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RESEARCH ARTICLE

A prospective study of the efficacy of a combination of autologous dendritic cells, cytokine-induced killer cells, and chemotherapy in advanced non-small cell lung cancer patients

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Abstract Dendritic cells (DC) play a crucial role in the induction of an effective antitumor immune response. Cytokine-induced killer (CIK) cells, a subset of T lymphocytes, have the capacity to eliminate cancer cells. This study was to evaluate the correlation between the frequency of DC/CIK immunotherapies following regular chemotherapy, the time-to-progression (TTP), and overall survival (OS) of advanced non-small lung cancer patients. Sixty patients with III_B–IV non-small-cell lung carcinoma (NSCLC) were enrolled from August 2007 to December 2009 and were randomized into two groups. All 60 patients received four courses of navelbine–platinum (NP) chemotherapy. In one group, 30 patients were treated with adoptive autologous DC/CIK cell transfusion twice every 30 days. In the other group, the patients received immunotherapies more than twice every 30 days. The adverse effects, TTP, and OS were evaluated between the two groups. Median survival time of all 60 patients was 13.80 months. The 1-, 2-, and 3-year overall survival rates were 60.0, 21.7, and 15.0 %, respectively. The 1-, 2-, and 3-year overall survival rates of patients receiving more than two immunotherapies were 63.3, 30.0, and 23.3 %, and the rates of those receiving two immunotherapies were 56.7, 13.3, and 6.7 %, respectively. The difference between the two groups was statistically significant ($P=0.037$). Compared with patients in the fewer immunotherapies group, TTP in the group receiving more immunotherapies significantly prolonged, with the median improving from 6.2 months (95 % CI, 5.35–9.24) to 7.3 months (95 % CI, 5.45–6.95; $P=0.034$). The adverse effects of chemoimmunotherapy were tolerable. Advanced NSCLC patients can benefit from the combination of DC/CIK immunotherapies following conventional chemotherapy. More than two immunotherapies improved TTP and OS of those patients in this study.

Keywords Chemoimmunotherapy · Dendritic cell · Cytokine-induced killer cell · Non-small cell lung cancer

Introduction

Lung cancer is the most common cancer worldwide. However, it is often diagnosed at the advanced stage. Non-small-cell lung carcinoma (NSCLC) accounts for approximately 85 % of all lung cancers [1], and chemotherapy remains the current standard treatment for patients at advanced stage. However, chemotherapy has limited benefits and multiple side effects [2]. Therefore, developing a new and effective therapy is necessary to improve the survival time of advanced NSCLC patients.

Immunotherapies designed to eliminate tumor cells may be one of the promising approaches due to its specificity. Immunotherapies aim to stimulate and redirect the cellular immune response in cancer patients to target and eliminate tumor cells [3]. Increasing evidence suggests that immunotherapies can increase the survival and quality of lung cancer patients [4, 5].

DCs are the most potent antigen-presenting cells in the body and also capable of promoting the generation of helper T cells and cytotoxic T cells (CTLs). These cells are effective T cell stimulators that are able to induce a tumor-specific immune response. Vaccination of DCs with tumor-associated antigens (TAAs) can induce protective immune responses [6]. Several studies have shown that specific CTLs can be induced by human DCs pulsed with TAAs in cancer patients [7, 8]. Carcinoembryonic antigen (CEA) is a widely used serological marker for lung cancers because of its overexpression in advanced NSCLC. Therefore, we selected CEA605-613, restricted with HLA-A2, as the antigen to stimulate DCs.

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CIK cells are predominantly CD3+CD56+ type II natural killer T-cells. They are generated from peripheral lymphocytes by using a cytokine cocktail including anti-CD3 monoclonal antibody, interleukin (IL)-2, and interferon gamma (IFN-γ). CIK cells show an enhanced cytotoxic activity, higher proliferation rate, and lower toxicity compared with lymphokine-activated killer cells [9]. CIK cells that are activated by DC stimulation show increased antitumor activities. DC and CIK together can change the expression of surface molecules to each other, leading to a dramatic increase of IL-12 secretion as well as cytotoxic activity [5, 10, 11].

