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BRIEF REPORT

Nimotuzumab combined with gemcitabine and cisplatin as second-line chemotherapy for advanced non-small-cell lung cancer

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Abstract

Objective: To evaluate the efficacy and safety of nimotuzumab combined with gemcitabine and cisplatin as second-line chemotherapy for advanced non-small-cell lung cancer (NSCLC) and to investigate the association of the status of KRAS gene mutation and epidermal growth factor receptor (EGFR) genotype with clinical outcome.

Methods: Twenty-eight patients with advanced NSCLC were enrolled in this single center, uncontrolled pilot clinical study. All the patients developed drug resistance or disease progression after first-line chemotherapy of either a docetaxel + cisplatin regimen or a vinorelbine + cisplatin regimen and then received nimotuzumab combined with gemcitabine and cisplatin as second-line chemotherapy. Eight cases were stage IIIB and 20 were stage IV. An i.v. dosage regimen of 200 mg of nimotuzumab was given as a single dose, injected into the patient at days 1, 8 and 15; i.v. gemcitabine was injected at a dose of 1000 mg/m^2 at days 1 and 8 and cisplatin (25 mg/m^2 i.v.) at days 1, 2 and 3. Each patient received four or more therapeutic cycles. The efficacy and toxic reactions were evaluated, as well as time to progression and overall survival.

Results: In total, 28 patients with advanced NSCLC received 101 therapeutic cycles. The mean cycle number was 3.6. Median time to progression was 4.9 (2.5–6.5) months; median overall survival and 1-year survival rate were 9.8 months and 48.5%, respectively. There was one case of complete response, six cases of partial response, 11 cases of stable disease and 10 cases of progressive disease. Response rate was 25%, and clinical benefit rate was 64.3%. Major toxic reactions were bone marrow suppression and gastrointestinal reaction. Only one patient developed grade I acneiform eruption.

Conclusion: Nimotuzumab combined with gemcitabine and cisplatin as second-line chemotherapy for advanced NSCLC was active and well-tolerated in this setting. Patients with EGFR amplification and KRAS gene wild type had a better prognosis. Prospective, randomized, controlled, large-scale clinical studies are needed to confirm the results.

Non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related mortality and the incidence increases year by year.[1–4] The majority of patients are at middle or late stage at the time of presentation, that is stage IIIB (locally advanced stage) or stage IV (metastatic).[5–8] Through years of numerous clinical studies, the administration of platinum-based combination chemotherapy regimens, which standard chemotherapy for advanced NSCLC, have been constantly

improved. However, many studies indicated that the efficacy has reached a plateau.[9–17] The emergence of molecular-targeted drugs brought new hope for treatment of NSCLC. Besides the application of small molecule tyrosine kinase receptor antagonists like Iressa and Tarceva, depending on a patient's epidermal growth factor receptor (EGFR) gene mutation determination, cetuximab, a human–mouse chimeric anti-EGFR monoclonal antibody, may be combined with chemotherapy for an increased response rate (RR) in advanced NSCLC, prolonging survival.[18–23] However, acneiform eruption associated with cetuximab significantly impairs patient's life quality. Some patients have even had to stop therapy due to intolerance.[24–27]

Nimotuzumab is a human anti-EGFR monoclonal antibody.[28] It was reported that nimotuzumab combined with radiotherapy and chemotherapy achieved a certain efficacy and was well tolerated in the treatment of advanced nasopharyngeal carcinoma and head/neck squamous cell carcinoma, with low incidence of rash.[29,30] This study analyzed the clinical data from 28 advanced NSCLC patients treated in the First Department of Internal Medicine, Tianjin Medical University Cancer Institute and Hospital between February 2009 and June 2010. All the patients were resistant to first-line chemotherapy and received nimotuzumab combined with gemcitabine and cisplatin as second-line chemotherapy, so as to explore new drug treatment of advanced NSCLC and find a therapeutic regimen with high efficacy and low toxicity.

Materials and methods

Case selection

For the 28 patients enrolled in this study, their pathological sections were reviewed by more than two senior pathologists and histopathological type was determined in accordance with the World Health Organization (WHO) criteria of 2009. Patients provided written informed consent before entry into the study, then the tumors samples were obtained and EGFR and KRAS status were assessed. Imaging confirmed stage IIIB or IV, and all patients had evaluable lesions with a diameter $\geq$1.5 cm. These patients had not received any chemotherapy, radiotherapy or other antitumor therapy within the previous month; hepatic and renal functions were basically normal; systemic functional status score (ECOG) were 0–2 points; peripheral white blood cell $\geq 4.0 \times 10^9$, platelet $\geq 100 \times 10^9$; expected survival >3 months. All the patients developed tumor progression after two cycles of first-line chemotherapy.

