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ORIGINAL RESEARCH

Cytokine-induced killer cells/dendritic cells and cytokine-induced killer cells immunotherapy for the treatment of esophageal cancer in China: a meta-analysis

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Background: Immunotherapy based on cytokine-induced killer cells or combination of dendritic cells and cytokine-induced killer cells (CIK/DC-CIK) showed promising clinical outcomes for treating esophageal cancer (EC). However, the clinical benefit varies among previous studies. Therefore, it is necessary to systematically evaluate the curative efficacy and safety of CIK/DC-CIK immunotherapy as an adjuvant therapy for conventional therapeutic strategies in the treatment of EC.

Materials and methods: Clinical trials published before October 2016 and reporting CIK/DC-CIK immunotherapy treatment responses or safety for EC were searched in Cochrane Library, EMBASE, PubMed, Wanfang and China National Knowledge Internet databases. Research quality and heterogeneity were evaluated before analysis, and pooled analyses were performed using random- or fixed-effect models.

Results: This research covered 11 trials including 994 EC patients. Results of this meta-analysis indicated that compared with conventional therapy, the combination of conventional therapy with CIK/DC-CIK immunotherapy significantly prolonged the 1-year overall survival (OS) rate, overall response rate (ORR) and disease control rate (DCR) (1-year OS: P=0.0005; ORR and DCR: $P<0.00001$). Patients with combination therapy also showed significantly improved quality of life (QoL) (P=0.02). After CIK/DC-CIK immunotherapy, lymphocyte percentages of $CD3^+$ and $CD3^-CD56^+$ subsets ($P<0.01$) and cytokines levels of IFN-γ, -2, TNF-α and IL-12 ($P<0.00001$) were significantly increased, and the percentage of cluster of differentiation $(CD)4^+CD25^+CD127^-$ subset was significantly decreased, whereas analysis of $CD4^+$, $CD8^+$, $CD4^+/CD8^+$ and $CD3^+CD56^+$ did not show significant difference ($P>0.05$).

Conclusion: The combination of CIK/DC-CIK immunotherapy and conventional therapy is safe and markedly prolongs survival time, enhances immune function and improves the treatment efficacy for EC.

Keywords: cytokine-induced killer cells, dendritic cells, esophageal cancer, immunotherapy, meta-analysis

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Introduction

Esophageal cancer (EC) is a global common cancer, with 450,000 new cases and 400,000 estimated deaths per year.[1,2] The incidence of EC has increased exponentially over the past few decades and the 5-year survival rate remains bleak.[3] At present, surgery, radiotherapy and chemotherapy are most widely used for EC.[4] However, their

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application is limited by the failure to thoroughly eliminate tumor cells, drug resistance and other adverse effects.[5,6] Therefore, a more effective and safer therapeutic method is urgently required.

In recent years, immunotherapy has been rising rapidly and is considered the fourth powerful therapeutic method after surgery, radiotherapy and chemotherapy.[6] Cancer immunotherapy is accomplished in multiple ways, including manipulation of the immune system through the use of immune agents, such as vaccines,[7] cytokines,[8] checkpoint inhibitors (including anti-programmed death 1 [PD-1]/PD-ligand 1 [PD-L1] antibodies and anti-cytotoxic T-lymphocyte-associated antigen (CTLA)-4 antibodies),[9,10] kinase inhibitors (such as apatinib and gefitinib)[11,12] and immune cells.[13–19] However, their applications have the following hurdles. Simply activating the immunity via vaccination is not able to thoroughly eliminate tumor cells because cancer patients are usually in immunosuppression.[19] Promotion of molecule-targeted treatment for tumors is also confined only to cancer patients bearing specific antigen-expressing cells.[13] Notably, adoptive cellular immunotherapy has been flourishing in cancer treatment. Its effectiveness relies on the application of dendritic cells (DCs),[14] tumor-infiltrating lymphocytes (TILs),[15] natural killer (NK) cells,[16] cytotoxic T lymphocytes (CTLs),[17] cytokine-induced killer (CIK) cells[18] and other immune cells. CIK cells, which consist primarily of the $CD3^+CD56^+$ subset, are induced by interferon (IFN)-γ, interleukin (IL)-1, cluster of differentiation (CD)3 monoclonal antibodies (OKT3) and IL-2 in vitro.[5] Compared with other immune cells, CIK cells are easy to obtain from peripheral blood and umbilical cord blood mononuclear cells, and they possess higher in vitro proliferation capacity, stronger antitumor activity and broader antitumor spectrum.[6] The tumoricidal ability of CIK cells is implemented by inducing tumor cell apoptosis through direct contact and secretion of cytokines such as IL-2, tumor necrosis factor (TNF)-α and IFN-γ.[20] CIK cells have shown promising prospects in immunotherapy for cancers. On the one hand, the cytotoxicity of CIK cells is not affected by immune inhibitors such as cyclosporin A (CsA) and FK506.[21] On the other hand, CIK cell-mediated cytotoxicity does not rely on the major histocompatibility complex (MHC). As in most cancers, these cells do not express MHC or human leukocyte antigen (HLA); this property of CIK cells is a great advantage over other immune cells in adoptive cell therapy.[22]

