Enhanced antitumor effects and improved immune status of dendritic cell and cytokine-induced killer cell infusion in advanced cancer patients

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Abstract. Little progress has been made in the treatment of advanced cancer. Dendritic cells (DCs) plus cytokine-induced killer (CIK) cells have exhibited antitumor effects. Thus, the aim of the present study was to evaluate the clinical efficacy of DC-CIK cell treatment in patients with advanced cancer. A paired study including 57 patients treated with DC-CIK cells (DC-CIK group) and 33 patients treated with best supportive care alone (BSC group) was performed. The patients in the DC-CIK group were matched to those in the control group in terms of sex, age, tumor type and clinical stage. T-cell subsets were detected and overall survival (OS) was compared between the two groups. The results demonstrated that $CD4^+/CD25^+$ and $CD8^+/CD28^-$ subsets significantly decreased following DC-CIK immunotherapy ($P<0.05$). The $CD3^+$, $CD3^+/CD8^+$, $CD8^+/CD28^+$ and $CD3^+/CD56^+$ T-cell subsets were significantly increased in the DC-CIK group compared with the BSC group, while the $CD8^+/CD28^-$ subset was significantly decreased. Univariate analysis demonstrated that a lower $CD8^+/CD28^-$ and a higher $CD8^+/CD28^+$ ratio were associated with prolonged OS in advanced cancer patients. In addition, DC-CIK treatment administration, age (>60 vs. <60 years), clinical stage and the frequency of CIK treatment significantly affected the OS of patients in the DC-CIK group. A $CD8^+/CD28^-$ ratio of <21.12 was found to decrease the hazard ratio (HR) of OS to 0.50 [95% confidence interval (CI): 0.29-0.87] and a $CD8^+/CD28^+$ ratio >9.04 was found to decrease the HR of OS to 0.45 (95% CI: 0.21-0.98). No serious side effects were observed in the DC-CIK group. Taken together, these data indicate that DC-CIK infusions were able to change the ratios of the T-cell subsets, which increased the T helper cell and cytotoxic T lymphocyte subsets, while it decreased regulatory T lymphocyte subsets. Thus, this method of immunotherapy was found to improve the imbalance in the immune system and prolong the OS in patients with advanced cancer.

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Introduction

Cancer is among the leading causes of morbidity and mortality worldwide, with an estimated 14 million new cases and 8.2 million cancer-related deaths annually. Over 60% of the cases globally occur in developing countries, which account for ~70% of cancer-related mortality worldwide (1). Given their poor overall condition, patients with late-stage cancer are mostly not eligible for conventional anticancer treatments such as surgery, chemotherapy, or radiotherapy, and best supportive care (BSC) is currently considered as the only option for patients with a relatively poor prognosis. Several efforts have been made to improve the survival of patients with advanced cancer (2). However, the results thus far have been unsatisfactory. Therefore, further efforts must be made to improve the current therapeutic modalities and to explore novel therapies for advanced cancer, in order to improve patient care and prolong survival.

Immunotherapy has been shown to be effective in the treatment of a number of malignant tumors, with adoptive cellular immunotherapy being considered a promising and effective modality (3-8). Several types of immune cells, such as lymphokine-activated killer cells (9), tumor-infiltrating lymphocytes (10) and anti-CD3 monoclonal antibody-induced killer cells (11) have shown efficacy in advanced cancers. Among these cells, cytokine-induced killer (CIK) cells have several advantages compared with traditional immune cells, such as proliferating rapidly *in vitro*, exhibiting intensified antitumor activity and a broader spectrum of targeted tumors, and being associated with less severe side effects, which qualifies them as one of the most promising treatments, particularly for patients with advanced cancer (6,12-17). It is noteworthy that the antitumor activity of CIK cells may be activated

and enhanced by dendritic cells (DCs), which are the main antigen-presenting cells (18,19). DCs present tumor antigens through MHCII molecules, preventing the immune escape of tumor cells. CIK cells are also able to recognize DCs in a T-cell receptor-independent manner, and the DC-CIK interaction stimulates the proliferation and antitumor activity of CIK cells through secreting interleukin (IL)-12, interferon (IFN)-γ, and other cytokines (20). DCs plus CIK cells not only enhance the antitumor effect, but also regulate and improve the immune function in cancer patients (20-22). Lately, a report from the International Registry on CIK cells found that adjuvant immunotherapy with CIK cells may prevent recurrence and improve the quality of life and progression-free survival rates in cancer patients (6). Our previous study also indicated that DC-CIK treatment may be able to recover cellular immunity and improve the Eastern Cooperative Oncology Group (ECOG) performance status and quality of life in patients with advanced cancer (23,24).

