Prospective Study of Chemotherapy in Combination with Cytokine-induced Killer Cells in Patients Suffering from Advanced Non-small Cell Lung Cancer

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Abstract. *The present study evaluated the clinical efficacy of chemotherapy in combination with cytokine-induced killer (CIK) biotherapy compared to the chemotherapy alone. Fifty-nine advanced non-small cell lung cancer (NSCLC) patients were randomly divided into two groups, group A (chemotherapy alone, including docetaxel 75 mg/m^2, day 1; cisplatin, 25 mg/m^2, days 1-4, tri-weekly) and group B (chemotherapy plus CIK cell transfusion). Autologous CIK cells were induced from the patients' peripheral mononuclear cells in vitro and separated by cytometry and then transfused back the patients. The host cellular immune function, clinical curative effects and quality of life (QOL) were examined and were compared between the two groups. The host immune function was enhanced and QOL was improved in the patients treated by chemotherapy plus CIK biotherapy compared to the patients treated by chemotherapy alone. The overall response rate (ORR) was 43.3% and 44.8% in groups A and B, respectively. The disease control rate (DCR) was higher in group B than in group A (89.7% vs. 65.5%, p=0.030). The time to progression was 4.67 months (95% CI 3.98-6.02 months) in group A and 6.65 months (95% CI 4.70-7.30 months) in group B and the median survival time was 11.0 months (95% CI 7.88-14.1 months) in group A and 15.0 months (95% CI 11.04-18.96 months) in group B. Compared to patients in group A, the patients in group B had significantly longer progression-free survival (p=0.042) and overall survival (p=0.029). No severe side-effects occurred in the CIK cell transfusion patients. It was concluded that chemotherapy plus CIK cells has potential benefits compared to chemotherapy alone in patients suffering from advanced NSCLC and autologous CIK cell transfusion has no obvious side-effects.*

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Lung cancer is one of the major causes of cancer deaths with a 5-year survival rate of only about 15% (1). About 80% of lung malignancies are non-small cell lung cancer (NSCLC) (2), and more than 50% of these patients have advanced local invasion and/or long distance metastasis, which needs further post-operative treatment including chemotherapy, radiotherapy and immunotherapy. Meta-analysis of several randomized trials has demonstrated a modest survival advantage for treatment with cisplatin-based regimens in patients with advanced stages of NSCLC (3, 4). The second-generation chemotherapy regimens with cisplatin or carboplatin plus newer agents, such as taxanes (paclitaxel and docetaxel), gemcitabine, vinorelbine, gemzar and irinotecan have shown consistent increased overall response rates (ORRs) from 30 to 40% and 1-year survival rates from 35 to 40% (5-7), in randomized trials. Nevertheless, most patients will eventually exhibit disease progression after initial response and the overall survival (OS) of NSCLC patients remains very poor. Improvement of quality of life (QOL) has also been limited because of toxic side-effects of the chemotherapy drugs (8-10). Therefore, developing a new treatment modality is necessary to improve the OS and QOL in NSCLC patients.

It has been suggested that biotherapy is an important and effective additive therapy for cancer patients, especially for the patients at later stages (11). Immune response cells such as lymphokine-activated killer (LAK) cells, tumor-infiltrating lymphocytes (TILs), cytokine-induced killer (CIK) cells and anti-CD3 monoclonal antibody-induced killer (CD3-AK) cells may function as killers of the residual tumor cells which were resistant to the chemotherapy (12). However, the therapeutic effectiveness of adoptive immunotherapy using LAK, TIL and CD3-AK is limited because of their inherently low cellular antitumor activity *in vivo* (13). It has been demonstrated that CIK cells proliferate abundantly *in vitro* and can kill tumor cells directly (14). Moreover, CIK cells can regulate and increase host cellular immune function *in vivo* (15, 16). The high lysis activity in tumor cells is mainly due to the high proliferation potential of $CD3^+CD56^+$cells among the CIK cells (17, 18). CIK cells

are therefore suitable for immunotherapy against residual tumor cells. The combination of CIK cells and chemotherapy has been used in clinical practice and showed synergistic effects on the malignant cells and had potential benefits in the patients with recurrent carcinomas (12).

