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Original Article

Clinical effect of crizotinib on lung cancer patients with EML4-ALK fusion gene mutation

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Abstract: Objective: To investigate the clinical efficacy of crizotinib in the treatment of non-small cell lung cancer (NSCLC) with echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) fusion gene mutation. Methods: Forty-eight NSCLC patients with EML4-ALK fusion gene mutation were collected and divided into the experimental group (exp group) and the control group (con group), with 24 patients in each group. The experimental group received crizotinib twice daily, and the control group received intravenous chemotherapy of pemetrexed combined with cisplatin three times a week. The systemic nutrition, immune status, incidence of adverse reactions, KPS life quality score and patient satisfaction after 3 months, and survival rate of patients within 6 months were compared between the two groups. Results: There was no significant difference in nutritional status between the two groups before treatment (P>0.05). After treatment, the prealbumin and albumin levels in the experimental group were significantly higher than those in the control group. The CD4+/CD8+ ratio in the experimental group was markedly higher than that in the control group, and the incidence of adverse reactions was obviously reduced in the experimental group. The KPS life quality score and patient satisfaction after 3 months in the experimental group were significantly higher than those in the control group, with statistically significant differences (all P<0.05). The survival rate in the experimental group at 6 months after surgery was 50%, which was notably higher than the survival rate of 30% in the control group, and the difference was statistically significant (P<0.05). Conclusion: Crizotinib is more effective than intravenous chemotherapy in the treatment of NSCLC with EML4-ALK fusion gene mutation. It may be widely used in clinical practice.

Keywords: EML4-ALK fusion gene, non-small cell lung cancer, crizotinib, intravenous chemotherapy

Introduction

The incidence of lung cancer is increasing year by year. According to statistics, the incidence of lung cancer in the world is as high as 46.5%, among which the incidence of non-small cell lung cancer (NSCLC) accounts for 80%~85% [1-3]. NSCLC is different from small cell lung cancer, which is characterized by slow growth of cancer cells and late spread [4, 5]. At present, the main clinical treatment of NSCLC is chemotherapy, but the side effects are severe, and the long-term survival time of patients is short [6, 7]. Therefore, the treatment method based on chemotherapy alone without targeted therapy is not enough, which makes the patient's prognosis not ideal [8].

Echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) fusion gene is the newly discovered mutation site in NSCLC [9-11]. Studies have shown that the EML4-ALK fusion gene is a NSCLC-driven gene, in which the ALK fusion site occurs in exon 20 [12]. Currently, EML4-ALK fusion gene plays an important role in normal development and function of the respiratory system. Mutation of EML4-ALK fusion gene could promote the occurrence of respiratory diseases, such as NSCLC [13].

Crizotinib is a molecularly targeted drug that acts on the EML4-ALK fusion gene and mainly suppresses the activity of ALK on the fusion gene, thereby inhibiting the occurrence and development of NSCLC [1, 14]. At present, clinical treatment of tumors is not limited to surgery, chemotherapy and radiotherapy, but also includes biomolecular targeted therapy, which targets molecules in specific signal transduc-

tion systems in tumors and inhibits carcinogenic sites at the cellular and molecular level, thereby inhibiting tumor progression [15, 16]. There are few studies on the effect of crizotinib on the NSCLC with EML4-ALK fusion gene. In this paper, the clinical effect of crizotinib on NSCLC patients was studied, providing another clinical therapeutic scheme for NSCLS.

Materials and methods

The general information

Forty-eight NSCLC patients with EML4-ALK fusion gene mutation from June 2017 to June 2018 in Dezhou Second People's Hospital were collected and divided into the experimental group (exp group) and the control group (exp group), with 24 patients in each group. The average age of the patients was 57.2 ± 6.4 years old, including 22 males and 26 females. All patients signed the informed consent, and this study was approved by the Ethics Committee of Dezhou Second People's Hospital.