Current data from clinical studies on the antitumor effects and prognostic benefits of DC-CIK cells are limited, particularly for patients with advanced cancer. The aim of the present study was to evaluate the clinical efficacy of DC-CIK cell treatment in patients with advanced cancer.

Patients and methods

Patients. A paired study was performed to compare the clinical outcomes of advanced cancer patients received either autologous DC-CIK immunotherapy or BSC alone. Between June 2012 and January 2014, a total of 90 patients with advanced cancer were recruited in the present study from the Beijing Shijitan Hospital Cancer Center (Capital Medical University, Beijing, China). A total of 57 patients underwent DC-CIK immunotherapy (DC-CIK group) and 33 patients were administered BSC alone (BSC group). All the patients had a definitive histological or cytological diagnosis and were unresponsive or intolerant to conventional anticancer treatments, such as surgery, chemotherapy or radiotherapy. The characteristics of the patients are summarized in Table I. The criteria for patient selection were as follows: i) Patient aged 18-85 years; ii) chemoradiotherapy-free for ≥3 months; iii) expected survival duration of >3 months; iv) ECOG performance status of 0-2; v) white blood cell (WBC) count >3,500/μl; vi) hemoglobin level >80 g/dl; vii) platelet count >100,000/μl; viii) serum aspartate aminotransferase (AST)/alanine aminotransferase (ALT) <2.0 the upper limit of normal; ix) no cardiac arrhythmias, congestive heart failure or severe coronary artery disease; x) no active autoimmune disease or T-cell lymphoma; and xi) no pregnancy or lactation. The subjects in the two groups were matched for sex, age, stage, pathology, tumor size and metastasis at the first visit.

Informed consent was obtained from all the patients. After enrollment, a complete medical history was taken and physical examination was conducted by professional oncologists for each patient. This study was approved by the Ethics Committee of the Beijing Shijitan Hospital.

Treatment. Patients in the control group received BSC alone, which included active symptom control, pain management, and the multiprofessional attention to the individual's overall physical, psychosocial, spiritual and cultural needs. Patients in the DC-CIK group received autologous DC-CIK cells with an interval of 1 month in addition to BSC. For each cycle of treatment, the patients received three intravenous infusions of DC-CIK cells with 1-day intervals. Patients without disease progression were eligible for maintenance treatment. For each cycle of treatment, the patients received a median of $6.47x10^8$ (range, $5.35x10^7$-$2.98x10^9$) of autologous DC cells and $7.35x10^9$ (range, 3.00-$17.25x10^9$) of CIK cells. The median number of CIK cell immunotherapy cycles was 2 (range, 1-13 cycles). All the patients were seen biweekly or monthly by oncology specialists, and clinical examinations were performed monthly, including physical examination, T-cell subsets, routine blood count, serum AST and ALT, blood urea nitrogen and creatinine, and electrocardiogram. Additional care was provided if needed.

Clinical assessment of response and toxicity. All the patients were followed up at the outpatient clinic or the oncology ward from the date of initial treatment to March 31, 2016, or to the date of death. Clinical response was determined according to the National Cancer Institute's Response Evaluation Criteria in Solid Tumors, version 1.1 (https://ctep.cancer.gov/protocoldevelopment/docs/recist_guideline.pdf). Patients were assessed by oncologists after each cycle of treatment, including color Doppler ultrasound, computed tomography scan, magnetic resonance imaging and technetium bone scan. Overall survival (OS) was calculated from the time of treatment initiation until death, and patients who remained alive were censored at the time of the last follow-up. Adverse events were evaluated according to the World Health Organization (WHO) criteria (25).