It is generally accepted that the combination of immunotherapy with chemotherapy can reduce tumor recurrence and the metastatic rates of malignant tumors [5, 12]. However, there is no data on the frequency of immunotherapy-combined chemotherapy influencing the clinical efficacy of advanced NSCLC. Our previous study has shown that compared with chemotherapy alone, the combination of DC/CIK immunotherapies and conventional chemotherapy can alleviate the toxicity profile and prolong time-to-progression (TTP) in advanced NSCLC patients [13]. This study aims to evaluate the impact of the frequency of DC/CIK immunotherapies on advanced NSCLC patients receiving regular chemotherapy.

Patients and methods

In this study, all patients are histologically proven NSCLC according to the WHO criteria and have elevated CEA level in the peripheral blood, ranging from 6 to 476 ng/ml (cutoff value, 5 ng/ml). Mononuclear cells obtained from the patients were phenotyped for HLA-A2 allele by flow cytometry. Only HLA-A2 positive patients were included in this study. All patients had an Eastern Cooperative Oncology Group performance status score of ≤2. All patients had adequate bone marrow (white blood cells, $>4.0\times10^9$/L; hemoglobin, >120 g/L; blood platelet, $>100\times10^9$/L), liver, and renal functions. None of the patients had any malignancies other than lung cancer and only newly diagnosed patients with no prior chemotherapeutic, radiotherapeutic, or surgical treatment were included. The exclusion criteria included metastatic disease in the central nervous system, autoimmune disease, and active acute or chronic infections. From August 2007 to December 2009, 60 patients were enrolled in this study.

The mean age of the patients was 51 years old, and 38 of 60 patients were female. Forty-eight patients (80 %) had adenocarcinomas and the other 12 patients (20 %) had squamous cell carcinomas. The Eastern Cooperative Oncology Group (ECOG) performance status was as follows: 8 patients (13.3 %) with ECOG performance status 0, 42 patients (70 %) with ECOG performance status 1, and 10 patient (16.6 %) with status 2. Thirty-one patients were stage IIIB (51.6 %) and 29 patients were stage IV (48.3 %) at the time of enrollment. The patients were randomized into two groups with 30 patients in each group. Thirty patients received immunotherapies twice and 30 patients received more than twice. The characteristics of the population are outlined in Table 1.

The patients were monitored for the progression of their tumor by computed tomography every 4 weeks. Toxicity was graded according to the National Cancer Institute common toxicity criteria v.2.0 grading system. This study was approved by the Ethical Committee of Shanghai Chest Hospital, and written informed consents were obtained from all the patients. The primary end point of the study was the assessment of toxicity and the secondary end point was TTP and overall survival (OS).

Treatments

All 60 patients received four cycles of navelbine with platinum chemotherapy every month. During each cycle, navelbine (25 mg/m^2) was given on days 1 and 8, and cisplatin (75 mg/m^2) was given on day 1. Peripheral blood was collected for the preparation of DC/CIK before every chemotherapy cycle. DC/CIK immunotherapy was administered via intravenous infusion after chemotherapy. Patients received DC/CIK transfusion at an interval of 1 month. In one group, DC/CIK immunotherapy was administered twice and in the other

Table 1 Patient characteristics

Characteristic	Chemoimmunotherapy
Number	60
Mean age (range)	51 (43–72)
Gender	
Male	22
Female	38
ECOG performance status	
0	8
1	42
2	10
Stage status	
III B (T4 alone)	9
IIIB (N3 alone)	12
IIIB (T4 N3 both)	10
IV	29
Tumor histology	
Adenocarcinoma	48
Squamous cell	12
Frequency of immunotherapy	
Two	30
Three	14
Four	11
Above four	5

ECOG Eastern Cooperative Oncology Group

Table 2 Average number of immune cells and adverse effects of patients who received immunotherapies twice

