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ORIGINAL ARTICLE

Adjuvant dendritic cells vaccine combined with cytokine-induced-killer cell therapy after renal cell carcinoma surgery

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Summary

Purpose: *To observe the efficacy and side effects of adjuvant dendritic cells' (DCs) vaccine combined with cytokine-induced killer cell (CIK) therapy after renal cell carcinoma (RCC) surgery (RCCS).*

Methods: *DCs vaccine and CIK that loaded the autologous tumor cell lysate were prepared in vitro. Four hundred and ten RCC patients were recruited, and the study group was given DCs-CIK immunotherapy, while the control group was given IFN-a therapy.*

Results: *Disease progression (recurrence, metastasis or death) showed significant differences between the two groups in clinical stage I and II patients, as well as in highly and moderately differentiated disease ($p<0.05$), while there was no significant difference between the two groups in patients with poorly differentiated disease ($p>0.05$). The 3- and 5-year overall survival rates of the DCs-CIK group (96% and 96%, respectively) exhibited significant difference compared to the IFN-a group (83% and 74%, respectively ($p<0.01$). Progression-free survival (PFS) between the two groups was significantly different ($p<0.01$). Tumor stage and DCs-CIK treatment were independent factors concerning prognosis of RCC ($p<0.05$). There was no severe toxicity observed in the DCs-CIK treatment group.*

Conclusions: *Adjuvant post-RCCS DCs-CIK treatment prolonged PFS and reduced mortality, showing better overall activity compared to interferon treatment.*

Key words: *CIK, DCs, immunotherapy, renal cell carcinoma, tumor lysate*

Introduction

RCC is one of the common malignant tumors of the genitourinary system [1]. Surgical treatment has a good therapeutic effect in early-stage RCC patients, but about 20% patients have already perirenal infiltration or distant metastasis when firstly diagnosed, and more than 30% of the patients will develop local recurrence or distant metastasis after surgery [2]. RCC is not sensitive to traditional chemotherapy, but it is a tumor with high immunogenicity. Over the years, interleukin-2 (IL-2), interferon-α (IFN-α) and other cytokines have been used as important adjuvant therapies post-RCCS and in advanced RCC. However, clinically, IFN-α therapy achieves only 15-20% partial remissions and 3-5% complete remissions. In recent years, the application of vascular endothelial growth factor (VEGF) or mammalian target of rapamycin (mTOR) targeting therapies in metastatic RCC showed certain important effects, but their serious side effects limited their clinical applications.

DCs are the most powerful professional antigen-presenting cells, and tumor vaccines based on DCs might lead to production of specific antitumor immunity through various ways [3]. Animal and *in vitro* experiments have confirmed that DCs that were loaded with tumor antigen could induce a potent antitumor response [4], and proved effica-

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Table 1. General clinical information of enrolled RCC cases

	Study group (DC-CIK, N=154) N (%)	*Control group (IFN-a, N=256) N (%)*	*p value*
Gender			0.839
Male	105 (68.2)	177 (69.1)	
Female	49 (31.8)	79 (30.9)	
Diseased side			
Left	90 (58.4)	126 (49.2)	
Right	64 (41.6)	130 (50.8)	0.07
Surgical method			0.265
Radical nephrectomy	128 (83.1)	223 (87.1)	
Partial nephrectomy	26 (16.9)	33 (12.9)	
Differentiation grade (Fuhrman grading)			0.002
High	72 (46.7)	95 (37.1)	
Moderate	70 (45.5)	109 (42.6)	
Low	12 (7.8)	52 (20.3)	
Tumor stage (TNM)			0.001
I	105 (68.2)	141 (55.5)	
II	37 (24.0)	54 (21.1)	
III	9 (5.8)	17 (6.6)	
IV	3 (2.0)	43 (16.8)	
Local lymph node metastasis			0.659
No	146 (94.8)	240 (93.8)	
Yes	8 (5.2)	16 (6.2)	
Distant metastasis			0.001
No	152 (98.7)	215 (84.0)	
Yes	2 (1.3)	41 (16.0)	

cious in the clinical treatment of malignant melanoma and prostate cancer [5,6]. In recent years, research over DC vaccines in RCC also yielded significant results. Baek et al. [7] used TuLy-DC to treat 6 cases of metastatic RCC and found that TuLy-DC could induce specific immune responses. Kraemer et al. [8] treated 30 metastatic RCC patients with the tumor lysate-sensitized DC therapy, achieving a partial response rate of 40%.