Materials and Methods

Patients. Fifty-nine patients (aged from 38-78 years old) were included in the present study. All the patients were histologically or cytologically confirmed with NSCLC at stage IIIA to stage IV. The patients had not been treated with taxanes (paclitaxel and docetaxel) and had not received radiotherapy within 3 months when they were enrolled into the present study. The patients' white blood cell counts were more than 3,000/mm^3, platelets >100,000/mm^3 and hemoglobin >10 g/dl. The patients previously treated by surgery were required to have progressive disease. The exclusion criteria were as follows: active metastases in the central nervous system; inadequate hepatic function (serum bilirubin, aspartate aminotransferase or alanine amino-transferase level >1.5 times above the normal range; alkaline phosphatase >2.5 times normal) or renal dysfunction (creatinine clearance <50 ml/min); serious concomitant systemic disorders incompatible with the study; second primary malignancy except *in situ* carcinoma of the uterine cervix or adequately treated basal cell carcinoma of the skin; pregnancy or breast feeding; active cardiac disease requiring therapy (exception: controlled cardiac failure) and any psychological condition that precluded adequate treatment and/or follow-up. Informed consent was obtained from all the patients and the local Ethics Committee approved the protocol. After enrolment, all the patients provided a complete medical history and underwent a physical examination, including documentation of ECOG (Eastern Cooperative Oncology Group) performance status. The patient characteristics are summarized in Table I.

Induction and examination of CIK cells. Blood (50-100 ml) was drawn from the patients using heparin as anticoagulant. The peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Conray density gradient centrifugation, as described previously (19). The viability of the PMBCs was assessed by trypan blue exclusion. The PBMCs (2.0×10^6/ml) were plated onto 6-well dishes (Nunc, Roskilde, Denmark) and cultured with Medium I containing RPMI-1640 in the presence of human interferon-gamma (IFN-γ, 1.0×10^6 U/L, Shanghai Fosun Pharmaceutical (group) Co., Shanghai, China), recombinant human interleukin 2 (IL-2, 5.0×10^5 U/L, Shangdong Quanguang Phgroupaceutical Co., Quanguang, China), 10% inactivated human serum, 25 mM HEPES and 2 mM L-glutamine. The cells were incubated in a humidified atmosphere with 5% CO_2 at 37˚C. After 24 hs, monoclonal antibody (MAb) against CD3 (100 μg/l; Antibody Diagnostic Inc., New York, NY, USA) and IL-1α (1.0×10^5 U/l, Promega Biological Products, Ltd., Shanghai, China) were added. After another 48 h, the supernatant was removed by aspiration and the cells were cultured in Medium II (Medium I in the absence of IFN-γ). The medium was then changed every three days. The cells were identified and sorted by flow cytometry (FACS; Beckman-Coulter, Fullerton, CA, USA) on day 1, 7, 14, 21, 28 and 35. The cytotoxic activity of the CIK cells was determined by co-incubation with the NK-sensitive K562 cell line (American Type Culture Collection, ATCC, Manassas, VA, USA) as described previously (19).

Table I. *Characteristics of patients*.

	Chemotherapy alone	Chemotherapy plus CIK
Patients (No.)	30	29
Gender (M/F)	23/7	24/5
Mean age (range) (years)	61(38-74)	60(41-78)
Karnofsky performance status		
90-100	14 (46.7%)	14 (48.3%)
70-80	16 (53.3%)	15 (51.7%)
Histology		
Adenocarcinoma	13	12
Squamous cell carcinoma	17	16
NSCLC, type not specified	0	1
Stage IIIA/ IIIB /IV (cases)	0/22/8	1/21/7
Tumor size (mean diameter, cm)	3.12	3.20
Pleural effusion (cases)	5	7
First treatment (cases)	24	25

CIK: cytokine-induced killer biotherapy.

