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Original Article

Safety of dendritic cell and cytokine-induced killer (DC-CIK) cell-based immunotherapy in patients with solid tumor: a retrospective study in China

Shuo Wang[1*], Yuguang Song[1*], Qi Shi[1*], Guoliang Qiao[2], Yanjie Zhao[1], Lei Zhou[1], Jing Zhao[1], Ni Jiang[1], Hongyan Huang[1]

[1]*Department of Medical Oncology, Beijing Key Laboratory for Therapeutic Cancer Vaccines, Capital Medical University Cancer Center, Beijing Shijitan Hospital, Capital Medical University, Beijing 100038, China;*
[2]*Department of Surgical Oncology, Massachusetts General Hospital, No. 55, Fruit Street, Boston, MA 02114, USA.*
[*]*Equal contributors.*

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Abstract: Systematic assessment of adverse side effects of Adoptive T cell therapy, especially cytokine-induced killer cell and dendric cell treatment Dendritic cells-Cytokine-induced killer (DC-CIK) therapy, especially when combined with chemotherapy, has not been reported. Totally 1100 consecutive patients (2504 trail cycles) enrolled in DC-CIK treatment trials at Beijing Shijitian Hospital between August 2012 and August 2022 were retrospectively reviewed. The 370 patients (34%)/815 cycles enrolled in our trial combined with chemotherapy. In total, 548 (cases)/870 (cycles) patients experienced AEs. The AE class was mainly composed of Neurological 34 cycles (4%), Musculoskeletal 28 cycles (3%), Immunopathies 5 cycles (1%), Hematological 521 cycles (60%), 224 general disorders and administration site conditions cycles (26%), Gastrointestinal 209 cycles (24%), Skin 15 cycles (2%), and 119 Metabolism and Nutrition disorders cycles (14%). The AE class of gastrointestinal (vomiting, P=0.025), nutritional (anorexia, P=0.016), and hematological disorders (anemia P<0.0001, leukopenia P<0.0001) appeared in the DC-CIK treatment and were mainly correlated with chemotherapy. Multiple logistic regression analysis suggested that regardless of whether DC-CIK was combined with chemotherapy, multi-line treatment was more prone to nausea, anorexia, fatigue, anemia, and leukopenia than first-line treatment. However, correlation analysis verified that increasing the number of cycles of DC-CIK treatment alone could reduce the incidence rate of fatigue (P=0.001), anorexia (P<0.0001), and anxiety (P=0.01). Most of the adverse side effects that occurred during autologous DC-CIK treatment were associated with combined or previously applied chemotherapeutic treatment, which also indicated that autologous DC-CIK anti-tumor therapy was safe.

Keywords: Immunotherapy, adverse events, DC-CIK immunotherapy

Introduction

Our previous study demonstrated that cytokine-induced killer cell and DC cell treatment exerts an adjuvant immunomodulatory effect by prolonging survival in patients with different cancer types who undergo curative treatment, especially those with advanced malignancies [1-7]. Unlike other adoptive T-cell immunotherapy approaches, such as T-cell receptor (TCR) and chimeric antigen receptor (CAR) T-cell therapy, dendritic cell-cytokine-induced killer cell (DC-CIK) therapy is a potent stimulator of tumor-specific T-cell responses. DC-CIK therapy also stimulates ex vivo-expanded T lymphocytes, which mediate non-MHC-restricted cytotoxicity, and that have a natural killer/T-cell phenotype characterized by both CD56 and CD3 expression [8]. Based on this advantage, DC-CIK therapy has been widely used in adoptive T-cell immunotherapy (ACT). Currently, 171 registered studies on ACT-treated tumors have been published, of which 38 have been registered for the DC-CIK treatment of tumors (www.clinicaltrials.gov). Moreover, when combined with chemotherapy, tumor cleavage produces neoantigens that stimulate more specific effects of T-cell activation to attack tumors. The results showed that DC-CIK combined with chemotherapy could prolong patient survival and

improve prognosis [9, 10]. At the same time, chemotherapy combined with DC-CIK has a good therapeutic effect compared with standard first-line antitumor therapy in a variety of tumors (Table S1). Recently, many immune checkpoint inhibitors, especially those that block the PD-1/PD-L1 pathway, have shown remarkable clinical success in a variety of cancers [11-13]. However, as an adoptive immunotherapy, DC-CIK antitumor activity is restricted by immunosuppressive pathways in the tumor microenvironment, and inhibitory receptors are also expressed on CIK cells, which could achieve a better curative effect in combination with immunological checkpoint inhibitors for antitumor treatment [14, 15].

Immunotherapy agents have been associated with a unique spectrum of toxicities reported in some articles and have the potential for immune-related and cytokine-related adverse effects [16]. Unlike traditional cytotoxic chemotherapy agents that cause toxicity in rapidly proliferating tissues, such as the bone marrow and the gastrointestinal tract [17], or molecularly targeted agents that produce toxicity in organs based upon expression of the target [18], immunotherapy agents, especially anti-PD1, can result in adverse effects that affect any organ system [19]. In recent years, TIL therapy for patients with advanced melanoma showed a 52-72% objective response rate based on the Response Evaluation Criteria in Solid Tumors (RECIST), and 19 of 93 patients (20%) achieved complete tumor regression after 3 years [20]. However, the TIL treatment reported by Rosenberg exhibited toxicity due to high-dose IL-2 but still achieved certain therapeutic effects; this was the most common manifestation of capillary leak syndrome and resulted in a hypovolemic state and extravascular fluid accumulation [21, 22]. CAR-T-cell therapy is also associated with various cytokine storm-related adverse effects [16, 23]. However, one advantage of nonspecific, non-MHC-restricted, and lower cytokine interventions in DC-CIK therapy appears to be a lack of significant side effects, as reported in various clinical trials [24].

In addition, various cytokines play important roles in tumor-stroma interactions [25]. Some are directly suppressive, while others have a positive effect on treatment, as shown in the following studies on the role of cytokines in tumors. Ana M Vuletić et al. found that IL-4-induced NK cell cytotoxicity and that increased activating NKG2D receptor expression may indicate an important antitumor effect of IL-4 with a potential application for immunotherapy in MM patients [26]. Katarina Mirjačić Martinović et al. explored whether TGF-β1 serum values are negatively correlated with NK cell activity, as analyzed by CD107a, IFN-γ, NKG2D, and NKp46 expression in metastatic melanoma patients; their conclusion indicated that the association of high levels of TGF-β1 with NK cell inhibition represents the primary mechanism of tumor immune evasion [27].

To identify DC-CIK-related adverse effects, we summarize the findings in patients who were treated with DC-CIK in recent years and analyze whether they experienced obvious side effects after treatment. We will also continue to study whether DC-CIK combined with chemotherapy enhances or reduces these therapy-related side effects.

Method

Patients and treatment

We retrospectively reviewed consecutive patients treated at the Beijing Shijitan Hospital between August 2012 and August 2022. We enrolled 1100 patients (a total of 2504 treatment cycles) in our study. Patients who received DC-CIK cell therapy as the main treatment were included. All treatment decisions were at the physician's discretion, including the schedule and duration of DC-CIK cell therapy, scheduling of patient visits, and the method and frequency of clinical assessments.