Preparation of DC and CIK cells. CIK cells were prepared as previously described (23). Briefly, peripheral blood mononuclear cells (PBMCs) were mobilized by granulocyte colony-stimulating factor (G-CSF) until WBC ≥10,000/μl, lymphocytes + monocytes ≥15%. Apheresis was performed from the patients using the COBE Spectra cell separator (COBE BCT, Lakewood, CO, USA) and repeated until $\geq 4.5x10^6$/kg $CD34^+$ cells were collected. PBMCs were separated by the Ficoll-Hypaque centrifugation method and incubated for 2 h, and the adherent cells were cultured *in vitro* to generate autologous DCs in the presence of IL-4, tumor necrosis factor-α and granulocyte-macrophage (GM)-CSF (Boehringer, Mannheim, Germany). For the culture of autologous CIKs, PBMCs were cultured in AIM-V medium containing the recombinant cytokines IL-2, IFN-γ and monoclonal anti-human CD3 antibody (50 ng/ml; IM1650, Boehringer). The cells were incubated in a humidified atmosphere with 5% CO_2 at 37˚C. IL-2 and IFN-γ were added to the medium every 5 days.

Cell growth was observed under the microscope, and DC phenotypes were determined by flow cytometry of CD80, CD86, HLA-DR, CD1a and CD11c (Beckman-Coulter, Shanghai, China). The DC suspension contained >80% of $CD80^+/CD86^+$ cells prior to infusion. The CIKs expressed CD3 and CD56 (Beckman-Coulter). After culture *in vitro* for 7-10 days, DCs and CIKs were harvested and administered intravenously 3 times with 1-day intervals.

Detecting the phenotype of DCs, CIK cells and T-cell subsets. The phenotype of DCs and CIK cells was determined prior

Table I. Patient characteristics.

Characteristics	DC-CIK group	BSC group	P-value
No. of patients	57	33	
Sex (n)	0.230		
Male	27	20	
Female	30	13	
Age (years)	0.874		
Mean ± standard deviation	60.00±16.30	60.52±11.64	
<60 (n)	31	16	
≥60 (n)	26	17	
Tumor types (n)	0.450		
Digestive system cancer	19	11	
Lung cancer and mesothelioma	12	11	
Breast cancer	8	3	
Head and neck cancer	4	2	
Male genitourinary system cancer	4	1	
Female genitourinary system cancer	3	0	
Lymphoma	3	5	
Sarcoma	2	0	
Glioblastoma	1	0	
Melanoma	1	0	
Stage (n)			0.105
IV	40	19	
III	15	11	
II	2	3	
DC-CIK treatment cycles (n)			
1	33	-	
2	11	-	
3	7	-	
≥4	6	-	

DC-CIK, dendritic cell/cytokine-induced killer cell immunotherapy; BSC, best supportive care.

to infusion. T-cell subsets were assessed at the beginning of the first treatment and redetected monthly. Briefly, 2 ml of heparinized peripheral blood was drawn from each patient and PBMCs were separated by the Ficoll-Hypaque centrifugation method. A total of 100 μl PBMCs were incubated in the dark with primary antibody at 4˚C for 15 min. After hemolysis for 10 min, the samples were centrifuged for 10 min at 450 x g at room temperature, and then washed twice in phosphate-buffered saline and subjected to flow cytometric analysis (Becton-Dickinson, Franklin Lakes, NJ). The primary antibodies included: CD4-FITC (A07550), CD8-PE (IM1650), CD3-PerCP (A07749), CD25-PE (A07774), CD28-FITC (IM1236U), CD3-FITC (A07746) and CD56-PE (IM2073U) (Beckman-Coulter). All the antibodies were mouse anti-human monoclonal antibodies, with 1:10 dilution.

The cell phenotypes were analyzed by flow cytometry (FC500, Beckman-Coulter) and CXP analysis software (Beckman-Coulter) was used. Lymphocyte subset levels were reported as percentages of the total population.