CIK can be extracted and amplified easily, showing high antitumor activity, thus it was considered to be the preferred solution for a new generation of antitumor adoptive cytoimmunotherapy method [9]. Olioso et al. [10] reported that using CIK therapy in 5 cases of metastatic RCC, 3 cases obtained complete remission, and in 2 cases the disease stabilized.

Based on the existing researches, our hospital registered the randomized controlled phase III clinical trial (ID: NCT00862303) at Clinical Trials Gov in 2009, and administered the combined therapy of DC vaccine and CIK, as RCCS adjuvant treatment, the preliminary findings of which are reported below.

Methods

This study was conducted in accordance with the declaration of Helsinki and after approval from the Ethics Committee of Fuzhou General Hospital. Written informed consent was obtained from all participants.

Inclusion criteria

Age >18 years; WHO-ECOG performance status 0-1; patients with radical or partial nephrectomy, and pathologically diagnosed RCC; expected survival more than 3 months.

Subjects

Four hundred and ten RCC patients were recruited from the department of urinary surgery, Fuzhou General Hospital of Nanjing Military command, from

March 2009 to November 2012. The subjects that met the inclusion criteria were randomly divided into two groups: the study group and the control group. The study group received post-RCCS adjuvant DCs-CIK bioimmunotherapy, while the control group received post-RCCS adjuvant IFN-α therapy. The whole cohort of RCC patients included 282 male and 128 female patients, aged 22 to 82 years (mean 51.3±15.3), among whom 351 cases were subjected to radical nephrectomy, and 59 cases to partial nephrectomy. Pathological types: 341 (83.2%) cases clear cell carcinoma, 15 (3.7%) cases papillary carcinoma, 21 (5.1%) cases chromophobe cell carcinoma, and 33 (8.0%) cases undifferentiated carcinoma. Twenty four (5.9%) cases had local lymph node metastasis, 43 (10.5%) cases had distant metastasis [17 (41%) cases with bone metastasis, 15 (3.7%) cases with lung metastasis, 7 (1.7%) cases with brain metastasis and 4 (1.0%) cases with liver metastasis]. Tumor stage (TNM staging criteria of AJCC, 2002 Edn) distribution was as follows: stage I: 246 (60.0%) cases, stage II: 91 (22.0%), stage III: 26 (6.3%), and stage IV: 47 (11.5%). The follow-up time ranged from 13 to 57 months (median 35;Table 1).

Acquisition and preparation of cells

50 ml of peripheral anticoagulated whole blood were collected from each patient before surgery and every 10 days before treatment. The density gradient centrifugation method and human lymphocyte separation medium (Canada CEDARLANE) were used to separate the mononuclear cells; then the lymphocyte serum-free medium (AMMS) was added for 3-h culture. The suspended cells and adherent cells were then collected for future use.

Preparation of autologous tumor antigen-loaded DCs vaccine

The surgically obtained RCC tissues of all patients were minced and passed through a 0.22 μm mesh filter, followed by sterilization and preparation of single cell suspension. The tumor cells were then obtained through centrifugation and were subjected to repeat freezing and thawing in liquid nitrogen. After centrifugation, the supernatant was obtained for protein content determination of tumor cell lysate, and was then reserved at -80°C for further use.

The wall-adherent DCs were then cultured with lymphocyte serum-free medium, and the cytokines were then added with the following final concentrations: granulocyte-macrophage colony-stimulating factor (GM-CSF) 1000IU/ml, IL-4 1000 IU/ml and tumor necrosis factor-α (TNF-α) 500IU/ml; half of the amount of medium was changed every 3 days, and an equal amount of fresh cytokines was complemented (all of the cytokines were purchased from the Academy of Military Medical Science (Peiking, China). On the 7th day of culture, the prepared tumor antigens were added into the DC culture system at a final concentration of 20 μg/ml, and then the mixture was cultured at 37°C for 2-3 days after which the autologous tumor antigen-loaded DCs were obtained.