EGFR protein analysis

Serum EGFR protein levels were measured by commercial Human EGFR ELISA Kit (Uscn Life Science & Technology Company, Missouri, TX, USA) according to the manufacturer's recommended protocol. Each sample was assayed in triplicate. The level of EGFR protein was calculated according to standard substance provided by manufacture.

EGFR expression fluorescence *in situ* hybridization (FISH) analysis

Gene copy number per cell was investigated by FISH using the LSI EGFR Spectrum Orange/CEP 7 Spectrum Green probe (Vysis, Abbott Laboratories, IL, USA), according to published protocol. Sections were incubated at 56°C overnight, deparaffinized by xylene and dehydrated in 100% ethanol. After incubation in pretreatment buffer at 80°C for 10 min, the sections were digested with proteinase K (0.25 mg/mL in 2 × saline-sodium citrate buffer (SSC), pH 7.0) at 37°C for 15 min, rinsed in 2 × SSC (pH 7.0) at room temperature for 5 min, and dehydrated using ethanol in a series of increasing concentrations (70%, 85%, 100%). The EGFR/CEP 7 probe set was applied to each slide, and the hybridization area was covered with a glass cover slip and sealed with rubber cement. The slides were incubated at 73°C for 5 min for co-denaturation of chromosomal and probe DNA and were then placed in a humidified chamber at 37°C overnight. Posthybridization washes were performed in 2 × SSC/0.3% NP40 (pH 7.0–7.5) at 73°C three times. After the samples were dehydrated in ethanol, DAPI (4′, 6′-diamidino-2-phenylindole) was applied for chromatin counterstaining.

For result analysis, patients were classified into six FISH strata with ascending number of copies of the EGFR gene per cell according to the frequency of tumor cells with the specific number of copies of the EGFR gene and chromosome 7 centromere: (i) disomy ($\leq$2 copies in >90% of cells); (ii) low trisomy ($\leq$2copies in $\geq$40% of cells, 3 copies in 10–40% of the cells, $\geq$4 copies in <10% of cells); (iii) high trisomy ($\leq$2 copies in $\geq$40% of cells, 3 copies in $\geq$40‰f cells, $\geq$4 copies in <10% of cells); (iv) low polysomy ($\geq$4copies in 10–40% of cells); (v) high polysomy ($\geq$4copies in $\geq$40‰f cells); and (vi) gene amplification (defined by the presence of tight EGFR gene clusters and a ratio of EGFR gene to chromosome of $\geq$2 or $\geq$15copies of EGFR per cell in $\geq$10% of analyzed cells).[30]

K-RAS gene mutation detected by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP)

Plasma KRAS gene mutation was detected by PCR-RFLP in 20 NSCLC patients. It was performed according to the manufacturer's recommended protocol.

Clinical data

A total of 28 NSCLC patients were enrolled, 19 men and nine women; the median age was 51 years (32–66). According to

Table 1 Clinical data of 28 patients with advanced non-small cell lung cancer

Number	28
Median age (years)	51 (32–66)
Sex (male/female)	2.1/1 (19/9)
Stage	
I	0
II	0
IIIA	0
IIIB	8
IV	20
Pathology	
Squamous carcinoma	15
Adenocarcinoma	11
Mixed subtype adenocarcinoma	1
Large cell carcinoma	1

the WHO pathological classification of 2009, there were 15 cases of squamous carcinoma, 11 cases of adenocarcinoma, one case of mixed subtype of adenocarcinoma (signet ring cell adenocarcinoma) and one case of large cell carcinoma (clear cell carcinoma). There were eight stage IIIB patients and 20 stage IV patients (Table 1).

Treatment

Nimotuzumab 200 mg i.v. injection was administered on days 1, 8 and 15; cisplatin 25 mg/m^2 i.v. injection, on days 1–3; and gemcitabine 1000 mg/m^2 i.v. injection, on days 1 and 8; one cycle consisted of 21–28 days. During chemotherapy, prophylactic antiemetic treatment with serotonin receptor antagonist was routinely applied. After chemotherapy, granulocyte colony stimulating factor (G-CSF) was administered once bone marrow suppression (grade II or above) was observed. In cases that had an adverse reaction (grade III or above), dosage of chemotherapy drugs was reduced by 15–30% in the next cycle. (Table 2).