All study variables were collected from the available hospital records, including electronic prescribing oncology pharmacy applications and patient medical history, as well as other complementary sources (pathology, laboratory, and radiology records). Information included patient age, sex, relevant medical history events, cancer history (histological tumor type, sites of metastasis, date and stage at initial diagnosis, date of advanced disease diagnosis, date of disease progression, and death), and DC-CIK-based treatment (dose, schedule, line, and treatment cycles).

Generation of DC-CIK cells

DC-CIK cells were prepared as described in our previous studies [7]. Briefly, when routine blood examination revealed a return to normal conditions, 50-60 ml of heparinized peripheral blood was obtained from each patient over a 2-week period. Peripheral blood mononuclear cells (PBMCs) were separated using a COBE Spectra cell separator (COBE BCT, Lakewood, CO, USA) until CD34+ cells reached ≥4.5 × 10^6/kg. The cells were cultured in X-VIVO 15 medium containing 2% autologous serum and allowed to adhere for 1 h. The suspended cells were then collected and induced to become CIK cells with 1000 U/ml rhIFN-for the first 24 h, followed by stimulation with 100 ng/ml OKT-3, 1000 U/ml rhIL-2, and 100 U/ml IL-1a. Adherent cells were cultured in DC medium. On the sixth day, another 10 ng/ml TNF-α was added to the DCs to induce maturation. The next day, the CIK cells were mixed with DCs (DC-DIK cells) at a ratio of 20:1 and cultured in fresh medium containing 1000 U/ml rhIL-2 for another seven days. At 14 days, DC-CIK cells were harvested, and their number, viability, phenotype, and whether contamination was present were analyzed. Cultured cells that met the lot release criteria were infused intravenously over 20 min (**Figure 1A**). However, from the 50 ml of peripheral blood that was extracted directly for cell culture and recovery of frozen PBMCs collected by the COBE Spectra cell separator, only cultured CIK cells were used for reinfusion (**Figure 1B**, **1C**).

DC-CIK combined with other antitumor programs

The enrolled patients received intravenous and splanchnic infusions of autologous DC-CIK cells at Beijing Shijitan Hospital. All participants received at least one cycle (**Figure 1D**) of infusion, and some continued to receive cycles until they experienced disease progression, unacceptable adverse effects, or withdrew consent. For patients who received multidisciplinary synthetic therapy, most received this therapy combined with chemotherapy, and fewer patients received synthetic therapy combined with targeted therapy, immune checkpoint inhibitors, and radiotherapy and were recruited into our trial according to physician recommendation. If patients achieved an objective response or stable disease after treatment, they were considered eligible to receive additional cycles of maintenance treatment. The scheme of DC-CIK as a basic treatment combined with other systemic antitumor (chemotherapy and immune checkpoint inhibitor) treatments is shown in **Figure 1F**, **1G**. The DC-CIK program combined with targeted therapy involves the daily application of targeted drugs (gefitinib 250 mg/day or oxitinib 80 mg/day) during DC-CIK treatment (**Figure 1E**).

Adverse events

Toxicity was assessed by the study investigators and data were sourced from electronic patient records at every treatment cycle. If the patient was treated with only one cycle, we continued to follow-up the patient for one month after the treatment cycle. Peripheral blood samples were collected for routine blood tests and to examine biochemical liver function, renal function, ions, and coagulation function on the days before and after the DC-CIK treatment cycle. AEs were toxicities with a potential immunological basis that were considered by investigators to be related to the study treatment. The severity (Common Terminology Criteria for Adverse Events version 4.0 [CTCAE v4.0]), management, and resolution of AEs of all grades (G) were reviewed. Treatment-related AEs were monitored during the treatment and observation periods, and the observed grade was recorded for each patient and cycle.

Statistical analysis

Two-tailed paired Student's test was carried out to compare liver and kidney function and blood routine among peripheral blood indicators before and after DC-CIK treatment. Binary logistic analysis was used to explore the adverse reactions caused by DC-CIK combined chemotherapy, as well as the adverse reactions in different treatment lines. The results are reported as hazard ratios (HRs) and 95% confidence intervals (CIs). An HR>1 indicated an elevated risk with respect to the reference category. Correlation analysis based on the Kendall coefficient calculated the number of different chemotherapy lines or quantitative dendritic cell/cytokine-induced killer cells correlated with adverse side effects. Correlation analysis of the application of the last chemotherapy line to the side effects of DC-CIK treatment based on the Kendall coefficient calcu-