Patient	Average number of DC administrated (10^6)	Average number of CIK administrated (10^8)	Serum CEA	Toxicity of skin	Noninfective fever
1	4.5	8.6	↓	0°	0°
2	8.9	11.1	→	I°	II°
3	8.6	10.8	→	0°	I°
4	9.5	9.8	→	I°	0°
5	6.1	12.5	→	I°	0°
6	4.7	9.0	↑	0°	0°
7	10.6	15.3	↓	I°	I°
8	4.9	10.1	→	0°	0°
9	11.2	16.2	↓	I°	II°
10	6.5	10.2	→	0°	0°
11	6.9	9.8	→	0°	0°
12	9.4	11.7	↑	0°	I°
13	5.2	10.6	→	0°	II°
14	10.7	17.6	↑	II°	0°
15	8.9	12.5	↓	0°	0°
16	12.1	16.9	↓	III°	0°
17	4.8	9.1	→	0°	0°
18	4.8	9.7	→	0°	0°
19	7.9	10.9	↑	0°	II°
20	12.5	12.1	↑	II°	I°
21	5.1	10.8	→	0°	0°
22	5.6	10.9	↑	0°	0°
23	4.3	9.6	→	I°	0°
24	7.8	13.5	↓	I°	0°
25	6.5	14.1	↑	0°	0°
26	11.0	12.1	→	0°	0°
27	9.8	14.6	↓	II°	0°
28	10.2	14.6	↑	II°	0°
29	7.0	10.1	↑	0°	0°
30	4.9	8.6	↓	0°	I°

The toxicity of immunotherapy was graded by the National Cancer Institute (1999) classification criteria version 2.0

DC dendritic cell, *CIK* cytokine-induced killer, *CEA* carcinoembryonic antigen

Table 3 Average number of immune cells and adverse effects of patients who received more immunotherapies

Patient	Average number of DC administrated (10^6)	Average number of CIK administrated (10^8)	Serum CEA	Toxicity of skin	Non-infective fever
1	9.9	12.3	→	I°	I°
2	7.6	13.6	↓	0°	I°
3	11.3	14.1	↑	I°	III°
4	8.4	7.9	→	II°	II°
5	7.1	16.2	→	0°	0°
6	7.5	12.4	→	0°	II°
7	8.1	15.2	↑	0°	0°
8	5.5	16.7	↑	0°	IV°
9	11.2	13.9	↑	I°	I°
10	10.8	14.9	↓	0°	II°
11	6.7	9.4	→	I°	0°
12	7.8	12.9	→	0°	III°
13	10.5	11.5	↓	0°	0°
14	10.8	11.1	↓	I°	0°
15	9.6	12.7	→	0°	0°
16	9.4	12.1	→	0°	0°
17	9.0	12.3	↑	I°	0°
18	11.1	13.5	↑	I°	0°
19	8.7	9.9	↓	0°	0°
20	10.9	10.9	→	0°	II°
21	11.2	11.6	↑	II°	II°
22	10.9	12.1	↓	III°	0°
23	7.8	9.1	↓	0°	0°
24	9.0	9.9	→	II°	0°
25	12.0	12.9	↑	I°	0°
26	6.4	8.1	↓	0°	I°
27	6.9	8.8	↓	I°	0°
28	8.8	8.9	→	0°	0°
29	9.1	11.2	↓	0°	0°
30	11.9	12.1	↑	I°	II°

The toxicity of immunotherapy was graded by the National Cancer Institute (1999) classification criteria version 2.0

DC dendritic cell, *CIK* cytokine-induced killer, *CEA* carcinoembryonic antigen

group, patients received more than two immunotherapies until they refused to continue. Clinical examinations of these patients were performed by oncologists weekly or biweekly. The evaluation included complete blood count and liver and kidney function examinations. Clinical efficacy was determined based on the National Cancer Institute's Response Evaluation Criteria in Solid Tumors (RECIST) [14]. The overall response rate (ORR) and disease control rate (DCR) were evaluated. Complete response (CR), partial response (PR), stable disease (SD), and progressive disease were reported based on RECIST. The ORR was the sum of CR and PR, whereas the DCR was the sum of CR, PR, and SD. Responding and stable patients were followed up once every 2 months until disease progression or as clinically indicated.