Efficacy and toxicity assessment

Imaging examinations (computed tomography, magnetic resonance imaging and positron emission tomography-computed tomography) were performed at the end of every two cycles of chemotherapy. Efficacy was assessed according to RECIST standard and classified into complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). RR was defined as CR+PR. Clinical benefit rate (CBR) was defined as CR+PR+SD. Time to progression (TTP) was defined as time from initiation of treatment to tumor relapse or progression. Overall survival (OS) was defined as the period from the treatment start date to the date of patient death, last follow up, or observation end. Toxic reactions were classified into grades 0-IV according to WHO standard.

Statistical analysis

SPSS version 11.0 software was used (SPSS Inc, Chicago, IL, USA) for statistical analyses. Group analyses were done by age, sex, performance status, tumor location, histological subtype, EGFR expression, tumor length and width. Survival time was calculated from the date of the first treatment until the date of death (with an overall two-sided type I error at a significance level of 95% and statistical power of 80% to detect differences). TTP and OS data were estimated with the Kaplan–Meier method.

Role of the funding source

The sponsor of this study had no role in the study design, data collection, data analysis, or data interpretation. The corresponding authors had full access to all data in the study, and had the final responsibility to submit for publication.

Results

EGFR analyses and KRAS mutation detection

In our study, we detected the EGFR protein level (with ELISA), EGFR gene copy number (with FISH) and KRAS mutation in patients whose specimens (tissue or blood or both) were available. EGFR protein level was measured in 20 patients and the range was between 4.6 and 623.5 fmol/L. F for analyzing the copy number of EGFR amplification 15 patients' tissue specimens were available. The FISH positive rate was 80.0% (12/15). EGFR FISH positive patients had survival benefit after treatment with nimotuzumab in combination with chemotherapy, and the TTP ranged from 3.8 months to 6.5 months (Table 3). KRAS mutation was analyzed in 20 patients' tissue or blood specimens and the mutation rate was 15.0% (3/20). One patient was resistant to nimotuzumab plus chemotherapy and another two patients showed stable disease.

Table 2 Nimotuzumab, gemcitabine, cisplatin (NGC) regimen for 3 weeks cycle

NGC regimen	Dose	Usage	Delivery time
Nimotuzumab	200 mg	Dissolved in 500 mL normal saline, i.v. drip over 4 hours	Days 1, 8, 15
Gemcitabine	1000 mg/m^2	Dissolved in 100 mL normal saline, i.v. drip for 30 min	Days 1, 8
Cisplatin	25 mg/m^2	Dissolved in 250 mL normal saline, i.v. drip over 30 min	Days 1–3

Table 3 EGFR status and responses of the patients

Case	TTP (m)	OS (m)	EGFR high copy number
1	4.2	10.0	Amp
2	5.1	13.7	Amp
3	2.5	5.8	Non-amp
4	4.0	8.1	Amp
5	5.0	20.0	Amp
6	4.6	12.3	Amp
7	2.8	6.2	Non-amp
8	5.6	14.8	Amp
9	6.0	16.5	Amp
10	3.2	15.2	Non-amp
11	6.5	22.6	Amp
12	5.8	21.8	Amp
13	5.6	18.5	Amp
14	3.8	9.4	Amp
15	6.3	19.6	Amp

Amp, amplification; EGFR, epidermal growth factor receptor; m, months; non-Amp, non-amplification; OS, overall survival; TTP, time to progression.

Short-term efficacy and survival

Twenty-eight patients received 101 cycles of chemotherapy in total (average cycle number: 3.6). Median OS (MST) and 1-year survival rate (SR) were 9.8 months and 48.5%, respectively. Median TTP was 4.9 (2.5–6.5) months; there was one case of CR, six cases of PR, 11 cases of SD and 10 cases of PD. PR was 25% and CBR was 64.3% (Figs 1, 2).

Toxic reactions

The major toxic reactions were bone marrow suppression and gastrointestinal reaction. Bone marrow suppression mainly resulted in neutropenia and thrombocytopenia, which were reversible. Gastrointestinal reactions mostly manifested as grade I/II nausea and vomiting. Only one patient developed grade I acneiform eruption. The incidence of rash was 3.6% (Table 4).