Statistical analysis. Data were analyzed using the SPSS 16.0 software package (SPSS Inc., Chicago, IL, USA). T-cell subsets were expressed as mean ± standard deviation. The independent samples t-test and paired t-test were used to compare the changes in T-cell populations between the two groups. The OS rate and survival curves were calculated by the Kaplan-Meier method. Associations between OS and potential prognostic factors were estimated using the log-rank test in univariate analyses. The significant variables were further analyzed by the Cox hazard proportional regression model with adjustments for age, sex and tumor type. All tests were two-sided and the significance level was set at 0.05.

Table II. T-cell subsets in the peripheral blood before and after DC-CIK cell treatment.

T-cell subsets	Before treatment (%)	After treatment (%)	P-value
CD^{3+}	68.67±11.23	82.55±12.59	0.000
$CD3^+/CD4^+$	34.23±11.97	40.22±17.09	0.005
$CD3^+/CD8^+$	32.27±12.21	42.42±17.83	0.000
$CD4^+/CD25^+$	4.48±3.05	3.23±2.65	0.024
$CD8^+/CD28^-$	23.39±10.09	17.97±9.18	0.000
$CD8^+/CD28^+$	14.11±8.46	27.00±15.10	0.000
$CD3^+/CD56^+$	10.88±7.76	15.56±16.24	0.047

Values are presented as mean ± standard deviation. DC-CIK, dendritic cell-cytokine-induced killer cell immunotherapy.

Results

Patient characteristics. Of the 57 patients in the DC-CIK group, 27 were male and 30 were female; the mean patient age was 60 years (range, 6-81 years). The patient characteristics are summarized in Table I. For each cycle of treatment, the patients received three intravenous infusions of DC-CIK cells with 1-day intervals. The median number of CIK cell immunotherapy cycles was 2 (range, 1-13 cycles).

Of the 33 patients in the BSC group, 20 were male and 13 were female; the mean patient age was 61 years (range, 42-85 years). The patients were administered BSC at the Department of Oncology of our hospital between June 2012 and January 2014.

The patient characteristics, such as sex, age, tumor type and clinical stage, were comparable between the two groups (Table I).

Comparison of T-cell subsets before and after DC-CIK infusion. To investigate the immunomodulatory effects of

DC-CIK cell treatment, the T-cell subsets in the 57 patients in the DC-CIK group were analyzed prior to and 1 month after DC-CIK cell infusion. The $CD3^+$, $CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD8^+/CD28^+$ and $CD3^+/CD56^+$ T-cell subsets were significantly increased following DC-CIK treatment ($P<0.05$). Conversely, the $CD4^+/CD25^+$, $CD8^+/CD28^-$ subsets were significantly decreased following DC-CIK immunotherapy ($P<0.05$) (Table II).

Comparison of the changes in T-cell subsets between the DC-CIK and BSC groups. Next, the changes in the T-cell subsets in peripheral blood between the DC-CIK and BSC groups we observed. The T-cell subsets were analyzed prior to and 1 month after the first treatment in patients from the two groups. Although no significant difference in the T-cell subsets were observed between the two groups at the beginning of the first treatment (data not shown), the $CD3^+$, $CD3^+/CD8^+$, $CD8^+/CD28^+$ and $CD3^+/CD56^+$ T-cell subsets were significantly increased in the DC-CIK group compared with the BSC group, while the $CD8^+/CD28^-$ subset decreased significantly. No significant differences in the $CD3^+/CD4^+$ and $CD4^+/CD25^+$ subsets were observed between the two groups before and after treatment (Table III). Thus, these data indicated that DC-CIK cell treatment improved cellular immune function in advanced cancer patients.

Association between cycles of DC-CIK infusion and T-cell subset changes. The effect of the frequency of DC-CIK infusion on the changes in T-cell subsets was further evaluated, and it was observed that the T-cell subsets changed after 1 cycle of DC-CIK immunotherapy. $CD3^+$, $CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD8^+/CD28^+$ and $CD3^+/CD56^+$ subsets were significantly increased, while $CD4^+/CD25^+$ and $CD8^+/CD28^-$ were significantly decreased. However, no obvious changes in T-cell subsets were observed before or after >2 cycles of infusion (Table IV).