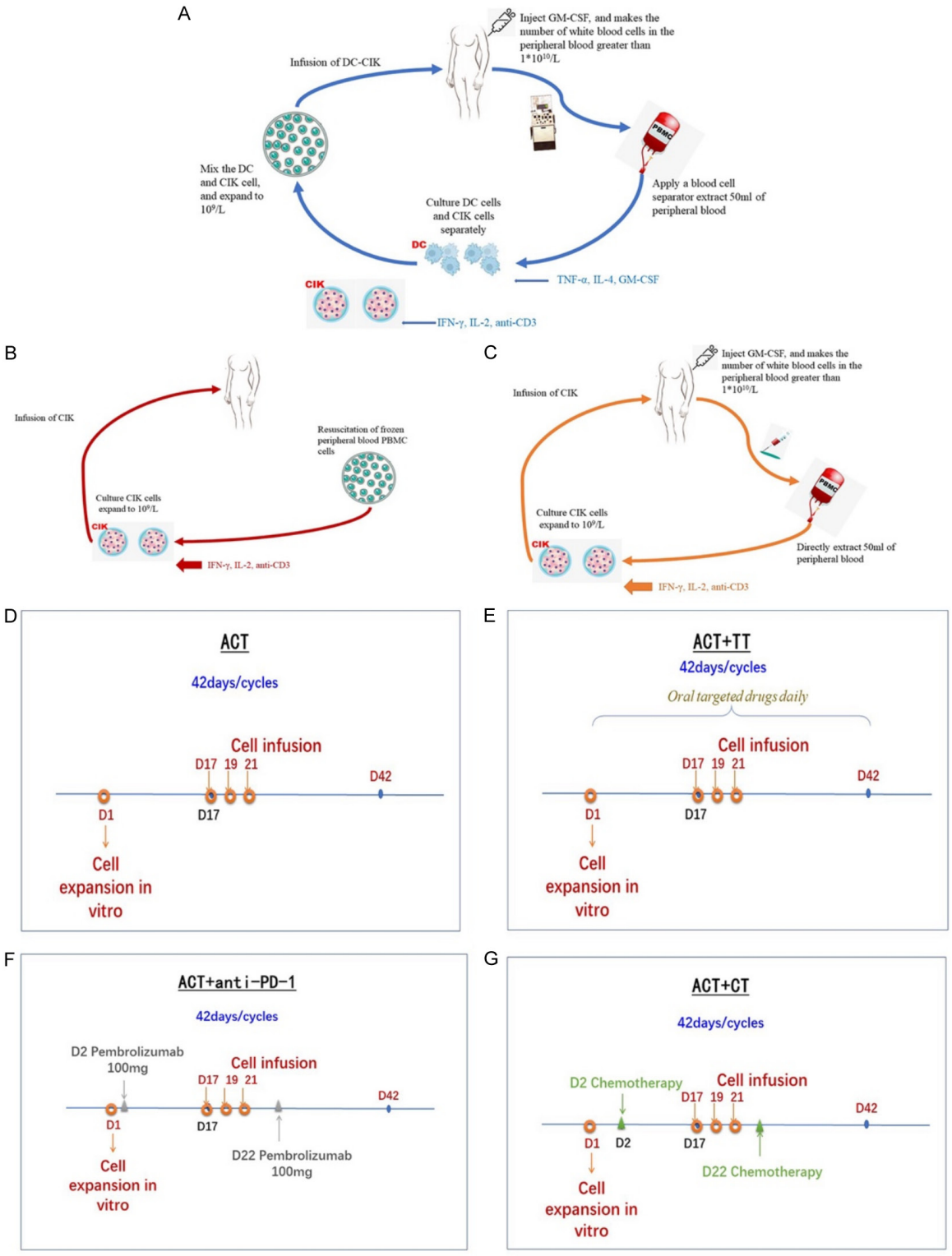

Figure 1. Different adoptive T cell transfer (ACT) approaches to harness the immune system to treat cancer. A: Extracting 50 ml peripheral blood by the COBE Spectra cell separator to collected PBMC for cultured DC-CIK cells to treat cancer. B: Extracting 50 ml peripheral blood directly for cell culture. C: Resuscitation of frozen PBMC collected by the COBE Spectra cell separator were only cultured with CIK cells for reinfusion. D-G: The scheme of DC-CIK as the basic treatment combined with other systemic anti-tumor (DC-CIK alone, combined chemotherapy, combined immune check point inhibiter and combined target therapy) treatment.

Table 1. Patient demographics

Characteristic	Number of patients, *n (%)*	Number of cycles
Age		
Median (range) in years	58 (16-96)	
Infusion ACT cell numbers		
DC median (range)	$6.2*10^7$ ($1.5*10^7$-$2.7*10^8$)/cycle	
CIK median (range)	$5.96*10^9$ ($3.1*10^7$-$30.2*10^9$)/cycle	
Infusion ACT cell type		
DC-CIK infusion	508 (46)	768
CIK infusion	591 (54)	1736
Treatment cycle for patient		
Median (range)	2 (1-30)	
One cycle	507 (46)	507
Multi-cycle	592 (54)	1997
Gender		
Male	539 (49)	1178
Female	560 (51)	1326
ECOG scoring		
0	577 (53)	1222
1	470 (43)	1095
2	52 (4)	187
Tumor type		
Head and neck	40 (4)	81
Lung	263 (23)	672
Urological	50 (5)	180
Gynecological	117 (10)	267
Gastrointestinal	172 (16)	273
Sarcoma	16 (1)	33
Colorectal	111 (10)	260
Breast	111 (10)	207
Nervous system	12 (1.5)	21
Hepatobiliary	110 (10)	237
Lymphoma	8 (1)	20
Pancreatic	84 (8)	243
Melanoma	5 (0.5)	10
Tumor stage		
I	46 (4)	114
II	140 (13)	225
III	209 (19)	844
IV	704 (64)	1321
Infusion mode		
Vein	1064 (97)	2416
Intra-cavity (pleural & ascites)	35 (3)	88
Exclusively DC-CIK therapy line		
First line	368 (33)	1102
Multi-line*	770 (70)	1402
Prior Chemotherapy (median lines)	*535 (2)*	*1309*
Prior Radiotherapy	*188*	*442*

DC-CIK Combination		
DC-CIK+ Chemotherapy	370 (34)	815
DC-CIK+ Target therapy#	20 (2)	46
DC-CIK+ Anti-PD1&	41 (4)	89
DC-CIK+ Radiotherapy	5 (0.4)	11

*: Other single- or multi-line treatments before DC-CIK refusion. #: 51 patients were treated with gefitinib, and 21 patients were treated with oxitinib. &: All patients were treated with paporizumab 100 mg *intravenous infusion* 21 d/cycle.

lated. All statistical evaluations were performed using SPSS software (Statistical Package for the Social Sciences, version 23.0, SPSS Inc.) and GraphPad Prism 5 (Version 7.00, GraphPad Software, Inc.). Statistical significance was set at $P<0.05$.

Result

Study population

In all, 1100 patients with 2504 treatment cycles were enrolled in our study to observe adverse side effects (**Table 1**). The average age of these patients was 58 years; this study included slightly more female than male patients (51% *vs.* 49%), with an ECOG performance status that was primarily 0 or 1. Half of the patients had undergone DC combined with CIK treatment (46%), while the remaining patients had received only CIK treatment (54%), and the median number of infused DC-CIK cells in each cycle was $5.96*10^9$. In this study, patients with various tumors undergoing DC-CIK-based single or combined treatment were enrolled; among them, most patients had lung cancers, including non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) (23%), followed by gastrointestinal tumors (16%), breast cancer (10%), and malignant hepatobiliary tumors (10%), while patients with melanoma (0.5%), sarcoma (1%) and nervous system neoplasms (1.5%) accounted for the lowest proportions. Moreover, these tumors were mainly stage IV (64%), and a small number of malignant tumors had invaded the pleura and peritoneum (3%) and were managed by thoracic and abdominal infusion treatment, while the other patients were administered an intravenous injection of DC-CIK cells. In this study, DC-CIK-dominated single or combined treatment was adopted; for combined treatment, most patients received chemotherapy (34%), while fewer received radiotherapy (0.4%). Most patients received DC-CIK multiline treatment (70%), either single DC-CIK treatment or other combined treatments; more patients received multicycle (≥2 cycles) DC-CIK treatment than single-cycle treatment (54% *vs.* 46%).