Preparation of DCs

Peripheral blood mononuclear cells (PBMCs) collected from 100-ml venous blood of patients were enriched by density

Table 4 Clinical outcomes of patients in two groups

Group	ORR (CR+PR)	DCR (CR+PR+SD)	CR	PR	SD	PD
Fewer immunotherapies	5 (16.6 %)	21 (70 %)	0	5	16	9
More immunotherapies	6 (20.0 %)	21 (70 %)	0	6	15	9

ORR overall response rate, *DCR* disease control rate, *CR* complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease

gradient centrifugation with Ficoll-Paque, resuspended in X-VIVO with 1 % autologous heat-inactivated serum, plated at a concentration of 5×10^6 cells/ml, and allowed to adhere to 10 cm^2 dishes. After 4 h, non-adherent cells were removed at 37 °C in a humidified incubator for the preparation of CIK cells, and the adherent cells were cultured in X-VIVO supplemented with 1 % heat-inactivated autologous serum in the presence of 1,000 U/mL granulocyte macrophage colony-stimulating factor (Leukomax; Novartis International AG, Basel, Switzerland) as well as 500 units/ml IL-4 (Strathmann Biotec AG, Hannover, Germany) at 37 °C for 7 days.

Peptide loading

The CEA peptide (CEA605-613) was from Biotech Company (Shanghai, China) with over 95 % high-performance liquid chromatography-grade purity. The preparations were tested to be free of endotoxin. DCs were incubated with the CEA peptide at a concentration of 10 μg/ml for 12 h on day 8. Harvested DCs were resuspended at a concentration of 1×10^6/ml in 0.9 % normal saline for intravenous infusion.

Generation of CIK

Non-adherent PBMCs were incubated at 37 °C in a humidified atmosphere of 5 % CO_2. On day 0, cells were treated with X-VIVO medium containing 1,000 units/ml of IFN-γ (Boehringer, Mannheim, Germany). After 24-h treatment, 50 ng/ml antibody against CD3 (Orthoclone OKT 3; Cilag GmbH, Sulzbach, Germany) was added to cell cultures. Cells were subcultured every 3 days in fresh complete medium containing 1,000 U/ml IL-2 (Four Rings Biopharmaceutical Company, Beijing, China) at 3×10^6 cells/ml. CIK cells suspended in 0.9 % normal saline were infused intravenously on day 14.

Statistical analysis

Data were analyzed by using the SPSS16.0 software package (Chicago, IL, USA). Statistical significance was assessed by using the log-rank test. OS was the time between the first day of treatment and the date of death or the last day on which patient was known to be alive. TTP was calculated from the

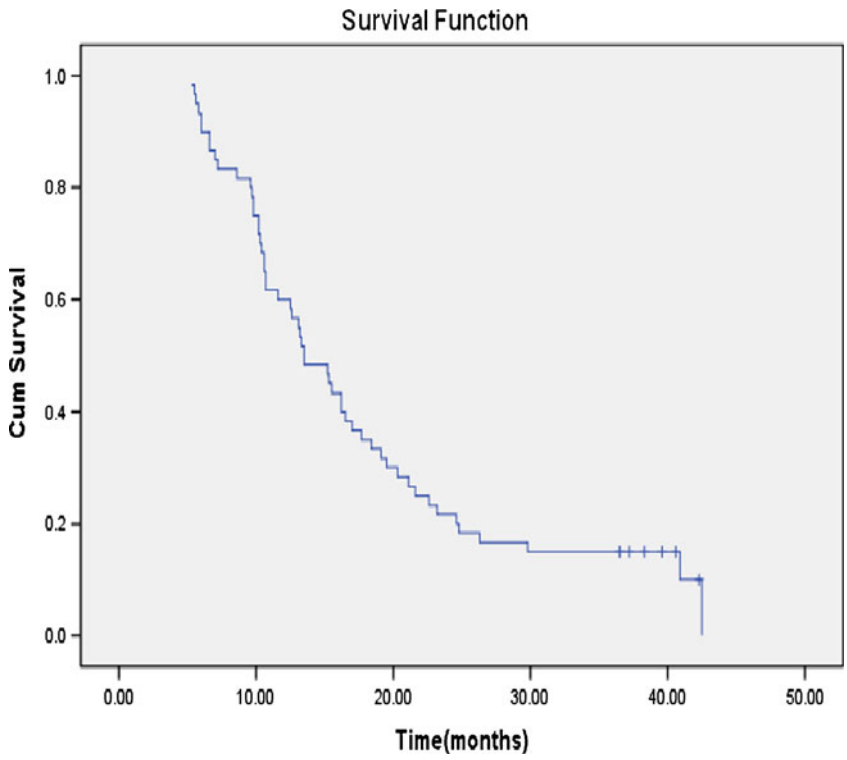

Fig. 1 The overall survival rates of all 60 patients. The 1-, 2-, and 3-year overall survival rates of all patients were 60.0, 21.7, and 15.0 %, respectively