Induced culture of CIK cells

The suspended cells were collected and adjusted with the serum-free medium to a cell concentration of 4×10^6 cells/ml; then the cell suspension was cultured at 37°C and 5% CO_2 for 2 hrs. The non-wall-adherent cells were collected, and the concentration was adjusted with the serum-free medium to 2×10^6 cells/ml. Following this, 1000 IU/ml of interferon-γ (IFN-γ) were added and cultured at 37°C and 5% CO_2 for 24 hrs; on the next day, 1 μg/ml mouse anti-human CD3 monoclonal antibody and 1000 IU/ml IL-2 were added and kept into the culture. Half of the amount of the medium was changed every 3 days, and an equal amount of IL-2 was complemented (all of these cytokines were purchased from the Academy of Military Medical Science).

Detection and identification of cells

Routine tests of bacteria, fungi and endotoxin were performed before the infusion.

Phenotype testing

The fluorescence-labeled mouse anti-human CD83, CD86, HLA-DR (COULTER, USA) were used to stain the mature DCs, which had been cultured for 9 days. Flow cytometry (COULTER, USA) was then used to detect the phenotype of DCs. The fluorescence-labeled anti-mouse human CD3 and CD (16+ 56+) (COULTER, USA) were used to stain the 14-day-cultured CIK cells, followed by the phenotype testing by the flow cytometry.

Immunotherapy program

The study group began the DC-CIK bioimmunotherapy 2-3 weeks postoperatively. DCs vaccine therapy: 1 ml of intradermally injected preparation in the inguinal regions bilaterally (the lymphatic drainage area), with a cell number of $2\text{-}5\times10^6$/ per day, for a total of 6 courses.

CIK therapy administered on the second of DCs vaccine treatment consisted of i.v. infusion of $3\text{-}6\times10^9$ cells (100 ml) in one h/once a day for a total of 5 courses. A 6-day treatment was considered as one course, and the second course started after one month interval, for a total of 3 courses.

The control group received conventional IFN-α therapy postoperatively, with 3×10^6 units, 3 times/week, for a total of 3 months.

Clinical efficacy and safety

The patients were followed up every 3 months after the operation with abdominal X-rays, abdominal B ultrasound, abdominal CT scanning and other imaging methods to see whether recurrence and/or metastasis

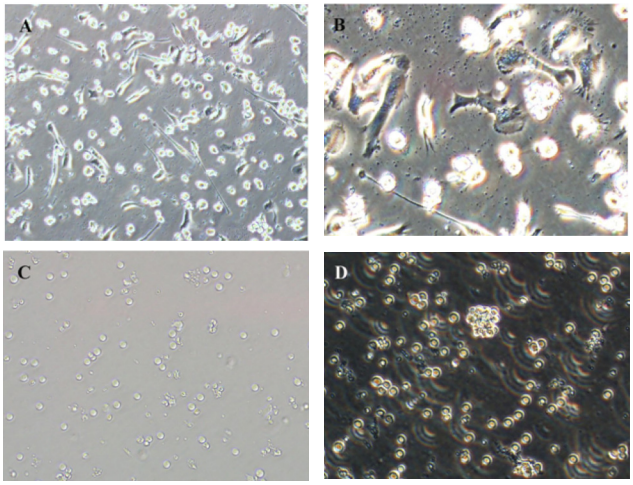

Figure 1. Cultured DCs and CIK cells. **A:** Human DCs cells (×100); **B:** Human DCs cells (×200); **C:** Human CIK cells (×100); **D:** Human CIK cells (×200).

developed. Because all of the patients in this study were operated there was no evaluable lesion, and imaging recurrence or metastasis or death were defined as progressive disease (PD). PFS and overall survival (OS) of the 2 groups were registered and compared.

Safety was assessed according to the National Cancer Institute (NCI) common toxicity criteria (CTC). Changes of patients' symptoms and signs were assessed, and before and after each vaccine treatment, peripheral blood leukocytes, liver function tests and kidney function tests were evaluated. All adverse reactions were closely observed, and their occurrence time, performance and outcome were recorded.

Statistics

Statistical analyses were performed by SPSS17.0 software (SPSS Inc, Chicago, Ill). Comparisons between the groups was assessed by the chi-square test. Kaplan-Meier with log rank test were used to compare difference of PFS and OS in different groups. Univariate and multivariate analyses were used to analyze the risk factors that could affect the disease outcome. A p value <0.05 was considered statistically significant.