Occurrence of AEs

In all, we observed AEs in 870 cycles and 548 cases: 419 (76%) in G1, 97 (17.3%) in G2, 28 (7%) in G3, and 4 (0.7%) in G4. Most cases (n=271) had hematological side effects; 220 cases had anemia, while 51 had leukopenia, which accounted for 41% and 9% of all AEs, respectively. Moreover, patients with severe side effects (≥G3) primarily had anemia and leukopenia; among them, 4 patients had G4 anemia and received transfusion of suspended red blood cells, while G3 leukopenia was also treated with GM-CSF to elevate the peripheral blood leukocyte count. The second most common side effect was digestive tract reactions, which occurred in 82 cases and accounted for 15% of all cases with side effects. Among them, 52 had nausea, and 30 had emesis. The above mentioned side effects were mainly seen in the combined chemotherapy group; among them, 217 cases had hematological side effects (accounting for 80% of all patients with hematological side effects), 187 cases had anemia (85% of all patients with anemia), and 40 cases had leukopenia (78% of all patients with leukopenia). Among the patients with severe hematological side effects, 81% were in the combined chemotherapy group. In addition, other side effects, such as general disorders, occurred in 101 cases (most of which were in the combined chemotherapy group), which accounted for 18% of all cases, among which fatigue and fever accounted for 67% and 55%, respectively. Side effects related to musculoskeletal and immunopathies were rare and were only seen in two patients. Finally, with regard to skin side effects, three patients had rashes, including two who received combined targeted therapy and one who received combined anti-PD1 treatment. However, other immune-related adverse effects, such as colitis, pneumonitis, hypophysitis, cardiac disease, myositis, and adrenal disease, which occurred more readily with immune

Table 2. Summarized adverse effects related with therapeutic approaches

AE class*	AE type	Grade	Number of cases	Number of patients with multiple cycles treatment	Number of cycles of multiple cycles	Number of patients with TNM stage IV	Immuno-suppressant (number of cases/total)	Only DC-CIK treatment (cases)	Combined with other treatment (cases)		
									Chemotherapy	Target therapy	Anti-PD1 therapy (Keytruda)
Neurological	Anxiety	G1	18	10	26	8	-	7/18	8/18	2/18	1/18
		G2	1			1	-	1/1	-	-	-
Musculoskeletal	Myalgia	G1	1	1	27	0	-	-	1/1	1/1	1/1
		G2	1			1	-	1/1	-	-	-
	Myositis	-	0	0	0	0	-	-	-	-	-
Immunopathies	Allergic reaction	G1	1	1	4	1	-	-	1/1	1/1	-
		G2	1			0	Claritin 1/1	-	-	-	1/1
Hematological	Leukocytosis	G1	23	17	58	16	-	4/23	1723	-	2/23
		G2	21	10	30	13	-	4/21	16/21	1/21	-
		G3	7	2	5	4	-	-	7/7	-	-
	Anemia	G1	132	59	150	89	-	6/132	121/132	-	5/132
		G2	64	24	48	42	-	8/64	52/64	-	4/64
		G3	20	9	18	12	-	6/20	14/20	-	-
		G4	4			3	-	-	4/4	-	-
General disorders and administration site conditions	Fatigue	G1	51	21	108	35	-	12/51	34/51	2/51	3/51
		G2	1			0	-	1/1	-	-	-
	Fever	G1	41	5	38	31	-	18/41	20/41	-	3/41
		G2	8	2	5	6	Corticosteroid 2/8	1/8	7/8	-	-
Gastrointestinal	Nausea	G1	52	41	135	41	-	14/52	32/52	2/52	4/52
	Emesis	G1	30	22	44	21	Corticosteroid 19/30	9/30	17/30	1/30	3/30
	Diarrhea	G1	3	2	10	2	Corticosteroid 1/3	-	2/3	-	1/3
	Colitis	-	0	0	0		-	-	-	-	-
Skin	Rash	G1	2	2	14	1	Corticosteroid 2/2	-	-	2/2	-
		G3	1	-	-	1	-	-	-	-	1/1
Metabolism and nutrition disorders	Anorexia	G1	65	45	99	29	Corticosteroid 12/65	22/65	30/65	6/65	7/65
Respiratory system	Pneumonitis	-	0	0	0	0	-	-	-	-	-
Endocrine	Hypophysitis	-	0	0	0	0	-	-	-	-	-
	Adrenal	-	0	0	0	0	-	-	-	-	-
	Diabetes	-	0	0	0	0	-	-	-	-	-
Kidney and urinary diseases	Nephritis	-	0	0	0	0	-	-	-	-	-
Cardiac	Myocarditis	-	0	0	0	0	-	-	-	-	-

*: AE: adverse effects.

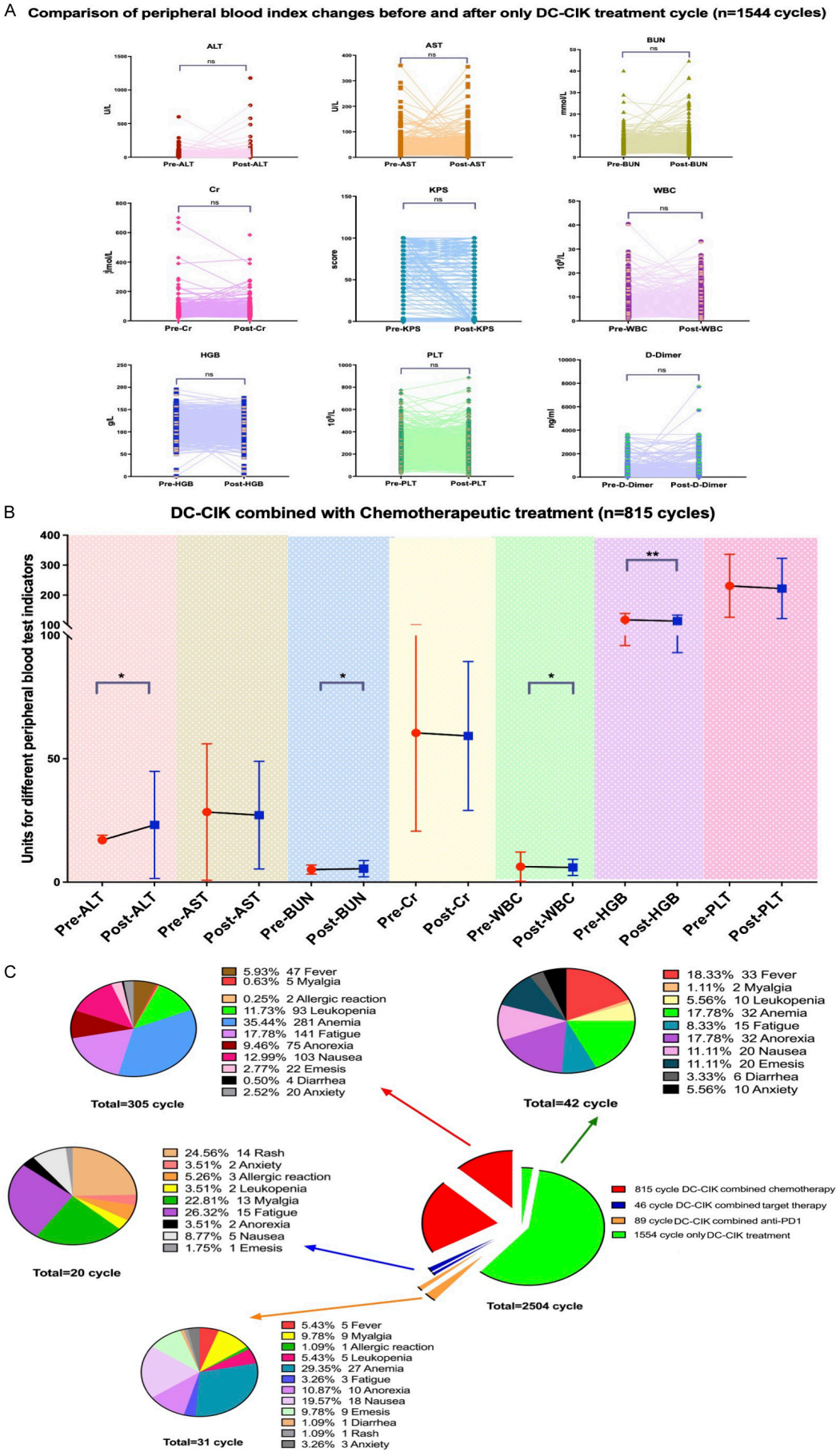
A
Comparison of peripheral blood index changes before and after only DC-CIK treatment cycle (n=1544 cycles)
ALT
AST
BUN
Cr
KPS
WBC
HGB
PLT
D-Dimer
ns
U/L
mmol/L
μmol/L
score
10⁹/L
g/L
ng/ml
Pre-ALT
Post-ALT
Pre-AST
Post-AST
Pre-BUN
Post-BUN
Pre-Cr
Post-Cr
Pre-KPS
Post-KPS
Pre-WBC
Post-WBC
Pre-HGB
Post-HGB
Pre-PLT
Post-PLT
Pre-D-Dimer
Post-D-Dimer
B
DC-CIK combined with Chemotherapeutic treatment (n=815 cycles)
Units for different peripheral blood test indicators
400
300
200
100
100
50
0
*
**
C
5.93% 47 Fever
0.63% 5 Myalgia
0.25% 2 Allergic reaction
11.73% 93 Leukopenia
35.44% 281 Anemia
17.78% 141 Fatigue
9.46% 75 Anorexia
12.99% 103 Nausea
2.77% 22 Emesis
0.50% 4 Diarrhea
2.52% 20 Anxiety
Total=305 cycle
18.33% 33 Fever
1.11% 2 Myalgia
5.56% 10 Leukopenia
17.78% 32 Anemia
8.33% 15 Fatigue
17.78% 32 Anorexia
11.11% 20 Nausea
11.11% 20 Emesis
3.33% 6 Diarrhea
5.56% 10 Anxiety
Total=42 cycle
24.56% 14 Rash
3.51% 2 Anxiety
5.26% 3 Allergic reaction
3.51% 2 Leukopenia
22.81% 13 Myalgia
26.32% 15 Fatigue
3.51% 2 Anorexia
8.77% 5 Nausea
1.75% 1 Emesis
Total=20 cycle
815 cycle DC-CIK combined chemotherapy
46 cycle DC-CIK combined target therapy
89 cycle DC-CIK combined anti-PD1
1554 cycle only DC-CIK treatment
Total=2504 cycle
5.43% 5 Fever
9.78% 9 Myalgia
1.09% 1 Allergic reaction
5.43% 5 Leukopenia
29.35% 27 Anemia
3.26% 3 Fatigue
10.87% 10 Anorexia
19.57% 18 Nausea
9.78% 9 Emesis
1.09% 1 Diarrhea
1.09% 1 Rash
3.26% 3 Anxiety
Total=31 cycle