Inclusion criteria: Patients diagnosed with NSCLC according to the 2017 CSCO guidelines for diagnosis and treatment of primary lung cancer [17]; with anaplastic lymphoma kinase (ALK)-positive locally advanced or metastatic NSCLC and positive EML4-ALK fusion gene mutation.

Exclusion criteria: Patients with small cell lung cancer; patients with distant tumor metastasis; patients whose primary tumor was located in other organs and then with pulmonary metastases; patients with important organ failure; patients who did not cooperate with treatment; pregnant and lactating women; children; patients who were allergic to crizotinib.

Treatment methods

The experimental group: Patients were given oral crizotinib (Sericol) 250 mg twice a day, and the treatment was stopped immediately if the patients had cardiac and gastrointestinal dysfunction. For the above-mentioned adverse reactions, symptomatic treatment was given.

The control group: Patients were given intravenous chemotherapy, which was intravenous infusion of 500 mg/m^2 pemetrexed with 75 mg/m^2 cisplatin three times a week.

Outcome measures

The systemic nutrition, immune status, incidence of adverse reactions, KPS life quality score and patient satisfaction after 3 months of treatment, and survival rate of patients within 6 months were compared between the two groups.

Systemic nutritional status: Changes in albumin and prealbumin levels in blood routine examination of patients in each group before and after treatment for 1 and 3 courses of chemotherapy were compared.

Immune condition monitoring: The patients' fasting peripheral blood was collected, anticoagulated with EDTA tubes and centrifuged at 3,000 rpm for 15 min at low temperature. Then the supernatant was discarded and the cell layer was retained. After that, 5 mL erythrocyte lysate (Beyotime, China) was added to each tube, and then placed in the dark for 10 min. After the erythrocytes were completely lysed, centrifugation was performed and supernatant was discarded, and 1 mL PBS was added. According to the different T cell subsets to be detected, 1 μL corresponding fluorescent-labeled monoclonal antibodies (CD4+ and CD8+, Biosciences, USA) were added to each test tube and mixed gently, then incubated in the dark for 15 min at room temperature. Flow cytometry (BD Company, Accuri C6, USA) was used for detection, and the CD4+/CD8+ T lymphocyte ratio was measured using the software supplied with the instrument.

Survival rate: The number of patients who survived for 6 months after treatment was recorded. Survival rate = survivor/total number of patients observed.

Adverse reactions: Common adverse reactions that occurred during the treatment were observed.

KPS life quality score: According to the patient's normal activity, condition of the illness and self-care degree, KPS regards the patient's health status as a total score of 100 points, with 10 points as a grade. The higher the score, the better the health, and the more tolerable to the side effects of treatment. The lower the score, the worse the health and the lower the tolerance.

Table 1. Comparison of general information between the two groups

	Experimental group (n=24)	Control group (n=24)	Statistics	P
Male/Female	10/14	12/12	0.336	0.562
Age (year)	56.70 ± 6.60	58.20 ± 5.40		
BMI (kg/m^2)	24.51 ± 3.53	24.84 ± 3.22		
Hypertension	15	12	0.762	0.383
Diabetes	6	7	0.105	0.745
NSCLC type			0.873	0.646
Adenocarcinoma	10	12		
Squamous cell carcinoma	5	6		
Large cell carcinoma	9	6		
NSCLC staging			0.957	0.620
T1	4	5		
T2	12	14		
T3-4	8	5		
NSCLC grade			0.398	0.819
Grade I	5	4		
Grade II	12	13		
Grade III-IV	7	7		

Note: NSCLC: non-small cell lung cancer.

Patient satisfaction: (very satisfied + satisfied)/ number of patients in each group.

Statistical analysis

All data were statistically analyzed using SPSS22.0 software, and the picture was drawn using Graphpad Prism 7 software. The count data were expressed as cases/percentage (n/%) and evaluated by the χ^2 test. The measurement data were analyzed by Kolmogorov-Smirnov test for normality analysis, and those conforming to the normal distribution were expressed by mean ± standard deviation ($\bar{x}$ ± sd). The two independent samples t test was used for intragroup comparison, and the paired sample t test was used for before-after comparison. Survival rate analysis was compared using log-rank test. P<0.05 was statistically significant.