Results

Phenotype identification

DCs showed the characteristics of wall-adherent growth, with polygonal, spindle and irregular cell shapes, and branched cell protrusions. CIK cells exhibited the characteristics of a suspended growth, with round cell shape, similar to the peripheral blood lymphocytes, but the volume was slightly bigger, the cytoplasm was plump, with good refraction, and the cell separation phase and colony-like cell clusters could be seen under the microscope (Figure 1).

The cultured DCs exhibited not only the specific surface marker CD83 of the mature DCs, but also expressed such costimulatory molecules as MHC-II antigen (HLA-DR) and CD86 (B7-2). The CIK cells were a heterogeneous cell group, with high expressions of CD3 and CD (16+ 56+) on the cell surface (Figure 2).

Disease progression situations

The follow up period of this research ranged from 13 to 57 months (median 35). In the study group (DCs-CIK group) we noticed 1 case of local recurrence, 1 case of metastasis and 5 deaths. At the same time no case of local recurrence, 5 cases of metastasis and 34 deaths were registered in the control group (INF-α group) ($p<0.01$).

Relationships of disease progression and clinical tumor stage

Intergroup comparison of stage I and II disease progression showed statistically significant difference ($p<0.05$). Comparison also showed that stage III patients of both groups had no statistically significant difference ($p>0.05$), in contrast to stage IV patients who showed such difference ($p<0.05$). Because the number of the cases was small, no clinical significance could be determined yet, still needing further clinical cases accumulation. It also indicated that the DCs-CIK treatment had clinical significance in early-stage RCC, and was better than the IFN-α therapy.

Relationships of disease progression and pathological grades

Statistical significance was shown between highly and moderately differentiated patients in both groups ($p<0.05$), while no statistical significance existed between the poorly differentiated patients of the two groups ($p>0.05$), indicating that DCs-CIK treatment had positive clinical significance in patients with highly and moderately differentiated tumors, and the effects were better compared to the IFN-α therapy. As for the poorly differentiated RCC patients, the DCs-CIK treatment was not better than the IFN-α treatment (Table 2).

Analysis of post-RCCS overall survival

During follow-up, 5 of 154 (3.2%) patients of the DCs-CIK group died, and the mean 3-and

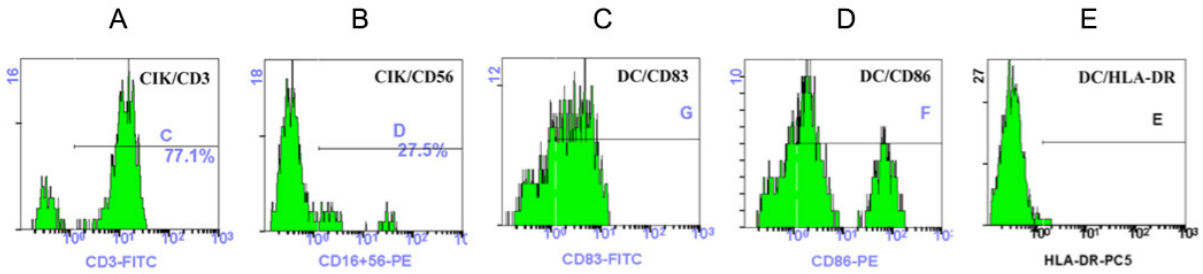

Figure 2. Identification of surface markers of DCs and CIK cells (flow cytometry).
Positive markers on the surface of human DCs and CIK.
Flow cytometry confirms the human DCs are a homogeneous cell population of CD83+ CD86+ HLA-DR+ cells (**C**, **D**, **E**).
Flow cytometry confirms the human CIKs are a homogeneous cell population of CD3+ CD56+ cells (**A**, **B**).

Table 2. Metastasis, recurrence and death of RCC patients

	Study group (DC-CIK) N (recurrence, metastasis and death)	*Control group (IFN-a) N (recurrence, metastasis and death)*
Tumor stage (TNM)		
I	105 (0, 0, 0) *	141 (0, 1,18)
II	37 (0, 0, 0) *	54 (0, 2, 6)
III	9 (0, 1, 3)	17 (0, 1, 4)
IV	3 (1, 0, 2) *	43 (0, 1, 6)
Differentiation grade (Fuhrman grading)		
High	72 (1, 1, 2) *	95 (0, 2,15)
Moderate	70 (0, 0, 2) *	109 (0, 2,13)
Low	12 (0, 0, 1)	52 (0, 1, 6)
Local lymph node metastasis		
No	146 (1, 0, 2)	240 (0, 4,33)
Yes	8 (0, 1, 3)	16 (0, 1, 1)
Distant metastasis		
No	152 (0, 1, 4) *	215 (0, 4,28)
Yes	2 (1, 0, 1) *	41 (0, 1, 6)