Figure 2. Comparison of changes in every peripheral blood values before and after DC-CIK only treatment and DC-CIK combined with chemotherapeutic treatment. A: After DC-CIK treatment alone, no obvious changes were observed in blood routine, liver-kidney functions and coagulation indexes compared with those before treatment. B: After DC-CIK and chemotherapy combined therapy, the BUN and ALT indexes were evidently elevated compared with those before treatment (ALT: P=0.03, BUN: P=0.042), while white blood cell and hemoglobin were remarkably reduced after treatment (WBC: P=0.047, HGB: P=0.0015). C: The adverse side effects occurrence of DC-CIK alone or combined with other anti-tumor treatments.

checkpoint inhibitor treatment, were not observed in patients who received DC-CIK-based antitumor therapy (**Table 2**).

Comparisons of changes in peripheral blood examination indices before and after each DC-CIK treatment cycle

Peripheral blood was extracted for routine blood tests, liver-kidney function tests, and coagulation indices, and changes before and after the treatment cycles were compared to observe the changes in the above indices after DC-CIK treatment. First, patients who received DC-CIK treatment alone were summarized, and they received 1544 cycles, as shown in **Figure 2A**. After DC-CIK treatment alone, no obvious changes were observed in white blood cells, hemoglobin, or platelets compared with before treatment, according to routine blood tests. In terms of biochemical parameters, differences in ALT and AST, which reflect liver function, as well as differences in BUN and CR, which reflect kidney function, were not statistically significant before and after DC-CIK treatment alone. Finally, with regard to coagulation function, D-dimer also showed no obvious change before and after DC-CIK treatment alone. In terms of general patient conditions, the Karnofsky Performance Status (KPS) score in patients who received DC-CIK treatment alone showed no obvious change before and after treatment. Subsequently, we analyzed the changes in peripheral blood indices before and after DC-CIK combined with chemotherapy. As presented in **Figure 2B**, the BUN and ALT indices after treatment were significantly elevated compared with those before treatment (ALT: P=0.03, BUN: P=0.042), while white blood cell and hemoglobin levels were markedly reduced after treatment (WBC: P=0.047, HGB: P=0.0015). With regard to the peripheral blood indexes in DC-CIK combined targeted therapy and DC-CIK combined with antiPD1 groups, the ALT, AST, BUN, CR, WBC, HGB and PLT before treatment showed no obvious differences compared with those after treatment.

Effect of DC-CIK cell treatment combined with chemotherapy on adverse side effects

In all, of the 2504 DC-CIK treatment cycles that were calculated, including 815 for DC-CIK treatment combined with chemotherapy, side effects were observed in 305 (37%) cycles, with hematological side effects, including leukopenia (n=93 cycles) and anemia (n=281 cycles), as the most obvious. Our results found that DC-CIK combined with chemotherapy had the most side effects. In addition, chemotherapy-induced gastrointestinal tract reactions also accounted for a large proportion, among which nausea was observed in 103 cycles. Forty-six cycles of DC-CIK combined with targeted therapy were administered, and of these, side effects occurred in 20 cycles (43%) and consisted predominantly of skin rash (n=14 cycles). Finally, of 89 cycles of DC-CIK combined with anti-PD1 treatment, side effects were observed in 31 cycles (34%). Of the 1554 cycles of DC-CIK treatment alone, side effects were observed in 42 cycles (3%) and were primarily hematological and digestive system side effects (**Figure 2C**).

Subsequently, we applied binary logistic regression analysis to observe the correlation of different DC-CIK combined treatments with each side effect. The results revealed that vomiting, anorexia, anemia, and leukopenia were mainly correlated with DC-CIK therapy combined with chemotherapy (**Figure 3A**), among which anemia and leukopenia were the most obvious (P<0.0001). Side effects including rash, fatigue, allergy, myodynia and insomnia had relationship with DC-CIK combined targeted therapy, which were dominated by rash and myodynia. Meanwhile, DC-CIK combined with antiPD1 treatment had no influence on each side effect.