Treatments. The patients were randomly divided between group A and group B. All the patients received chemotherapy of the TP regimen (docetaxel 75 mg/m^2, day 1; cisplatin, 25 mg/m^2, days 1-4, tri-weekly). Antianaphylaxis and antiemetic therapy was routinely prescribed. The group B patients received autologous CIK cell transfusion five times, five days after chemotherapy. The CIK cells (1.0×10^9) were transfused into the patients within 1 h every second day. Chemotherapy was continued until disease progression, development of unacceptable toxicity or completion of six cycles. The curative effects were evaluated after each treatment.

Curative effects. The distribution of T-cell subgroups, CIK cells ($CD3^+CD56^+$) and NK cells ($CD3^-D56^+$) was determined by FACS and the assessments of QOL was according to the Lung Cancer Symptom Scale (LCSS) (20, 21), which is a validated patient-rated questionnaire to evaluate the full benefits of treatment for lung cancer. The QOL evaluations were carried out at the beginning and at cycle 3. The LCSS includes six important symptoms (appetite, fatigue, cough, dyspnea, hemoptysis and pain) and three global items (symptoms from illness, daily activity and overall QOL), as determined by the patients and the health-care professionals. All the items were measured by visual–analog scales using 100 mm lines to assess the intensity of responses. Assessment for clinical response was carried out every two cycles of chemotherapy until disease progression or completion of therapy. Complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) were reported according to World Health Organization and International Union Against Cancer Criteria (22). The overall response rate (ORR) was the sum of CR and PR, whereas the disease control rate (DCR) was the sum of CR, PR and SD. The time to progression (TTP) was defined as the interval from the date of randomization to the date of documented disease progression and OS rates were measured according to the interval from the date of randomization to the date of death or last follow-up information for living patients.

Statistical analysis. T-cell subtype distributions are expressed as means±SD and were analyzed by the Student's *t*-test. Changes in LCSS were compared by the Mann–Whitney test. The ORRs and

Table II. *Determination of $CD3^+CD56^+$ CIK cells (Means±SD, n=29).*

	Day 1	Day 7	Day 14	Day 21	Day 28
$CD3^+$ (%)	46.6±10.3	74.3±4.1	89.6±6.5	85.2±5.3	70.6±5.6
$CD3^+CD56^+$ (%)	2.7±1.9	12.5±2.8	24.6±2.1	25.1±3.5	18.8±3.4

Table III. *Treatment efficacy in patients.*

	ORR	DCR	CR	PR	SD	PD
Chemotherapy alone	13 (43.3%)	19 (65.5%)	0	13	6	11
Chemotherapy+CIK	13 (44.8%)	26 (89.7%)*	0	13	13*	3*

*$p<0.05$ *vs.* chemotherapy alone. ORR, Overall response rate; DCR, disease control rate; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

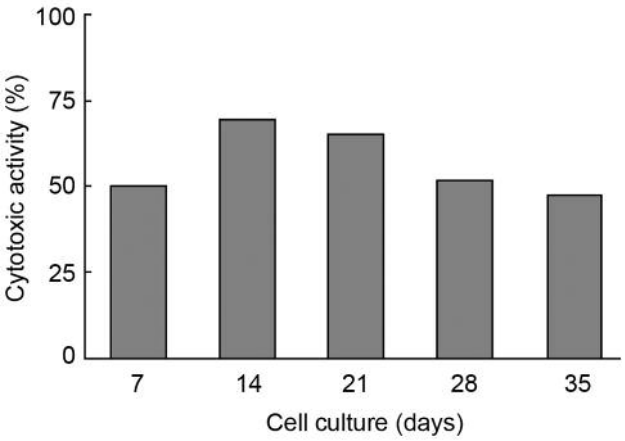

Figure 1. *Cytotoxic activity of CIK cells. The data are presented as means.*

DCRs of the two groups were compared by the Fisher's exact test. The survival curves were analyzed by the Kaplan–Meier method and log-rank test.