*Intergroup comparison, p<0.05

5-year OS rates were both 96±2%. In the IFN-α group 34 (13.3%) patients died, with a mean 3-year and 5-year OS rate 83±3% and 74±6%, respectively. The difference of OS between the two groups was statistically significant (p<0.01), indicating that DCs-CIK treatment could significantly improve the 3- and 5-year OS rates of RCC patients compared INF-a therapy (Figure 3).

Analysis of post-RCCS progression-free survival

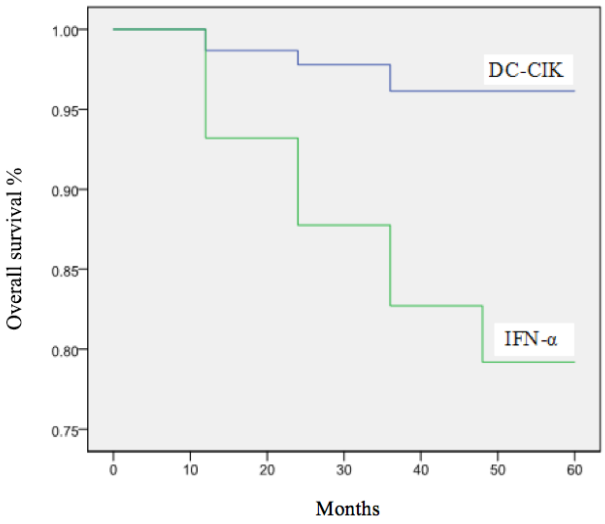

Figure 3. Analysis of post-RCCS overall survival according to treatment method (p<0.001).

Because all of the patients in this study were subjected to operation, there were no evaluable lesions, and the imaging-proven recurrence or metastasis, or death was determined as PD. Among the 154 cases of the DCs-CIK group, there were 1 case of recurrence, 1 case of metastasis and 5 cases of death (total PD cases 7), while among the 256 cases of the control group, there were 0 case of recurrence, 5 cases of metastasis and 34 cases of death (total PD cases 39). These results favored significantly the DCs-CIK group and (p<0.01) (Figure 4).

Post-RCCS risk regression analysis

Univariate analysis was used to analyze the impact of multiple variables on PD of RCC patients. The results showed that TNM stage, lymph node metastasis, distant metastasis and DCs-

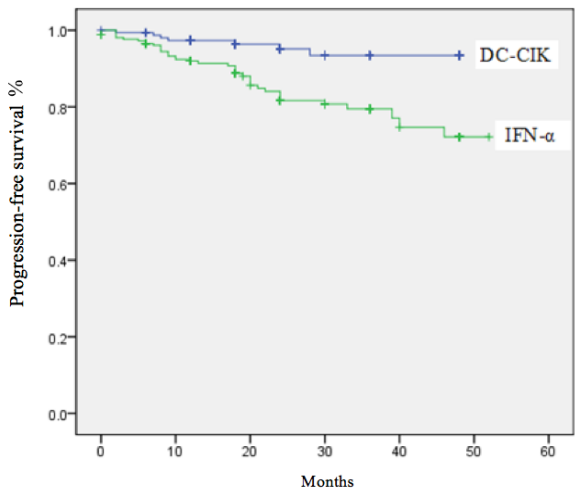

Figure 4. Analysis of post-RCCS progression free survival according to treatment method (p<0.01).

CIK therapy were significant prognostic factors (p<0.05), while the multivariate analysis showed that only TNM stage and DCs-CIK therapy showed statistical significance (p<0.05), indicating that DCs-CIK therapy was an independent prognostic factor for RCC patients (Table 3).

Adverse reactions

The main side effects of IFN-α therapy were flu-like symptoms (fever, fatigue and muscle pain), often occurring within the first 2 weeks of treatment. During treatment, 31% of the cases exhibited leukopenia, necessitating administration of G-CSF; 14% of the cases exhibited liver toxicity (mostly grade I-II); 7% of the cases developed proteinuria and 23 (9.0%) patients were withdrawn from treatment because of fever and liver dysfunction.