Effects of DC-CIK antitumor therapy at various cycles, various lines and various infused cell numbers on the incidence of side effects

We analyzed the side effects of different DC-CIK treatment cycles. First, findings in patients who received one cycle of DC-CIK

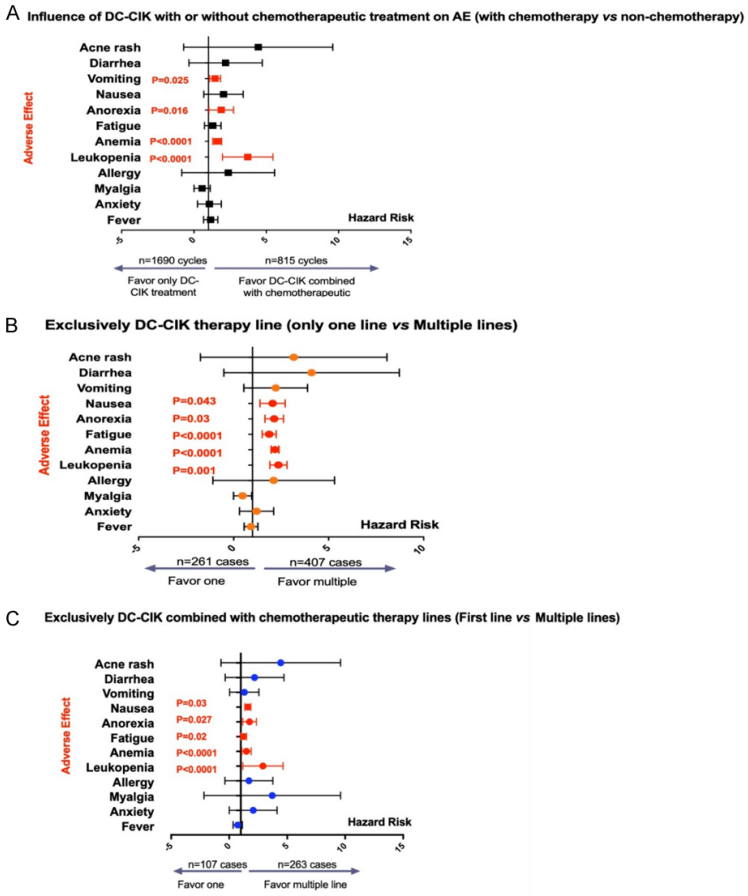

Figure 3. Effects of DC-CIK based anti-tumor therapy at various lines on the incidence of side effects. A: The correlation of adverse side effects with DC-CIK combined chemotherapy treatment. B: The correlation of adverse side effects with various DC-CIK only treatment lines. C: The correlation of adverse side effects with various DC-CIK combined chemotherapeutic treatment lines.

(n=507) were summarized, and we found that 592 patients had received multicycle treatment and that a total of 1997 cycles had been administered, with an average of two cycles. We applied a correlation analysis to understand the correlation between the number of DC-CIK treatment cycles and the occurrence of side effects. As shown in **Table 3**, with an increase in DC-CIK only treatment cycles, fatigue (P= 0.001), anorexia (P<0.0001), and anxiety (P= 0.01) symptoms were significantly reduced. However, the number of patients with anorexia (P=0.023), anxiety (P<0.0001), leukocytosis (P=0.01), anemia (P=0.00023), and nausea (P=0.0079) after multiple cycles of DC-CIK therapy combined with chemotherapy increased significantly.

Next, we analyzed whether various lines of antitumor therapy before DC-CIK-based treatment were associated with side effects in the first cycle. The results suggest that patients who received multiline DC-CIK-based treatment were more prone to nausea, anorexia, fatigue, anemia, and leukopenia than those who received first-line DC-CIK-based therapy, regardless of whether DC-CIK was combined with chemotherapy (**Figure 3B**, **3C**). Among these multiline DC-CIK-based treatment cases, 535 patients received prior chemotherapy treatment. Subsequently, we performed a correlation analysis between the increasing number of chemotherapy lines before DC-CIK treatment and the side effects, which suggested that treatment with increasing chemotherapy lines before DC-CIK more readily led to some adverse side effects, such as nausea, emesis, anorexia, leukocytosis, anemia, and fatigue (**Table 4**).

Each patient was infused with various numbers of cells, and some patients were infused with CIK cells alone, whereas others were infused with DC-CIK cells. To verify whether different infused cell numbers would affect the incidence of side effects, a correlation analysis was performed to analyze the number of infused CIK cells and each side effect. The results revealed no statistical relationship, and the number of infused DCs showed no statistical relationship with any side effect (**Table 5**).

Discussion

Over the past few decades, many innovations have been made in the development of anticancer drugs, especially targeted therapies, surgical techniques, chemotherapy, and radiation, which have demonstrated significant progress and improvements in the overall treatment landscape of cancer. However, despite these significant advances, most patients may relapse and experience serious side effects caused by chemotherapy, targeted therapy,

Table 3. Correlation analysis between the number of infusion cycles and the occurrence of adverse side effects

	DC-CIK only	DC-CIK combined with Chemotherapy
Fatigue	R=-0.074, P=0.001	R=0.043, P=0.062
Anorexia	R=-0.094, P<0.0001	R=0.059, P=0.023
Anxiety	R=-0.058, P=0.01	R=0.146, P<0.0001
Myalgia	R=0.003, P=0.22	R=-0.075, P=0.126
Allergic reaction	R=0.009, P=0.144	R=0.001, P=0.32
Leukocytosis	R=-0.013, P=0.092	R=0.054, P=0.01
Anemia	R=-0.029, P=0.094	R=0.072, P=0.00023
Fever	R=0.012, P=0.113	R=0.012, P=0.571
Nausea	R=-0.013, P=0.092	R=0.081, P=0.0079
Vomiting	R=-0.002, P=0.074	R=0.043, P=0.057
Diarrhea	R=-0.0019, P=0.24	R=-0.002, P=0.138
Rash	R=-0.049, P=0.139	R=0.036, P=0.274

R: Kendall coefficient; positive numbers indicate positive correlation and negative numbers indicate negative correlation.

Table 4. Discrimination correlation analysis of previous chemotherapeutic lines with DC-CIK treatment

AE type	*Prior chemotherapy lines*	
	Kendall coefficient	*P* value
Anxiety	R=0.031	P=0.208
Myalgia	R=0.043	P=0.078
Allergic reaction	R=0.031	P=0.208
Leukocytosis	R=0.089	P<0.0001
Anemia	R=0.072	P=0.003
Fatigue	R=0.074	P=0.003
Fever	R=0.034	P=0.087
Nausea	R=0.085	P=0.001
Emesis	R=0.052	P=0.034
Diarrhea	R=0.027	P=0.176
Rash	R=0.042	P=0.09
Anorexia	R=0.064	P=0.008

AE: adverse effects.

and even immunotherapy [27-31]. Indeed, various fatal side effects often occur during cancer treatment, and many issues associated with antitumor treatment have been identified [32].