Discussion

NSCLC accounts for approximately 85% of all lung cancers. Since most of these patients are at stage IIIB or IV at diagnosis, chemotherapy combined with nutritional supportive treatment is extremely important. However, the median survival of newly diagnosed patients receiving conventional chemotherapy is only 8–12 months. The relative 1-year survival rate is only 30–50%.[31,32] Efficiency and survival with second-line chemotherapy is even more disappointing.[33] Therefore, it is urgent to find a more effective treatment. In recent years, targeted therapy, combined or sequential with chemotherapy, has been developed for treatment of advanced NSCLC, and has proved to prolong patient's survival in IPASS and FLEX studies.[34,35]

EGFR, as the first member of ErbB family and highly expressed in 40–80% patients with NSCLC, has received particular attention recently.[36] As the first EGFR antagonist, cetuximab was used to treat advanced NSCLC in many clinical trials. Preliminary results showed cetuximab had a synergistic effect with cytotoxic drugs.[37] In multicenter, randomized, controlled studies using cetuximab combined with platinum-based chemotherapy, cetuximab group showed better efficacy than the chemotherapy-only group in terms of RR.[38–41] In 2008 at the American Society of Clinical Oncology annual meeting, a phase III study (FLEX) was reported which compared cetuximab combined with chemotherapy with chemotherapy alone as first-line treatment of advanced NSCLC for EGFR immunohistochemically-

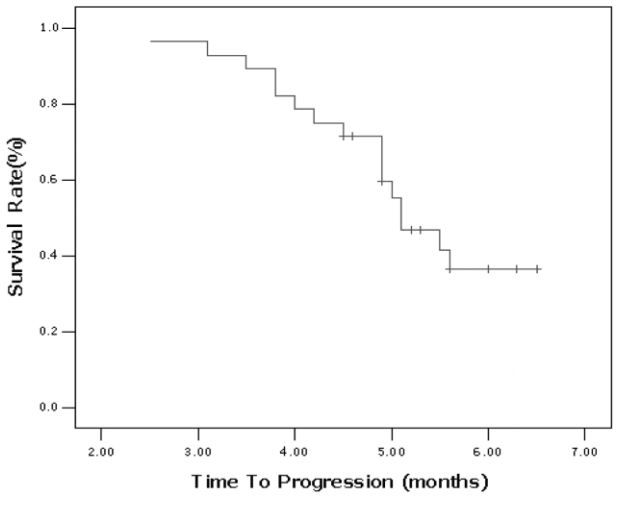

Figure 1 Kaplan–Meier curve with a median time to progression of 4.9 (2.5–6.5) months.

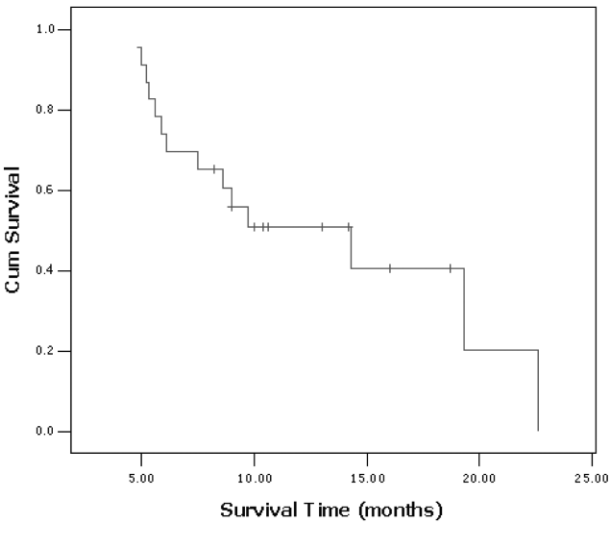

Figure 2 Overall survival curve with a median overall survival of 9.8 (4.8–22.6) months and 1-year survival rate of 48.5%.

Table 4 Adverse reactions of 28 patients with advanced non-small cell lung cancer treated to nimotuzumab, gemcitabine, cisplatin regimen as second-line therapy

Symptoms	0	I	II	III	IV	Incidence rate (%)	Incidence rate of III, IV grade (%)
Emesis and vomiting	10	12	5	1	0	64.28	3.57
Fever	26	2	0	0	0	7.14	0.00
Rash	27	1	0	0	0	3.57	0.00
Fatigue	9	10	6	3	0	67.86	10.71
Anemia	25	2	1	0	0	10.71	0.00
Leukopenia	12	6	9	1	0	57.14	3.57
Thrombocytopenia	9	7	11	1	0	67.86	3.57
Elevated transaminase	23	3	2	0	0	17.86	0.00
Renal dysfunction	25	2	1	0	0	10.71	0.00
Alopecia	24	2	2	0	0	14.29	0.00