The patients in the DCs-CIK group exhibited good tolerance, without serious adverse events. The main adverse reaction was transient low grade fever (below 38°C), which usually occurred within 2-6 hrs postinfusion, and was accompanied with fatigue; this side effect usually lasted 3-5 hrs, and subsided spontaneously (Table 4). No severe toxicity such as leukopenia, hypotension, allergy, pulmonary edema, liver and kidney dysfunction were observed, and no patient was withdrawn because of adverse effects.

Discussion

Biological treatments are the fourth type of cancer therapies, following surgery, radiotherapy and chemotherapy, and have become an important adjunctive treatment modality because RCC is not sensitive to traditional radiotherapy and chemotherapy, and cytokine therapy can only offer limited therapeutic effect. Based on the latest research progress of immunotherapy, we tried to perform adjuvant autologous tumor cell lysate-loaded DCs-CIK treatment after RCCS. The short-term results showed that the DCs-CIK treatment could significantly improve the 3- and 5-year PFS rates of RCC patients. Its effects were better than the IFN-α treatment, especially in patients who had early RCC stage with high and moderate differentiation; of note, the DCs-CIK immunotherapy did not exhibit any severe adverse reaction, and its tolerability was excellent.

Currently, there are no uniform standards for the clinical evaluation of immuno therapy, with most studies using the clinical efficacy evaluation criteria of radiotherapy and chemotherapy, which might help somehow the therapeutic evaluation of immunotherapy. Shimizu et al. [11] suggested that PFS or OS should be set as the endpoints of clinical immunotherapy trials and researches. Itoh et al. [12] summarized the different tumor vaccines applied in clinical trials from 2005 to 2008, and reported that the reduction of tumor volume should not be set as the research endpoint, while OS should be used as the observation indicator. Berntsen and colleagues [13] thought that the patients with minor residual lesions might obtain better benefits from this treatment. Accordingly, we speculated that immunotherapy might be much more effective in patients with small lesions or postoperative residual lesions, and it might play a leading role in preventing local reccurrence and metastasis. So we tried to apply the DCs-CIK treatment as postoperative adjuvant treatment of RCC patients. The results of the study group confirmed our hypothesis that the DCs-CIK therapy not only showed promising clinical efficacy, but it also exhibited better therapeutic effects in patients with early-stage RCC and with high and moderate differentiation. Therefore, agreeing with Shimizu et al. [11] and Itoh et al. [12] views, we abandoned the partial and complete remission rate, and used PSF and OS as the observation endpoint of immunotherapy.

In recent years, the RCC-specific immunotherapy was mainly focused in exploring the DC-based clinical vaccines [2]. Holtl and colleagues [14] co-cultured RCC lysates and DC, and administered them to 27 patients with metastatic RCC; all of them obtained some degree of disease remission, among them 2 patients achieving complete

Table 3. Cox proportional hazards regression model

	Single factor			*Multiple factors*		
	RR	*95%CI*	*p value*	*RR*	*95%CI*	*p value*
Gender	1.489	0.793 - 2.796	0.215			
Age	0.990	0.969 – 1.012	0.378			
Diseased side	0.839	0.446 – 1.511	0.559			
Surgical method	1.034	0.462 – 2.312	0.936			
DCs-CIK therapy	3.681	1.646 – 8.231	0.002*	3.130	1.372 – 7.136	0.007*
Tumor stage	1.598	1.272 – 2.006	0.001*	1.625	1.130 – 2.337	0.009*
Differentiation grade	0.871	0.578 – 1.311	0.507			
Pathological type	1.113	0.832 – 1.488	0.471			
Local lymph node metastasis	3.672	1.534 – 8.777	0.003*	1.881	0.724 – 4.891	0.195
Distant metastasis	2.377	1.146 - 4.928	0.020*	0.517	0.178 - 1.498	0.224

*: Intergroup comparison, p<0.05

Table 4. Adverse reactions

Adverse reactions	*Study group (DC-CIK, N=154) N (%)*	*Control group (IFN-a, N=256) N (%)*	*p value*
Fever	27(17.5%)*	89 (34.8)	0.001
Fatigue	22(14.3%)	55 (21.5)	0.071
Gastrointestinal reactions	15(9.7%)*	47 (18.4)	0.018
Leukopenia	0*	79 (30.1)	0.001
Liver toxicity	0*	36 (14.1)	0.001
Proteinuria	0*	18 (7.0)	0.001