Immunotherapy has made significant progress in this field of innovation. In recent years, it has become an increasingly important part of cancer treatment in addition to standard therapy. The method of cellular immunotherapy is based on 2 different principles [33]. On the one hand, the body's own immune system can be active and specific to stimulate immune cells by confrontation with autologous or allogeneic tumor antigens *in situ*, such as in anti-PD1 therapy. On the other hand, the specific affinity of autologous or allogeneic immune cells to tumor-associated antigens can be activated *in vitro* and subsequently directly applied to humans as cellular immunotherapy, such as TIL and DC-CIK therapies. Based on the above mentioned reasons, we investigated whether the infusion of DC-CIK cells would induce side effects. Our results suggest that DC-CIK therapy does not induce any side effects, regardless of the infused cell number or the DC-CIK ratio. Therefore, the use of autologous tumor antigen-specific cellular immunotherapy is particularly interesting because it promises effectiveness, a low rate of side effects, and continuous treatment options based on the use of the patient's own cells. Indeed, DC-CIK cells are currently emerging as an effective treatment option, especially when combined with standard adjuvant therapy [4-7, 34].

This study focused on the safety of DC-CIK therapy. First, we performed routine peripheral blood tests and determined liver-kidney function and coagulation function indices before and after DC-CIK treatment, and our results revealed that a single application of DC-CIK treatment would not induce any changes to the above mentioned indices. However, when combined with chemotherapy, the white blood cell and hemoglobin levels declined after completion of the treatment cycle, while the liver function index ALT and the kidney function index

Table 5. Influence of infused cell number of DC-CIK associated with adverse side occurrence

AE type*	*The numbers of transfused CIK cells*		*The numbers of transfused DC cells*	
	Kendall coefficient	*P* value	Kendall coefficient	*P* value
Anxiety	R=-0.003	P=0.899	R=-0.001	P=0.921
Myalgia	R=0.006	P=0.804	R=0.002	P=0.872
Allergic reaction	R=0.001	P=0.978	R=0.001	P=0.989
Leukocytosis	R=-0.006	P=0.809	R=-0.002	P=0.892
Anemia	R=-0.003	P=0.895	R=-0.001	P=0.931
Fatigue	R=-0.003	P=0.908	R=-0.001	P=0.956
Fever	R=-0.002	P=0.926	R=-0.002	P=0.932
Nausea	R=0.004	P=0.871	R=0.002	P=0.911
Emesis	R=0.002	P=0.918	R=0.001	P=0.934
Diarrhea	R=-0.001	P=0.982	R=-0.001	P=0.976
Rash	R=0.005	P=0.892	R=0.003	P=0.897
Anorexia	R=0.001	P=0.965	R=0.001	P=0.976

*: AE: adverse effect.

BUN were elevated after treatment. Changes in routine blood tests were also the most common side effects after chemotherapy [35]. Combined chemotherapy might cause adverse effects on liver and kidney function due to the toxicity and side effects of chemotherapy, which result in elevated ALT and BUN [36-39]. However, the peripheral blood detection indices after DC-CIK combined with targeted therapy and anti-PD1 treatment did not markedly change (Figure S1A, S1B). Based on the above results, DC-CIK treatment alone had no significant influence on the peripheral blood indices or on liver and kidney function.

Next, we observed the effects of different cycles and different lines of DC-CIK therapy on side effects, and interestingly, we found that multicycle DC-CIK treatment could not only improve the patient's fatigue, anorexia, and anxiety symptoms but did not increase the frequency of other side effects. We will continue to investigate these molecular mechanisms in the future. However, when used as a multiline treatment, the first cycle of DC-CIK-based treatment remarkably increased the toxicity and side effects at the gastrointestinal and hematological levels compared with first-line treatment. It is well known that most patients underwent ≥ first-line chemotherapy before they received multiline DC-CIK-based treatment, and the accumulated side effects and toxicity might result in corresponding complications after the first cycle of DC-CIK treatment. To verify the above mentioned hypothesis, we analyzed the correlations between the chemotherapy line before DC-CIK treatment and the adverse effects after DC-CIK treatment, and the results verified our speculation; in other words, the side effects that occurred in multiline DC-CIK treatment are primarily attributed to chemotherapy received before treatment.

To further verify the safety of DC-CIK treatment, we subsequently calculated the side effects induced by DC-CIK treatment alone and those induced by DC-CIK combined with different antitumor treatments, which suggested that the side effects induced by DC-CIK treatment alone accounted for the lowest proportion (only 3%), while those induced by combined chemotherapy, targeted therapy, and anti-PD1 treatment accounted for a higher proportion. To investigate whether these side effects were induced by a combination with other treatments, a regression analysis was performed on DC-CIK and different antitumor treatments. The results suggested that the combination of chemotherapy increased side effects, such as vomiting, anorexia, anemia, and leukopenia [40], while the combination of targeted therapy would induce additional side effects such as rash, fatigue, allergy, myalgia, and anxiety [41] (Figure S2A); the combination of anti-PD1 treatment did not markedly increase any side effects (Figure S2B). Recent research indicates that anti-PD1 can induce immune-related side effects [19]. However, this phenomenon was not observed here, which might be because of the lower dose of anti-PD1 used in this study,

which was insufficient to induce immune-related side effects compared with the standard dose. This finding also demonstrated that DC-CIK therapy combined with anti-PD1 treatment did not increase immune-related toxicity or side effects. Taken together, we discovered that most side effects were derived from the combination of DC-CIK therapy and other treatments.

Other studies have explored the association between DC-CIK cell therapy and side effects. A systematic review and meta-analysis published by Fenix KA indicated that compared with standard therapy, patients who received additional CIK cell therapy had favorable outcomes without increased toxicity, warranting further investigation into CIK therapy for the treatment of CRC [42]. Chang-Long Chen et al. found that grade 3 or 4 toxicities that were reversible or controllable were observed in two patients (assessed in 31 patients) with advanced solid tumors after DC-CIK treatment [15]. A meta-analysis reported by Shuai Wang et al. indicated that no more serious adverse events appeared in NSCLC patients who received DC-CIK cell immunotherapy than in those who received control therapies [43]. In their research on gastric cancer, Ying Mu et al. found that no serious side effects appeared in patients after the application of cellular immunotherapy based on CB-DC-CIK cells (cord blood-derived dendritic cells plus cytokine-induced killer cells) [44]. According to the results of the above studies, DC-CIK is relatively safe and does not cause significant side effects. This is consistent with our data and results. However, the above articles mainly focus on a single cancer type or include a small amount of data. Our article combines the patient data collected by our department over the years and focuses on the safety and side effects of DC-CIK therapy.

However, some side effects, such as diarrhea and fever, were not correlated with other treatments. In our analysis, the group that received DC-CIK cell treatment alone experienced only a few side effects, but to guarantee the safety of DC-CIK treatment, we analyzed 42 cycles after which side effects were observed. Our results revealed that most patients with side effects had received chemotherapy or radiotherapy before DC-CIK treatment (Table S2) and that the side effects were predominantly chemotherapy-induced bone marrow toxicity and digestive tract toxicity.

