was no significant difference in efficacy between patients detected with *EGFR* expression via IHC or FISH. However, patients detected with positive *EGFR* expression via FISH showed a trend toward higher PR and longer median PFS, which indicates that *EGFR* GCN may be a possible predictive biomarker. Because of the small sample size of our study, our data did not show statistical significance. Further studies are required to confirm the predictive value of nimotuzumab efficacy for *EGFR* GCN.

Our study had several limitations. It was a retrospective study with a small sample size, without randomization or a control group. Only 12% of patients with advanced LSCC received nimotuzumab as first-line treatment. As such, there may be bias in patient enrollment. Efficacy and safety could not be directly compared between treatment and control groups. *EGFR* mutation status was only assessed in only eight patients, and *EGFR* mutation was not detected. Most patients (85%) were smokers; therefore, it was difficult to analyze the effect of *EGFR* mutation and smoking history on nimotuzumab efficacy. The patient sample was a real world sample and the inclusion and exclusion criteria were comparable to those for chemotherapy. However, the results may not apply to specific patients, such as those with poor ECOG PS scores (≥ 3).

In summary, the ORR and DCR of nimotuzumab combined with chemotherapy as first-line therapy was considerably high in patients with advanced LSCC, and the safety profile was reassuring. *EGFR* GCN examined by FISH may be a possible predictive biomarker. Large-scale randomized controlled trials are required to confirm the efficacy of nimotuzumab combined with chemotherapy as first-line therapy for patients with advanced LSCC.

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Disclosure

No authors report any conflict of interest.

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