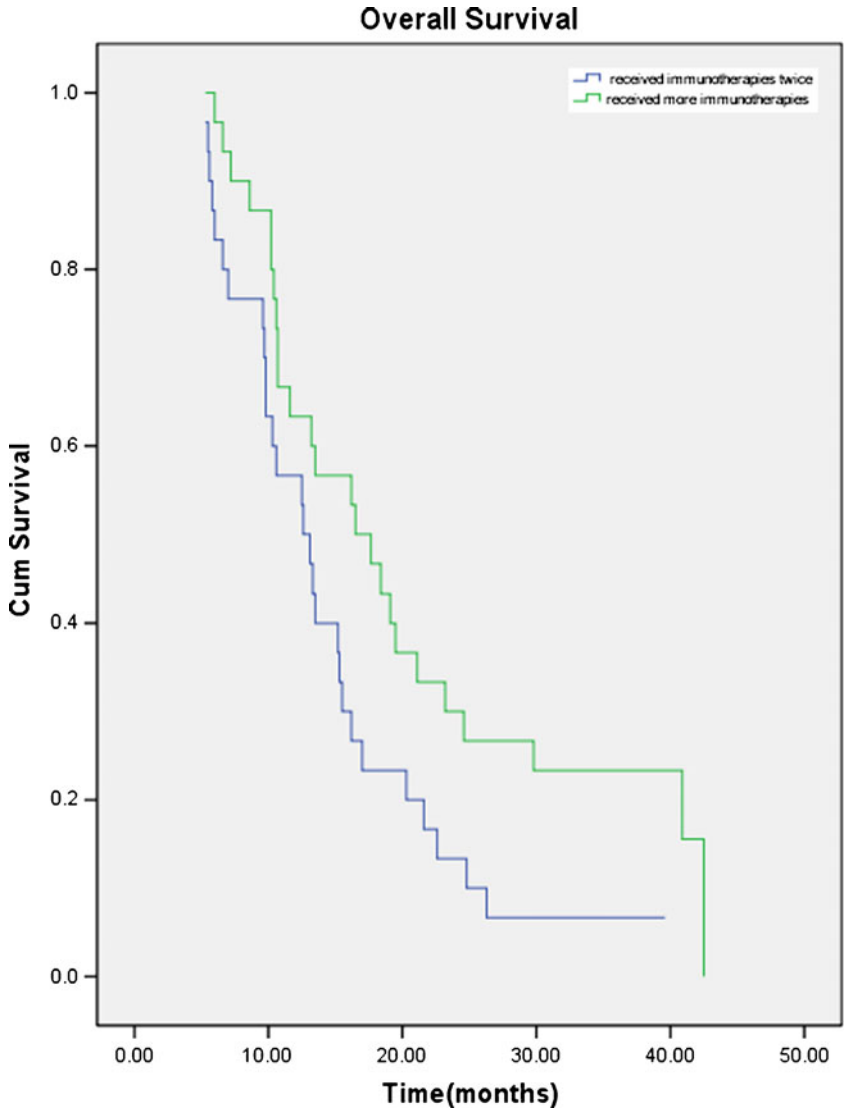

Fig. 2 The overall survival rates of two groups. The 1-, 2-, and 3-year overall survival rates of patients who received more immunotherapies were 63.3, 30.0, 23.3 % and the rates of who received twice were 56.7, 13.3, and 6.7 %, respectively. A statistically significant difference between the two groups was identified ($P=0.037$)

date of initiation of treatment to the date of disease progression or death. Correlations between the methods of treatment and TTP, OS were determined by Kaplan–Meier survival analysis. P values less than 0.05 (two tailed) were considered significant.

Results

Characteristics of chemoimmunotherapy

There were 30 patients that received DC/CIK immunotherapies twice followed chemotherapy, and 30 patients received more than twice. The numbers of patients receiving three, four, and above four times of DC/CIK transfusion were 14, 11, and 5, respectively (Table 1). The average number of DC infusions was 8.5±4.5 (10^6), and the average number of CIK infusions was 13.5±5.0 (10^8; Tables 2 and 3).