Association between OS and T-cell subset changes. To investigate the factors that affect the OS of patients with advanced cancer, a univariate analysis was conducted, demonstrating that a lower $CD8^+/CD28^-$ and a higher of $CD8^+/CD28^+$ ratio may be associated with longer OS. In addition, DC-CIK treatment administration, age (>60 vs. <60 years), clinical stage and the frequency of CIK treatment significantly affected the OS of patients in the DC-CIK group (Table V, Figs. 1 and 2).

The parameters that affected the prognosis of advanced cancer patients in this study were further analyzed through the Cox hazard proportional regression model with adjustments for age, sex and tumor type. A multivariate analysis demonstrated that a $CD8^+/CD28^-$ ratio <21.12 decreased the hazard ratio (HR) of OS to 0.50 [95% confidence interval (CI): 0.29-0.87] and a $CD8^+/CD28^+$ ratio >9.04 decreased the HR of OS to 0.45 (95% CI: 0.21-0.98) (Table VI, Figs. 3 and 4). Thus, taken together, these results demonstrated that T-cell populations may be associated with the OS of patients with advanced cancer, and DC-CIK immunotherapy may improve OS.

Side effects. No severe side effects were observed during DC-CIK cell treatment. Three patients in the DC-CIK group developed fever (<38.5˚C) that was spontaneously relieved 2 h after the infusion. No other serious adverse events, such as high fever, chills, rash or hemolytic anemia, were reported in patients receiving DC-CIK cell treatment.

Table III. Comparison of the changes in T-cell subsets between the BSC and DC-CIK groups.

T cell subsets	BSC group (%)[a]	DC-CIK group (%)[a]	P-value
$CD3^+$	2.09±10.89	13.88±13.72	0.000
$CD3^+/CD4^+$	0.85±10.54	5.99±15.55	0.367
$CD3^+/CD8^+$	0.97±7.38	10.15±18.00	0.006
$CD4^+/CD25^+$	-0.79±4.55	-1.25±4.07	0.247
$CD8^+/CD28^-$	1.96±8.68	-5.42±7.85	0.011
$CD8^+/CD28^+$	-0.22±5.20	12.89±14.76	0.000
$CD3^+/CD56^+$	-1.88±5.17	4.67±17.37	0.018

Values are presented as mean ± standard deviation. [a]Difference in T-cell subsets before and after treatment. BSC, best supportive care; DC-CIK, dendritic cell-cytokine-induced killer cell immunotherapy.

Discussion

Accumulating evidence supports cellular immunotherapy, which directly or indirectly regulates the biological interaction between the host and the tumor (26), as a potential strategy for the improvement of cancer treatment, with CIK cells representing a promising cellular immunotherapy associated with several advantages, such as MHC-unrestricted cytotoxic activity, increase in cytokine secretion, improvement of immune function and induction of apoptosis of cancer cells (27), and may thus be beneficial to patients with advanced cancer.

An increasing amount of studies demonstrated that CIK cell-based immunotherapy is a promising new treatment modality with the potential of improving the prognosis of cancer patients (18-24,26,27). A study from the International Registry reported the results of 11 CIK cell treatment trials for a variety of cancers, including hepatocellular carcinoma, gastric cancer and Hodgkin or non-Hodgkin lymphoma, and demonstrated a total response rate of 91/384 reported patients; 161 patients had stable disease and 129 patients had progressive disease. The disease-free survival rates were significantly higher in patients treated with CIK cells compared with those in the control group without CIK treatment (6). The present study demonstrated that the OS favored the DC-CIK arm compared with the BSC arm. The results indicated a 3-month prolongation in the median OS in favor of the DC-CIK treatment compared with the BSC alone arm (14.00 vs. 11.00 months, respectively). Thus, DC-CIK cell immunotherapy prolonged the OS of patients with advanced cancer.

The association between the T-cell subsets and the clinical outcome of advanced cancer was also investigated, as any successful host immune response against a tumor requires a well-balanced positive and negative regulation of lymphocytes. Imbalance of the host cellular immunity may trigger cancer progression disease progression and treatment

Table IV. Associations between cycles of DC-CIK infusion and T-cell subset changes.