Our study has some limitations. The number of patients who received immunotherapy is still small because immunotherapy was previously not very common in China. However, we will continue to collect these data, which may be published in the future.

In summary, we discovered that DC-CIK antitumor treatment is safe and is not associated with autoimmune disease, similarly to anti-PD1 treatment; DC-CIK is also not associated with toxicity and side effects caused by treatments that attack other organs. DC-CIK is an antitumor immunotherapy with remarkable therapeutic effects and a good safety profile.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hongyan Huang, Department of Medical Oncology, Beijing Key Laboratory for Therapeutic Cancer Vaccines, Capital Medical University Cancer Center, Beijing Shijitan Hospital, Capital Medical University, No. 10 Tieyi Rd, Beijing 100038, China. Tel: +86-010-63926116; E-mail: huangh1975@mail.ccmu.edu.cn

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Table S1. Clinical trial study of DC-CIK combined chemotherapy in various solid cancer

Tumor type	Literature	Survival analysis		Group		*P* value
				Chemotherapy	DC-CIK combined chemotherapy	
Lung cancer	Zhao, et al. [5]#	1-year rate	PFS	29.40%	47.60%	<0.001
			OS	58.20%	71.80%	0.028
Gastric cancer	[7]#	Days	PFS	92	136	<0.0001
			OS	141	212	<0.0001
Pancreatic cancer	[6]#	Disease control rates		33.30%	76.90%	0.001
Colorectal cancer	[45]	5-year rate	PFS	57.40%	41.30%	0.022
			OS	33.60%	19.40%	0.001
Breast cancer	[46]#	Month	PFS	3.7	10.2	<0.001
			OS	15.2	33.1	<0.001
Myeloma	[47]	Overall response rate		50%	70%	<0.05
Cervical cancer	[48]	3-year survival rates	OS	56.41%	80.00%	<0.05
Nasopharyngeal Carcinoma	[49]	Month	PFS	15	21	0.009
			OS	23	32	0.006

#: Data from the authors' group.

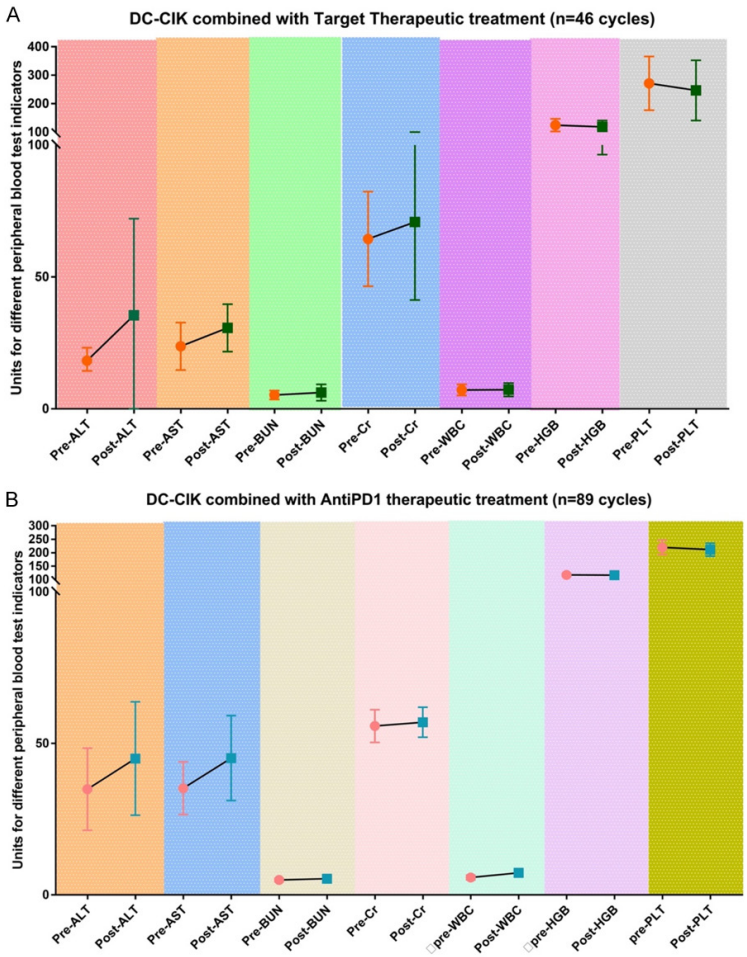

Figure S1. Comparison of changes in peripheral blood values before and after DC-CIK-based treatment. A: After DC-CIK combined target therapy, no obvious changes were observed in routine blood tests, liver kidney function, and coagulation indexes compared with those before treatment. B: After DC-CIK combined with anti-PD1, no obvious changes were observed in routine blood tests, liver kidney function, and coagulation indexes compared with those before treatment.

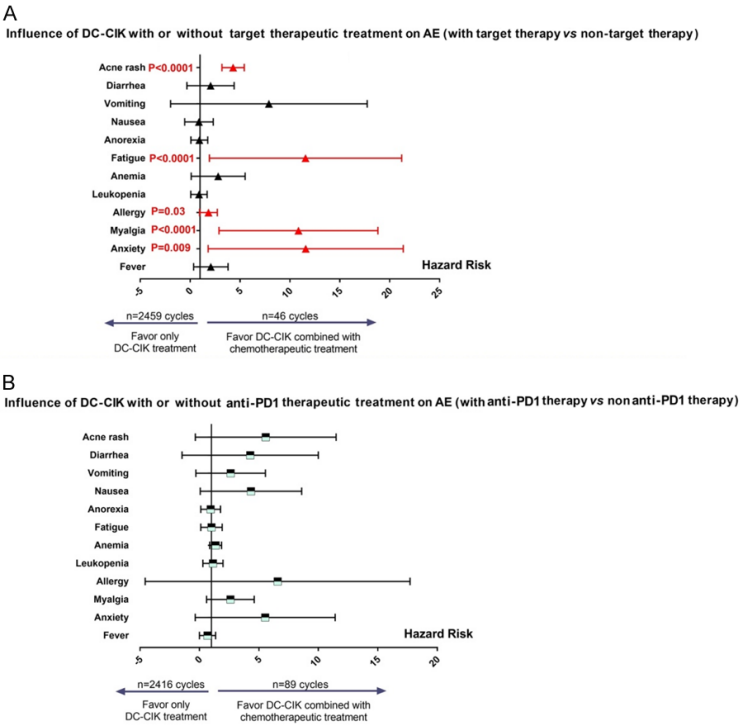

Figure S2. Effect of DC-CIK treatment combined with other anti-tumor treatments on adverse side effects. A: Correlation between adverse side effects and DC-CIK combined target therapy. B: Correlation between adverse side effects and DC-CIK combined with anti-PD1 treatment.

Table S2. Causal analysis of symptomatic patients of DC/CIK alone with previous therapeutic experiences

	Treatment cycle
Prior Chemotherapy	39/42
One line	12
Multi-lines	27
Prior Radiotherapy	13/42
Infection during treatment	31/42