Serum CEA level was considered as evaluated when the value increased 25 % than the baseline. The 25 % decrease of the CEA level than the baseline was defined as decreasement. Otherwise, it was defined as stable. In fewer immunotherapies group, CEA level decreased in 8 patients (26.6 %), while it remained stable in 12 patients (40.0 %). In patients receiving more immunotherapies, we found CEA level decreased in 10 patients (33.3 %), while it remained stable in 11 patients (36.6 %).

Adverse effects

Severe chemotherapy-induced hematological toxicities occurred frequently in the fewer immunotherapies group, including grades 3–4 leucopenia (80.0 vs. 76.6 %; $P=0.662$), and grades 3–4 thrombocytopenia (13.3 vs. 10.0 %; $P=0.326$). With regard to nonhemotologic toxicities, grades 2–4 nausea events were 26.6 vs. 16.6 %; ($P=0.083$). Grades 1–3 rash,

acne, and pruritus were considered immunotherapy related for only appearing after DC/CIK administering (36.6 vs. 46.6 %; $P=0.083$). Noninfective fever appeared at intervals after DC/CIK infusion (30.0 vs. 43.3 %; $P=0.043$; Tables 2 and 3).

TTP and OS

The clinical responses were assessed in fewer and more immunotherapies groups, respectively. The ORRs of fewer and more immunotherapies groups were 16.6 and 20.0 %, respectively ($P=0.326$). The DCR of two groups was both 70.0 % (Table 4).

All of the 60 included patients were assessed for TTP and OS. At the time of the analysis, there were 53 deaths, 7 patients were confirmed alive. Median survival time of all patients was 13.80 months. The 1-, 2-, and 3-year overall survival rate was 60.0, 21.7, and 15.0 %, respectively (Fig. 1).

The 1-, 2-, and 3-year overall survival rates of patients who received more than two immunotherapies were 63.3, 30.0, and 23.3 %; the survival rates of patients who received immunotherapies twice were 56.7,13.3, and 6.7 %, respectively. The difference was statistically significant ($P=0.037$; Fig. 2).

Compared with patients in fewer immunotherapies group, TTP in more immunotherapies group significantly prolonged, with the median improved from 6.2 months (95 % CI, 5.35–9.24) to 7.3 months (95 % CI, 5.45–6.95; $P=0.034$; Fig. 3).

Discussion

As the most effective antigen-presenting cells, DCs have been under extensive study as components of antitumor vaccines [15]. DCs can capture and present antigens including tumor-associated antigens, like CEA, activating naive T cells, regulating T cell response, expressing lymphocyte costimulatory molecules, and secrete cytokines to initiate cellular and humoral immune responses. DCs have the ability to promote the generation of helper T cells and CTLs. Therefore, they are essential in the initiation and maintenance of immunological responses [16]. The loading of tumor-associated antigens or tumor-derived peptides into DCs is crucial for their activation.

CIK cells, a subset of natural killer T lymphocytes, have the capacity of eliminating tumor cells. It is generally accepted that CIKs specifically lyze tumor cells as well as secrete IFN-γ and TNF-α [17]. With the help of DCs, the effective