DCs are the most potent antigen-presenting cells and are essential in CIK activation, proliferation, phenotype expression and cytokine secretion.[5,23,24] The cytotoxicity of CIK cells is remarkably enhanced when cocultured with DCs, indicated by the increased proportion of $CD3^+CD56^+$ cells and the improved levels of cytokines such as IL-2, IFN-γ, IL-12 and TNF-α.[6,23] Meanwhile, cocultured DCs also downregulate the expression of negative regulatory factors, including transforming growth factor (TGF)-β and IL-10, as well as the proportion of $CD4^+CD25^+$ regulatory T cells (Tregs) among CIK cells, which suppress the antitumor activity of CIK.[5,24] Several research reports have shown that the combination of DCs and CIKs (DC-CIK) is more effective and has indicated more promising clinical prospects than single CIK treatment.[6]

In EC treatment, there are emerging data indicating CIK or DC-CIK (CIK/DC-CIK) immunotherapy in combination with conventional therapy exhibited better therapeutic efficacy than conventional therapy alone.[25–37] However, CIK/DC-CIK immunotherapy clinical application is still in its infancy. In this research, we conducted a meta-analysis to investigate the efficacy and safety of CIK/DC-CIK combined with conventional therapy in comparison with conventional therapy alone for EC, in order to provide scientific evidence for future clinical trials.

Materials and methods

Search strategy and selection criteria

Literature reports were searched across Cochrane Library, EMBASE, PubMed, Wanfang and China National Knowledge Internet databases with the key terms "dendritic cells", "immunotherapy", "cytokine-induced killer cells" or "DC-CIK" combined with "esophageal cancer". No language limits were applied. The initial search was performed in April 2016 and updated in October 2016.

Studies selected in our research were randomized controlled clinical trials for EC. The included studies were all performed with comparison between the combination of CIK/DC-CIK and conventional treatment (defined as combination therapy group) and conventional regimens alone (defined as conventional therapy-alone group).

Data collection and quality assessment

Two authors independently searched and collected literatures from the databases according to our inclusion criteria, and they extracted the data from all the selected articles. Discrepancy was resolved by discussion with a third author. The collected information included the first authors' names, the years of publication, the numbers of subjects, patient ages, tumor stages, experiment regimens, in vitro cell culture conditions and dosages of the utilized immune cells.

The quality of the included articles was evaluated according to the *Cochrane Handbook*.[38]

Definition of outcome measurements

Treatment efficacy was assessed in terms of overall survival (OS), overall response rate (ORR; ORR = complete response rate + partial response rate), disease control rate (DCR; DCR = complete response rate + partial response rate + stable disease rate), patients' quality of life (QoL) and adverse events. OS was defined as the length of time from the initiation of treatment to death from any cause.[39] The immune function of EC patients before and after treatment was determined by the status of the lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD3^-CD56^+$, $CD3^+CD56^+$ and $CD4^+CD25^+CD127^-$) and cytokine secretion (IFN-γ, IL-2, TNF-α and IL-12).

Statistical analysis

Data were analyzed using Review Manager version 5.2 provided by Cochrane Collaboration. $P<0.05$ was considered statistically significant. Heterogeneity among the studies was assessed to determine the most suitable model.[40] A random-effects method was applied when heterogeneity existed; otherwise, a fixed-effects method was used. Cochran's Q-test was performed in order to evaluate homogeneity among studies, and $I^2<50\%$ or $P>0.1$ was considered homogeneous. Odds ratios (ORs) were the principal measurements for therapeutic effects and were presented with 95% confidence intervals (CIs).