	Before treatment	1 cycle	2 cycles	3 cycles	≥4 cycles
Cases, n		57	26	13	6
$CD3^+$	68.67±11.23	82.55±12.59[b]	80.87±15.01[a]	83.59±13.30	86.20±12.54[a]
$CD3^+/CD4^+$	34.23±11.97	40.22±17.09[b]	41.17±18.71	29.73±15.75	41.25±8.38[a]
$CD3^+/CD8^+$	32.27±12.21	42.42±17.83[b]	29.49±14.27	44.79±9.32	35.88±15.38
$CD4^+/CD25^+$	4.48±3.05	3.23±2.65[a]	2.35±1.49[a]	2.61±1.35	3.07±2.42
$CD8^+/CD28^-$	23.39±10.09	17.97±9.18[b]	16.98±6.92	22.07±10.95[a]	15.72±6.58[a]
$CD8^+/CD28^+$	14.11±8.46	27.00±15.10[b]	25.94±17.11	18.47±11.32	32.05±12.77[a]
$CD3^+/CD56^+$	10.88±7.76	15.56±16.24[a]	24.25±21.96	9.50±5.55	5.73±2.42

[a]Difference in T-cell subsets before and after treatment (P<0.05). [b]Difference in T-cell subsets before and after treatment (P<0.01). Values are presented as mean ± standard deviation. DC-CIK, dendritic cell-cytokine-induced killer cell immunotherapy.

Table V. Univariate analysis of the patient's clinical characteristics and overall survival.

Variables	Median OS (months)	Log-rank Relative risk (95% CI)	P-value
$CD8^+/CD28^-$			0.006
≥21.12	11.00	8.96-13.04	
<21.12	14.00	10.88-17.12	
$CD8^+/CD28^+$			0.007
≤9.04	10.00	7.08-12.92	
>9.04	13.00	10.82-15.18	
DC-CIK therapy			0.021
Yes	11.00	9.17-12.83	
No	14.00	11.54-16.46	
Age (years)			0.050
<60	15.00	10.54-19.46	
≥60	12.00	10.51-13.49	
Clinical stage			0.032
II	-	12.00-28.00	
III	19.00	13.34-20.54	
IV	12.00	11.05-15.44	
DC-CIK cycles (n)			0.040
0	11.00	9.17-12.83	
1	14.00	11.35-16.65	
≥2	14.00	10.56-17.45	

OS, overall survival; CI, confidence interval; DC-CIK, dendritic cell-cytokine-induced killer cell immunotherapy.

failure (28). Previous studies have demonstrated significant changes of peripheral blood lymphocyte cell subsets in patients with different malignant lesions and poor prognosis (29-32). Consequently, we also investigated the T-cell subsets before and after treatment in both groups, which indicated that DC-CIK infusions altered the ratios of T-cell subsets, increasing the T-helper and cytotoxic T lymphocyte (CTL) subsets, while decreasing regulatory T lymphocyte (Treg) subsets, particularly the $CD8^+/CD28^-$ subset.

Table VI. Multivariable analysis of the patients' clinical characteristics and overall survival.

Parameters	Hazard ratio	95% CI
$CD8^+/CD28^-$ <21.12	0.500	0.288-0.866
$CD8^+/CD28^+$ >9.04	0.435	0.209-0.907

CI, confidence interval.