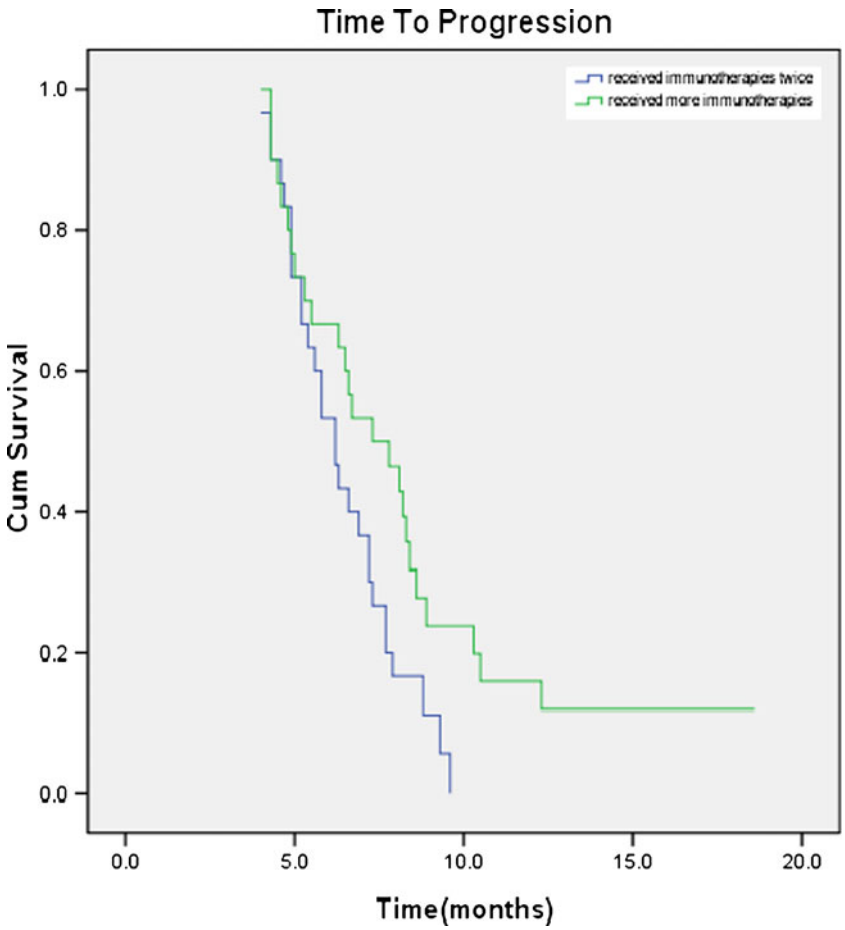

Fig. 3 Time to progression of two groups. Time to progression in more immunotherapies group showed statistically improvement compared with fewer immunotherapies group ($P=0.034$). The median time to progression is 7.3 (95 % CI, 5.45–6.95) and 6.2 (95 % CI, 5.35–9.24), respectively

T cell stimulator, CIK cells can substantially enhance the effect of tumor vaccines.

The capacity of DCs to establish a strong cellular immunity would have been greatly enhanced if combined with adoptively transferred CIK cells [18]. DC/CIK immunization generates effector $CD8^+$ T cells with high quality and avidity for tumor rejection. It also generates long-lived $CD8^+$ T cells for the prevention of relapse. The combination of DC and CIK may have potential therapeutic benefits in the long-term control of tumor progression.

Chemotherapy induces potent systemic antitumor effects by reducing the levels of immunosuppressive cytokines produced by cancer cells [19]. It also can induce apoptosis in tumor cells as well as the release of tumor antigens, which may be presented to T cells by DCs and activate the antitumor immunity [20]. Therefore, DC/CIK immunization following chemotherapy may have a profound influence on the subsequent immune response associated with tumor cell death.

Since patients can benefit from DC/CIK immunotherapy combined with chemotherapy, the optimum number of DC/CIK transfusion should be studied. The major objective of the study was to find out the optimum number of DC/CIK immunotherapies after conventional chemotherapy and how it affects the TTP and OS. We launched a clinical trial to evaluate chemoimmunotherapy containing CEA-pulsed DC and CIK following conventional chemotherapy. We found that there is a positive correlation between them. More immunotherapies following chemotherapy can prolong TTP significantly from 6.2 to 7.3 months and improved OS in patients with advanced NSCLC. Our findings indicate that DC/CIK immunization could synergize with chemotherapy to enhance systemic antitumor activity. The combination might establish better tumor control in the long term and it appeared that skin toxicity and non-infective fever were observed in the course of chemoimmunotherapy. However, they were generally mild, transient, and manageable with supportive care.

It should be noted that in this study, we recruited a limited number of advanced NSCLC patients. Further studies are required to rigorously test this treatment in the setting of a multicentered, randomized clinical trial.

In conclusion, our study provided direct clinical evidence supporting the notion that patients of advanced NSCLC can benefit from the repeated immunotherapies consisting of DC/CIK combination following conventional chemotherapy. Our findings may have implications for the development of further DC-based antitumor immunotherapeutic strategy.

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Conflicts of interest None

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