Results

Search results

A total of 1,405 articles were identified by initial retrieval. After title and abstract review, 1,381 articles were excluded because they did not focus on clinical trials (n=1,261), were in duplication and repetition (n=107) or were unrelated studies (n=13), and 24 studies remained as potentially relevant. After reading the full texts, 8 papers with insufficient data and 5 reviews or meta-analyses were excluded. Finally, 11 trials that included 994 EC patients met the inclusion criteria for our meta-analysis (Figure 1).

Patient characteristics

In all, 11 eligible trials including 994 EC patients were included in this analysis. All trials were conducted in mainland China. In total, 501 patients were treated by CIK/DC-CIK in combination with conventional therapy (combination therapy), while 493 patients were treated by conventional therapy alone. Detailed clinical information of the trials is presented in Table 1. DC and CIK cells used in the 11 trials were all obtained from autologous peripheral blood. In 4 trials, DC-CIK immunotherapy was applied, whereas in the other 7 trials, only CIK cells were used. In most studies, patients were transfused with $>1\times10^9$ immune cells, and other studies did not provide accurate cell numbers. Tumor size and injection modes were not analyzed in this article due to the lack of sufficient data in the included studies.

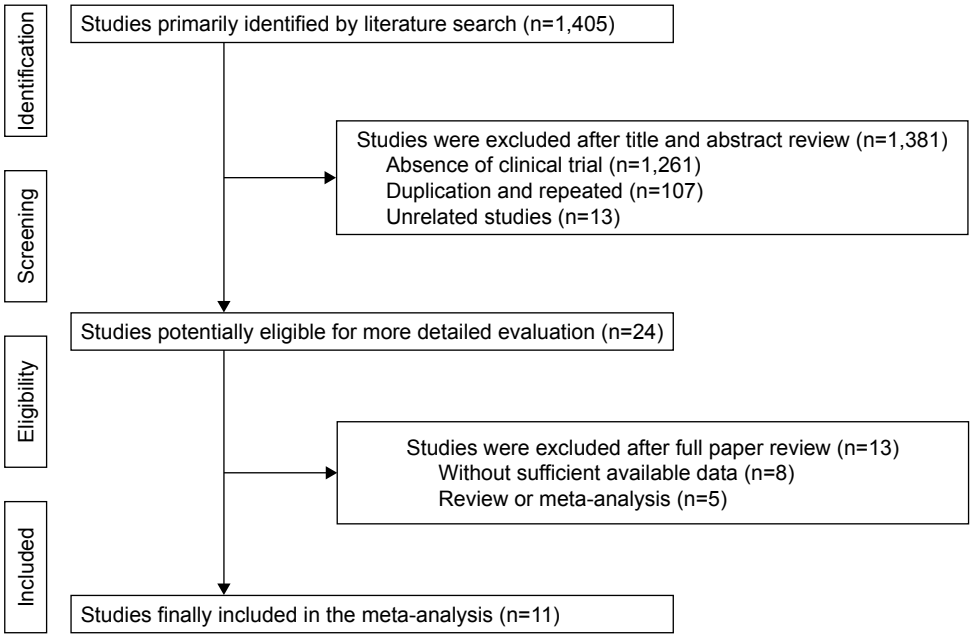

Figure 1 Flow diagram of the selection process.