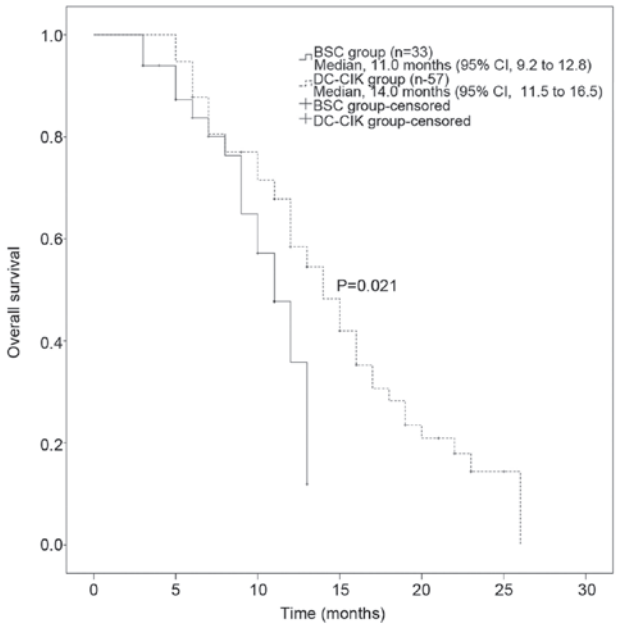

Figure 1. Kaplan-Meier curves indicating the OS of patients in the DC-CIK and the BSC groups. Patients in the DC-CIK group had a better OS compared with the BSC group. DC-CIK, dendritic cell/cytokine-induced killer cell immunotherapy; BSC, best supportive care; OS, overall survival; CI, confidence interval.

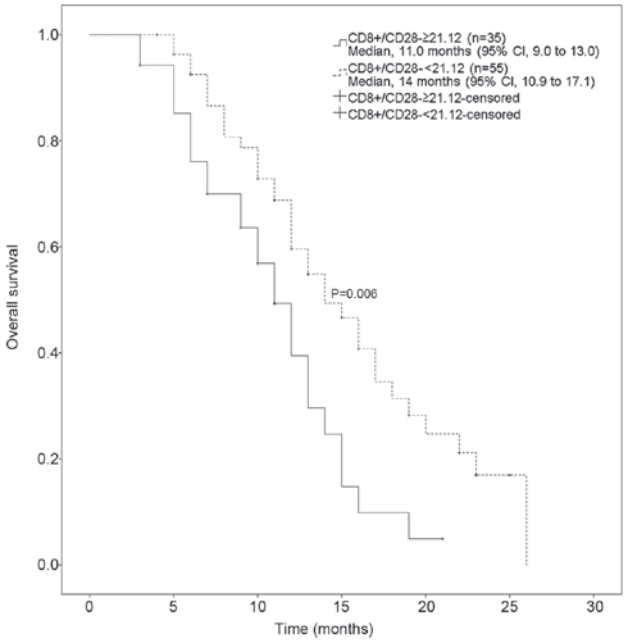

Figure 3. Kaplan-Meier curves indicating the impact of $CD8^+/CD28^-$ T-cell subsets on the OS of the patients. Multivariate analysis demonstrated that a $CD8^+/CD28^-$ ratio <21.12 decreased the HR of OS to 0.50 (95% CI: 0.29-0.87). OS, overall survival; HR, hazard ratio; CI, confidence interval.

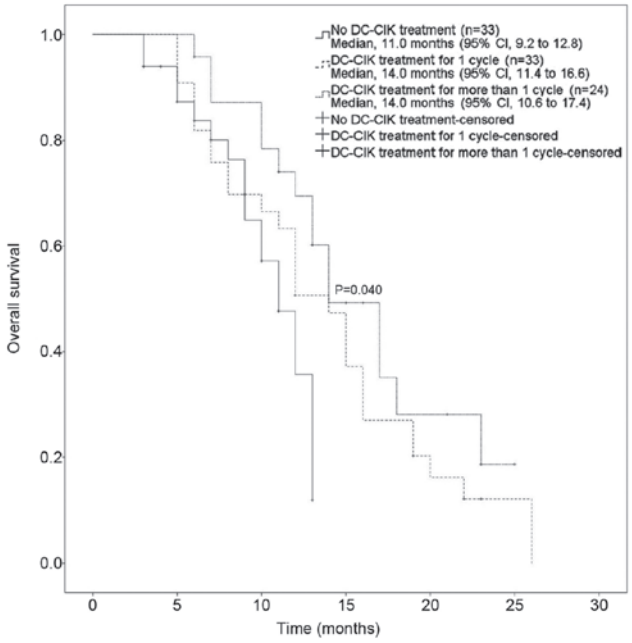

Figure 2. Kaplan-Meier curves indicating the impact of the frequency of DC-CIK therapy on the OS of patients. There was significant difference in the OS among the three groups (no immunotherapy, 1 cycle of DC-CIK infusion and ≥2 cycles of DC-CIK infusion). DC-CIK, dendritic cell/cytokine-induced killer cell immunotherapy; OS, overall survival; CI, confidence interval.