Results

Comparison of general information

There were no significant differences in gender, age, type of lung cancer, basic disease, stage and grade of lung cancer between the two groups. See **Table 1**.

Systemic nutritional status of patients

There was no statistically significant difference in albumin and prealbumin levels between the two groups before treatment (P> 0.05), while the albumin and prealbumin levels in the experimental group was significantly higher than that in the control group after 1 and 3 courses of treatment (all P< 0.05). See **Table 2**.

Immune status of patients

As shown in **Figure 1**, there was no statistically significant difference in the CD4+/CD8+ ratio between the two groups before treatment (P>0.05), while the CD4+/CD8+ ratio in the experimental group was markedly higher than that in the control group after 1 and 3 courses of treatment (P<0.05).

The incidence of adverse reactions

There were no significant differences in the incidence of hepatotoxicity, interstitial lung disease, prolonged QT interval, bradycardia, severe visual impairment and other adverse reactions between the two groups (P>0.05), while the total complications in the experimental group were obviously lower than that in the control group, and the difference was statistically significant (P=0.009). See **Table 3**.

The KPS life quality score

As shown in **Figure 2**, the KPS life quality score of the experimental group was clearly higher than that of the control group after 3 months of treatment, with statistically significant differences (P<0.05).

The patient satisfaction

The patient satisfaction in the experimental group was significantly higher than that in the

Table 2. Comparison of prealbumin and albumin levels between the two groups

	Experimental group (n=24)	Control group (n=24)	Statistics	P
Prealbumin				
Before treatment	345.2 ± 65.7	354.8 ± 66.4	0.503	0.617
After one course of treatment	302.3 ± 54.3*	259.3 ± 36.4*	3.222	0.002
After three courses of treatment	349.4 ± 58.8	300.5 ± 56.2	2.945	0.005
Albumin				
Before treatment	45.3 ± 7.6	46.7 ± 6.8	0.673	0.505
After one course of treatment	40.3 ± 5.4*	35.5 ± 6.2*	2.860	0.006
After three courses of treatment	45.2 ± 5.8	40.2 ± 5.5	3.065	0.004

Note: For intragroup comparison before and after treatment, *P<0.05.