*comparison between two groups, p<0.05.

remission. Marten et al. [15] selected 15 cases of metastatic RCC and injected the lysate-pulsed DC-prepared vaccine into the patients' inguinal lymph nodes or peri-tumoral tissues, achieving one partial response, and 7 disease stabilizations. Some studies [16,17] have shown that CIK treatment could induce complete or partial remission in RCC patients. Currently, the clinical trials about DC and CIK in the RCC treatment have the following characteristics: clinical phase I/II trials that mostly target advanced metastatic RCC; applied DC or CIK treatment alone; with small numbers of cases; initially showing some clinical efficacy; with low rate of side effects. As further large-sample prospective controlled clinical studies are needed, therefore, we designed this prospective randomized controlled phase III clinical trial, which was focused in the effects of combined DC-CIK treatment in early and middle stages of RCC. The short-term results showed that, no matter PFS or OS, DCs-CIK therapy was significantly better than the IFN-α therapy, thus further validating the clinical efficacy of DCs-CIK treatment in RCC. Of course, the follow-up time was short, and the number of cases was small. In a future follow-up report we will continue to add new cases and extend the follow-up observation time to make the results much more objective and accurate.

A study by Shi et al. [18] showed that the combination of CIK cells and DC vaccine could help relieve the immune incompetence of T cells in some cancer patients, thus revealing the synergistic antitumor effects. The study of Marten et al [19] showed that co-culture of CIK cells and DCs could significantly increase the specificity of DCs and the costimulatory molecule-presenting antigens, promote the secretion of IL-12 and augment the cytotoxicity of CIK cells. Theoretically, treatment with the combination of DC vaccine and CIK cells could produce dual antitumor effects of specific and nonspecific immunity. Accordingly, we designed the combination of CIK cells and DC vaccine therapy as adjuvant treatment of operated RCC patients. A similar report came from authors from the Zhongshan University in Guangdong, who conducted a clinical trial of DC-CIK combination therapy in 10 cases of metastatic RCC pa-

tients in 2006. The follow up period ranged from 6 to 20 months, one case achieved partial remission, and 6 disease stabilisation [20]. In 2012, DC-CIK combination treatment was applied in 46 patients with early and middle stage RCC, and revealed no difference in OS between the DC-CIK group and the IFN-a group [21]. Our results were different, possibly because the programs of Zhongshan University were different from ours in the following aspects: 1) cell preparation methods: after preparing CIK and tumor cell lysate-loaded DCs, the mixture was co-cultured at a ratio of 1:50-100 for 7 days; 2) infusion ways: the mixture of CIK cells and DCs was intravenously infused; 3) treatment began on the 4th postoperative week, once every other day, with a total of 5 times, and only applied for one course; 4) cell numbers: the mixture of CIK cells and DCs was 3×10^8 for each administration. Our program: CIK and DCs were prepared separately; DCs were intradermally injected, while CIK were intravenously infused; 6-day represented one course of treatment, with a total of 3 courses; cell numbers: DCs $2\text{-}5\times10^6$ for each administration (1 ml), CIK $3\text{-}6\times10^9$ for each administration Both the cell numbers and the courses were significantly more than the program of Zhongshang University. During the clinical treatment, we found that the key to the success was to achieve adequate cell numbers and full courses of treatment, which also might be the reason for the different results of the above 2 trials.

The side effects of DC vaccine and CIK cell therapy were mild, transient, and the safety has been confirmed by many phase I and II clinical trials [22,23]. In this study, the RCC patients exhibited no serious adverse reactions that could not be tolerated during treatment and follow-up. Only transient mild fever and chills were observed, and this discomfort might be induced by IL-2 contained in the infused CIK cells, as well as with the release of a large number of cytokines after the DC and CIK cells treatment. In short, the combined treatment of autologous renal cancer lysate-loaded DC vaccine and autologous CIK cells produced certain short-term clinical efficacy, as well as good tolerance, so the prospects of clinical application would be good. Selecting the appropriate therapeutic dose, timing, exploring the best settings of treatment course, improving and optimizing the preparation techniques of DCs vaccine and CIK cells would be the main directions of our future research.

Acknowledgements

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