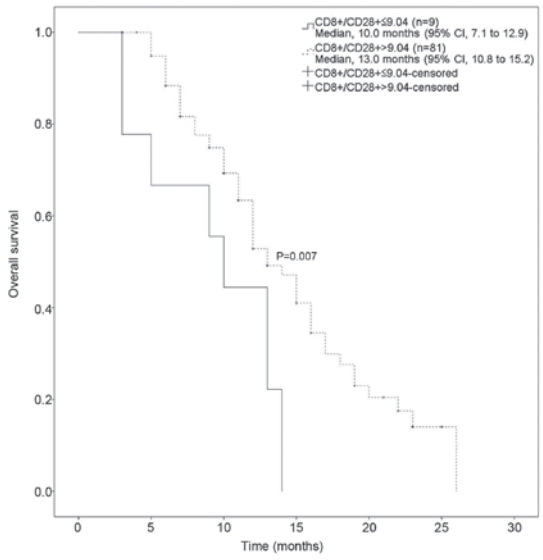

Figure 4. Kaplan-Meier curves indicating the impact of $CD8^+/CD28^+$ T cell subsets on the OS of the patients. Multivariate analysis demonstrated that a $CD8^+/CD28^+$ ratio >9.04 decreased the HR of OS to 0.45 (95% CI: 0.21-0.98). OS, overall survival; HR, hazard ratio; CI, confidence interval.

Emerging evidence supports the hypothesis that the improvement of the host immune status, including the anti-PD-1 antibody and anti-CTLA4 antibody, may favor the clinical outcome of cancer patients (33). Our previous data also indicated that elevated levels of $CD8^+/CD28^-$ suppressor T lymphocytes represent a novel independent predictor of progression-free survival in patients with metastatic breast cancer during post-chemotherapy follow-up (31). The present study revealed that T-cell subsets significantly affected the OS of patients with advanced cancer, and that DC-CIK treatment did not only improve the imbalance in the immune status, but also prolonged the OS in advanced cancer patients.

According to these results, there was a significant difference in the OS among the three groups (no immunotherapy, 1 cycle and ≥2 cycles of DC-CIK infusion). One cycle of DC-CIK infusion significantly altered the T-cell subsets,

but no obvious difference was observed among the groups receiving 2, 3 and ≥4 cycles of DC-CIK infusion, which was likely due to the small number of cases. A larger randomized clinical trial will be conducted in the near future to confirm the treatment benefit and the preferred treatment courses of DC-CIK cells for patients with advanced cancer.

No serious adverse events were observed in the patients receiving DC-CIK cell immunotherapy, which was consistent with the results of other studies (22). Therefore, DC-CIK cells are able to eliminate tumor cells without severe injury to normal tissues, which renders this treatment suitable for elderly and advanced-stage cancer patients.

It should be noted that there were several limitations to this study. First, the results were generated from a retrospective observational study, and a prospective paired study is required to confirm the clinical outcomes of DC-CIK cell immunotherapy. Second, only 90 patients were considered eligible for the present study. More cases are required and randomized clinical studies according to different tumor types must be performed to further analyze the treatment benefits of DC-CIK cells for patients with advanced cancer. Third, it must be mentioned that the $CD4^+/CD25^+$ T-cell subsets detected in this study may not represent $CD4^+/CD25^+FoxP3^+$ Tregs, as not only Tregs but different T-cell subsets were considered. Foxp3 expression will be included along with CD4 and CD25 to validate the reduction of Tregs in a following study.

The data presented herein demonstrated that T-cell subset changes were associated with the OS of advanced cancer patients, while DC-CIK cell immunotherapy may regulate and enhance the host's immune function, significantly improving the OS of patients with advanced cancer. No severe side effects were recorded during the immunotherapy process, indicating that this is a safe treatment modality. A larger randomized, prospective clinical trial is required to further validate the clinical efficacy of DC-CIK cell therapy for patients with advanced cancer, and to elucidate the detailed underlying mechanism.

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