Results

Induction of CIK cells. The proliferation and phenotype of the PBMCs after CIK induction varied between different individuals. The cell number increased more than 80-fold on average after 14 days' incubation. The CIK ($CD3^+CD56^+$) cells increased greatly by from 100-fold to more than 1,400-fold, with an average of 600-fold, reaching the highest at day 14 and day 21, and then slightly decreasing at day 28 (Table II). The cytotoxic activity of the CIK cells was highest at day 14 (69.3% ±9.2) (Figure 1).

Comparison between chemotherapy plus CIK cell transfusion (group B) and chemotherapy alone (group A). The ORR of group A and group B was 43.3% and 44.8% respectively, and there was no statistical difference between the two groups. The DCR was significantly higher in group B (89.7%) than in group A (65.5%) (Table III). The median TTP was 4.67 months (95% CI, 3.98-6.02 months) in group A, and 6.65 months (95% CI, 4.70-7.30 months) in group B. The median survival time (MST) was 11.0 months (95% CI, 7.88-14.1 months) and 15.0 months (95% CI, 11.04-18.96 months) in groups A and B, respectively. The 1- and 2-year survival was 50.0% and 13.3% in group A and 58.6% and 20.6% in group B, respectively. Compared to chemotherapy alone, chemotherapy plus CIK biotherapy significantly prolonged the progression-free survival (PFS) and OS (Figure 2). In group B, the proportions of CIK cells, NK cells, T-cell subgroups $CD3^+$, $CD4^+$ and the ratio of $CD4^+/CD8^+$ significantly increased and the proportion of $CD8^+$ cells was decreased after therapy compared to the pre-therapy levels, whereas there were no noticeable changes in group A (Table IV). All the patients completed the LCSS QOL questionnaire at the beginning and at cycle 3. In group A, the changes in LCSS were as follows: appetite 0 (40), fatigue 10 (35), cough 0 (15), dyspnea 0 (15), hemoptysis 0 (0), chest pain 0 (0), symptoms from illness 0 (0), daily activities 0 (15), and overall QOL 0 (10). In group B, the changes in LCSS were as follows: appetite 10 (25), fatigue 10 (15), cough 15(25), dyspnea 0(12.5), hemoptysis 0 (5), chest pain 0 (0), symptoms from illness 15 (25), daily activities 15 (25) and overall QOL 10 (10) (Figures 3 and 4). In contrast to group A, the patients in group B had significantly better QOL in terms of appetite and fatigue (Figure 3). The three global items (symptoms from illness, daily activities and overall QOL) were also significantly different between the two groups (Figure 4).

Side-effects of CIK cell transfusion. There were no severe or unusual side-effects recorded during or after CIK cell transfusions, except for temporary fever and headache, which could be relieved naturally within 24 h or be treated by simple allopathy.

Discussion

In the present study it was demonstrated that the host cellular immune responses were enhanced and the QOL was improved in the patients treated by chemotherapy together with CIK biotherapy. Although the ORRs were similar in these two groups of patients, the DCR of the patients treated by additional CIK biotherapy was higher than for those