positive patients. The results showed that patient survival was prolonged in the cetuximab combined with cisplatin/vinorelbine group, compared with the control group (NVB/Cis; 11.3 vs. 10.1) and the 1-year survival rate was increased (47% vs. 42%). The difference between the two groups had statistical significance ($P = 0.044$, hazard ratio 0.87), suggesting that cetuximab combined with chemotherapy reduced the risk of death by 13%. At present, few studies focus on cetuximab in combination with chemotherapy as second-line or further-line therapy for patients who have failed a platinum based doublet regimen. Kim *et al*. reported a study on cetuximab combined with docetaxel as second-line therapy for advanced NSCLC patients. The result showed that the response rate was 20% and the median OS was 7.5 months, which is higher than the standard second-line chemotherapy with docetaxel or pemetrexed.

However, in patients with advanced NSCLC additionally receiving human–mouse chimeric cetuximab, the side-effect rash (mainly acneiform rash) cannot be ignored. Some patients have even stopped the drug due to intolerable severe rash.[26,27] During administration of cetuximab, the incidence of grade III–IV rash is 11–22% and the occurrence shows dose-dependency.[42–44] Several clinical trials have been carried out trying to determine the correlation between rash and efficacy of chemotherapy; however, results have been inconclusive.[21,26,27,45]

Nimotuzumab is a human-derived anti-EGFR monoclonal antibody with high selectivity and a long half-life. Preclinical studies demonstrate that it could competitively inhibit endogenous ligands from binding to EGRF and block EGFR-mediated downstream signaling pathways, inhibiting tumor proliferation, promoting tumor apoptosis, preventing tumor angiogenesis and enhancing chemotherapy sensitivity.[46,47] In treatments of head and neck tumors, nimotuzumab has shown high efficacy and very low incidence of rash.[48,49] When applied in lung cancer, it has always been always combined with radiotherapy as reported in the literature. It was well tolerated by patients and enhanced radiotherapy sensitivity to some extent. Almost no rash was observed.[50]

In our study, 28 patients received nimotuzumab combined with chemotherapy. A total of 101 cycles of chemotherapy were administered, with a mean cycle number of 3.6. There was one case of CR, six cases of PR, 11 cases of SD and 10 cases of PD. RR was 25%; CBR was 64%, higher than the standard second-line chemotherapy with docetaxel or pemetrexed. The possible reasons may be: (i) 12 out of 15 patients had EGFR amplification and 17 out of 20 patients had KRAS wild type, which might contribute to patients' survival benefit; or (ii) the combination of nimotuzumab and gemcitabine may be a potential second-line therapy regimen and deserves to future study.

In our study, 28 patients received nimotuzumab plus chemotherapy as second-line therapy, MST and 1-year survival rate were 9.8 months and 48.5%, respectively. Median TTP was 4.9 (2.5–6.5) months, higher than the data of historial control. This may be because the FISH positive rate was 80.0% (12/15). EGFR FISH positive patients had survival benefit after treatment with nimotuzumab in combination with chemotherapy, and the TTP was 3.8 months to 6.5 months (Table 3), which is higher than for patients without EGFR gene amplification.

KRAS mutation has become an important contraindication for advanced colon cancer to use cetuximab. However, to date, no effective predictive biomarkers for cetuximab were identified in advanced NSCLC. Most recently, Byrne *et al*. evaluated the effect of K-RAS mutation and EGFR genotype on the response to cetuximab in combination with chemotherapy in FLEX trial. The results showed that there was no correlation between KRAS mutation, EGFR mutation and amplification (FISH positive) with progression-free survival or OS. In Our study, KRAS mutation was analyzed in 20 patients' tissue or blood specimens and the mutation rate was 15.0% (3/20). One patient was resistant to nimotuzumab plus chemotherapy and another two patients showed stable disease.

Concerning toxic reactions, only one of the 28 enrolled patients developed grade I acneiform eruption during the first cycle, which did not recur after the beginning of the

second cycle. Tolerance is significantly increased compared with cetuximab.

In conclusion, the results of this study show that nimotuzumab combined with gemcitabine-based chemotherapy is safe and tolerable. The mostly common toxicity is mild to moderate skin rash. For patients with advanced NSCLC, especially needing multiline treatment, nimotuzumab combined with gemcitabine and cisplatin as second-line chemotherapy was active and well tolerated in this setting Patients with EGFR amplification and KRAS gene wild type had better prognosis.

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Disclosure

No authors report any conflict of interest.

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