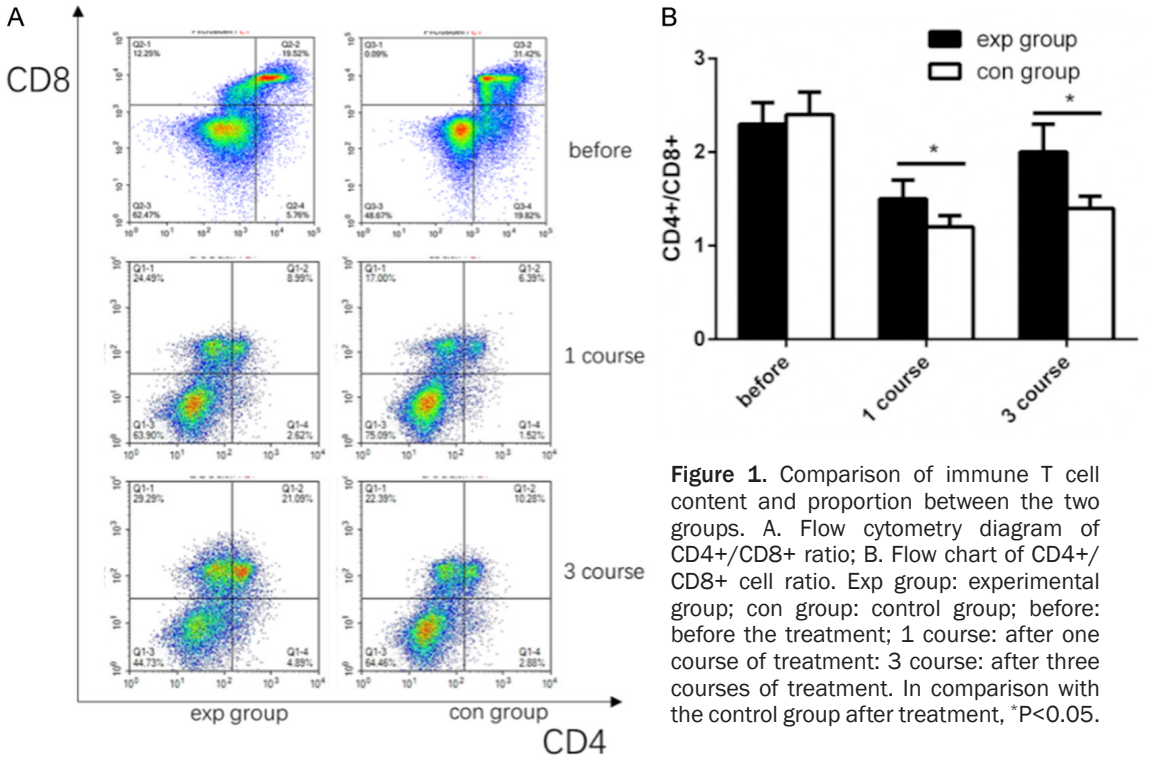

Figure 1. Comparison of immune T cell content and proportion between the two groups. A. Flow cytometry diagram of CD4+/CD8+ ratio; B. Flow chart of CD4+/CD8+ cell ratio. Exp group: experimental group; con group: control group; before: before the treatment; 1 course: after one course of treatment: 3 course: after three courses of treatment. In comparison with the control group after treatment, *P<0.05.

control group after 3 months of treatment, and the difference was statistically significant (P<0.05). See **Table 4**.

Comparison of survival time

The survival rate of the experimental group at 6 months after surgery was 50%, which was notably higher than the survival rate of 30% in the control group, and the difference was statistically significant (P<0.05). See **Figure 3**.

Discussion

Although the main treatment for NSCLC is still chemotherapy, the side effects caused by chemotherapy are intolerable for many patients, and the normal immune function is also severely affected [18, 19]. Therefore, it is urgent to find another way to fight cancer. Targeted molecular therapy is a hot topic in clinical studies at present, which inhibits the occurrence of diseases on specific targets and fights the occurrence and development of tumors to the maximum extent under the premise of minimal side effects [20]. Crizotinib is a FDA-approved ALK inhibitor that targets mutations in the ALK fusion genes [21]. Pemetrexed and cisplatin are common intravenous chemotherapy drugs for NSCLC, which suppress tumor growth by destroying cell metabolism and inhibiting cell

Table 3. The incidence of adverse reactions in the two groups

	Experimental group (n=24)	Control group (n=24)	Statistics	P
Hepatotoxicity	2	4	0.190	0.663
Interstitial lung disease	2	2	0.000	1.000
Extension of QT interval	0	1	0.000	1.000
Bradycardia	1	4	2.137	0.144
Severe visual impairment	2	5	0.669	0.413
Total adverse reactions	7	16	6.762	0.009

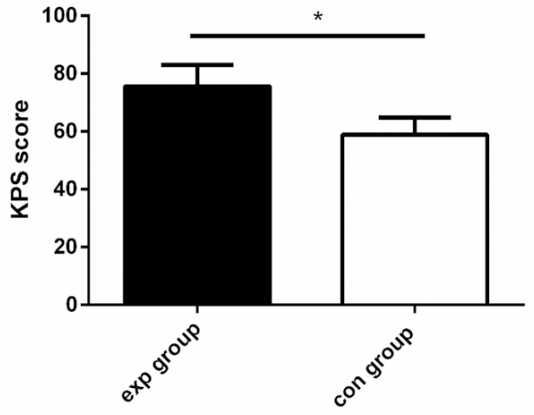

Figure 2. KPS quality life score after treatment in both groups. Exp group: experimental group; con group: control group. In comparison with the control group after treatment, *P<0.05.

growth [22, 23]. But they also have an inhibitory effect on normal organ cells and immune cells, and thus have an obvious inhibitory effect on immune function [24]. In this study, it was found that the immune function of the control group was significantly inhibited, while the targeted therapy had little effect on immune function. Dagogo-Jack I et al. found that the median survival time of patients with NSCLC was significantly prolonged and the immune function was not notably affected by the treatment with crizotinib [25].