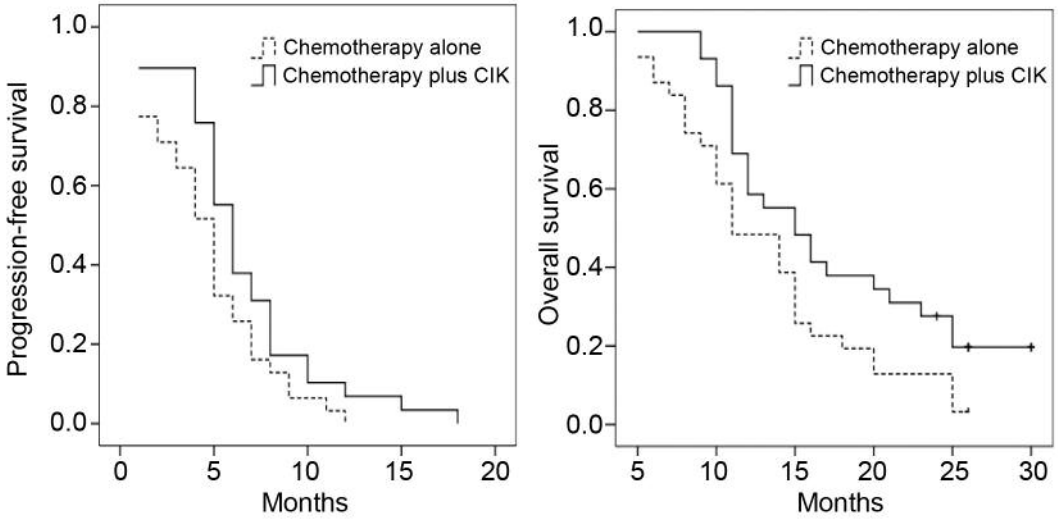

Figure 2. *The progression-free survival and overall survival curves. The median TTP was 4.67 months with chemotherapy alone and 6.65 months with chemotherapy plus CIK (p=0.042, log-rank test). The median survival time was 11.0 months with chemotherapy alone and 15.0 months with chemotherapy plus CIK (p=0.029, log-rank test).*

Table IV. *Cellular immune response after therapy (Means±SD, n=59).*

	$CD3^+$	$CD4^+$	$CD8^+$	$CD4^+/CD8^+$	NK	CIK
Chemotherapy+CIK						
Pre-therapy	49.3±10.9	32.2±10.3	33.5±11.1	1.0±0.1	15.6±3.5	0.8±0.3
Post-therapy	59.3±11.5*	39.6±9.7*	28.9±4.6*	1.4±0.2*	25.8±6.4*	4.1±2.1*
Chemotherapy alone						
Pre-therapy	49.8±11.2	32.0±8.1	33.1±9.7	0.9±0.1	16.1±4.1	0.7±0.2
Post-therapy	49.2±13.4	31.9±9.1	32.8±12.2	0.9±0.1	17.2±5.2	0.6±0.3

*$p<0.001$ *vs*. Pre-therapy.

treated by chemotherapy alone. Moreover, the PFS and OS were significantly prolonged in the group treated by chemotherapy plus CIK biotherapy. The proliferation and phenotype of the PBMCs after CIK induction differed between individual NSCLC patients. The number of CIK cells reached a peak from days 14 to 21 after being induced by cytokines, increasing by about 600-fold on average, coinciding with the highest cytotoxic activity of the CIK cells, therefore the 14-day incubated CIK cells were chosen for transfusion. There were no severe side-effects recorded during CIK cell transfusion, indicating that CIK cells together with chemotherapy are safe.

Cellular immunotherapy, which directly or indirectly regulates the biological interaction between the host and the tumor (23, 24), is an option for improving patient survival intervals and QOL. CIK cells represent a promising cellular immunotherapy. Heterogenous CIK cells were able to kill autologous and allogenous malignant target cells (18, 25). Interestingly, CIK cells have also been shown to be effective against multidrug-resistant and FasL-positive malignant cells (15, 26, 27). Theoretically, CIK cells may kill the residual tumor cells which resisted the chemotherapy and may be beneficial for the patients. As CIK cells are sensitive to cytotoxic drugs (12), the CIK biotherapies were performed 5 days after chemotherapy in the present study. The side-effects were slight and could be treated by simple allopathy. The results demonstrated that the combination of chemotherapy together with autologous CIK cells had beneficial effects compared to chemotherapy alone. Most of the patients treated by additional CIK biotherapy had a better QOL, higher DCR, longer TTP and OS. The efficacy of the treatment modality was confirmed in this prospective study. The percentage distribution of T-cell

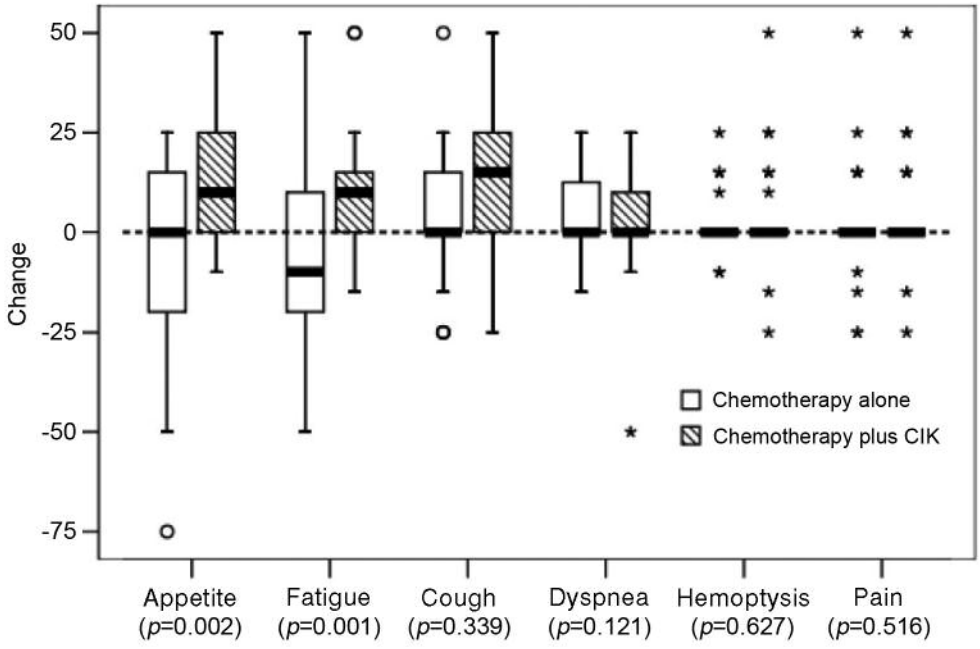

Figure 3. *The changes of six symptoms of LCSS. Data are presented with box-plots.*

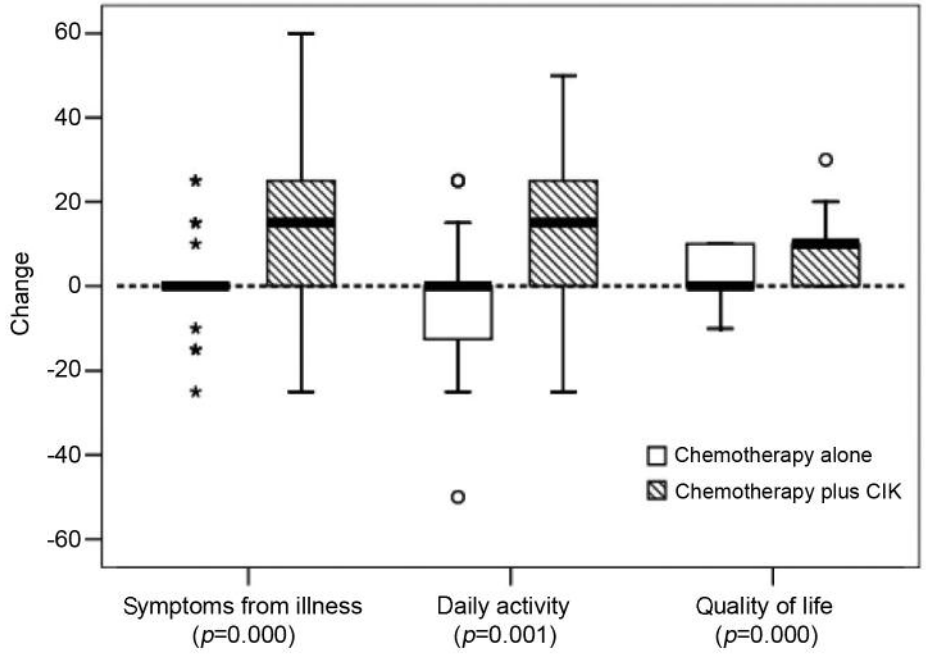

Figure 4. *The changes (%) of three global items of LCSS. Data are presented with box-plots.*

sub-groups, CIK cells and NK cells, in the blood was significantly changed in the chemotherapy plus CIK cell transfusion patients, indicating that the CIK biotherapy could improve the patients' host cellular response, which helped the patients to recover easily from chemotherapy and improved the QOL.

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