This study found that when crizotinib was used to treat NSCLC, there was no significant change in systemic nutritional status, and there was no obvious difference in the levels of prealbumin and albumin after 3 courses of treatment compared with those before treatment, while the systemic nutritional status of patients receiving intravenous chemotherapy was significantly decreased. The possible reason was that intravenous chemotherapy of pemetrexed combined with cisplatin inhibited the growth of tumor cells, but also inhibited the growth of normal cells and even hepatocytes, leading to a significant decrease in the secretion of albumin by hepatocytes [26]. Albumin is one of the important indicators reflecting the systemic nutritional status. It can maintain the plasma colloid osmotic pressure, and its decomposition products amino acids can synthesize other nutrients needed for life [27]. Butrynski JE et al. found that crizotinib was an ALK inhibitor with no blocking effect on mutations at other sites, and had no significant effect on the growth and development of normal cells [21].

Cellular immunity plays an important role in the immune effect of tumor. The cellular immune response is the specific cellular immune reaction mainly mediated by T cells, and T cells (CD4+/CD8+ T cells) play a crucial regulatory role in the anti-tumor effect of the body [14]. The higher the proportion of CD4+/CD8+ T cells, the stronger the cellular immune function of patients [18, 19]. Crizotinib can activate the immune function of patients, but the specific mechanism has not been studied [20]. This study found that the percentage of CD4+/CD8+ T cells in the experimental group was significantly higher than that in the control group after treatment. The possible reason was that crizotinib can enhance the anti-tumor effect of CD4+ T cells and thus improve the immune function of patients.

In this study, the KPS life quality score and patient satisfaction in the experimental group were notably higher than that in the control group after 3 months. Although the intravenous chemotherapy of pemetrexed combined with cisplatin plays an anti-tumor role, it has an inhibitory effect on the nutritional status and immunity of the whole body, and has obvious side effects. Therefore, this chemotherapy is not as effective as the targeted therapy in life quality and patient satisfaction.

This study investigated the clinical efficacy and mechanism of crizotinib, and there were no relevant reports in this field. However, the small number of participants in this study limited the further analysis of the results. In the later stage, the clinical effect of crizotinib through

Table 4. Comparison of patient satisfaction between the two groups

	Experimental group (n=24)	Control group (n=24)	χ^2	P
Very satisfied	4	4		
Satisfied	14	7		
Not satisfied	6	13		
Satisfaction	75.00%	45.83%	4.269	0.039

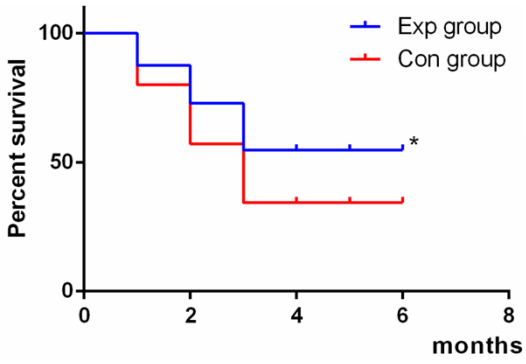

Figure 3. Comparison of survival time between the two groups. Exp group: experimental group; con group: control group. In comparison with the control group after treatment, *P<0.05.

which specific signal pathway in regulating NSCLC patients with EML4-ALK fusion gene mutation will be further discussed.

In conclusion, crizotinib is more effective than intravenous chemotherapy in the treatment of NSCLC with EML4-ALK fusion gene mutation, and can be widely used in clinical practice.

Disclosure of conflict of interest

None.

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