Table 1 Clinical information from the eligible trials used in the meta-analysis

Authors	Year	Stage	Exp regimens	Patients, Con/Exp groups	Age, years		Culture conditions	Cell dose (cycles)
					Con	Exp		
Chang et al[28]	2013	III–IV	CT, CIK	33/33	66 (median)	66 (median)	IFN-γ, IL-2, OKT-3	1×10^9/cycle (2 cycles)
Hu[29]	2012	III–IV	CM, CT, CIK	25/37	ND	ND	IFN-γ, IL-1, IL-2	$>1\times10^9$/cycle (ND)
Liu et al[30]	2011	III–IV	CT, CIK	20/20	62 (median)	62 (median)	IFN-γ, IL-2, OKT-3	$(0.6–1.6)\times10^{10}$/cycle (3 cycles)
Qu et al[31]	2015	IV	CT, CIK	100/100	56.3±7.5 (mean)	56.3±7.5 (mean)	IFN-γ, OKT-3, IL-2, IL-12	ND (ND)
Shu et al[32]	2015	II–III	RT, CT, CIK	30/30	59 (median)	57 (median)	IFN-γ, OKT-3, IL-2	5×10^{10}/cycle (ND)
Wang et al[33,41]	2014	I–IV	CT, DC-CIK[a]	62/62	ND	ND	IFN-γ, IL-2, OKT-3 (CIK) GM-CSF, IL-4, IFN-γ (DC)	2×10^{10}/cycle (ND)
Xi et al[34]	2015	II–IIIB	Surgery, CT, DC-CIK[a]	26/26	62 (median)	60 (median)	IFN-γ, IL-1, IL-2, OKT-3 (CIK) GM-CSF, IL-4, TNF-α, IL-1 (DC)	$3–4\times10^9$/cycle (2 cycles)
Xu et al[35]	2010	III–IV	CT, CIK	25/21	42 (mean)	45 (mean)	IFN-γ, IL-1α, IL-2, OKT-3	$>5\times10^9$/cycle (4 cycles)
Yan et al[26]	2015	I–IV	RT, DC-CIK[b]	34/34	71.6±2.2 (mean)	70.5±2.9 (mean)	IFN-γ, IL-1α, IL-2, OKT-3 (CIK) GM-CSF, IL-4 (DC)	5×10^9/cycle (CIK) 5×10^7/cycle (DC) (ND)
Yang et al[36]	2015	ND	CT, DC-CIK[a]	100/100	72.3±6.9 (mean)	70.2±7.3 (mean)	ND	ND (1–2 cycles)
Zhu and Zhang[37]	2014	ND	CT, CIK	38/38	59.8±1.4 (mean)	59.6±1.3 (mean)	ND	ND (ND)

Notes: The table summarizes patients' basic information regarding the tumor stage, treatment regimens, cases, age and details of the immunotherapy (culture conditions, cell doses and the treatment courses). [a]DCs cultivated with CIK before injection; [b]coinjection of DCs with CIKs.

Abbreviations: CD, cluster of differentiation; CIK, cytokine-induced killer cell; CM, Chinese medicine (Huisheng oral liquid); Con, control group; CT, chemotherapy; DC, dendritic cell; Exp, experimental group; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; OKT-3, CD3 monoclonal antibodies; ND, not determined; RT, radiotherapy; TNF, tumor necrosis factor.

Quality assessment

The assessment for risk of bias is shown in Figure 2A and B. The quality of the involved studies ranged from moderate to high: 9 studies were low in risk of bias, while the other 2 studies did not have a clear description of the randomization process. The allocation, performance, detection and attrition risks of the involved studies were low; 3 trials were considered to be of unclear risk owing to their selective reporting, while 3 other studies were considered as high risk as they did not show primary outcome data.

Efficacy assessments

This analysis indicated that OS, ORR and DCR were significantly improved in patients who underwent combination therapy compared to those treated by conventional therapy alone (Figure 3, 1-year OS: OR =2.59, 95% CI =1.52–4.40, P=0.0005; ORR: OR =2.18, 95% CI =1.57−3.02, $P<0.00001$; DCR: OR =3.83, 95% CI =2.47−5.92, $P<0.00001$). Moreover, the pooled results showed that patients in the combination therapy group had significantly improved QoL (Figure 4, QoL: OR =1.94, 95% CI =1.13−3.33, P=0.02). The fixed-effects model was applied in this analysis considering the slightly significant heterogeneity.

Immune function evaluation

The immune status of patients was examined before and after the treatment. After CIK/DC-CIK treatment, the proportions of $CD3^+$ and $CD3^-CD56^+$ in patients were significantly increased (Figure 5, $CD3^+$: OR =9.48, 95% CI =6.19−12.77, $P<0.00001$; $CD3^-CD56^+$: OR =6.57, 95% CI =2.00−11.14, P=0.005), $CD4^+CD25^+CD127^-$ proportion was significantly decreased ($CD4^+CD25^+CD127^-$: OR =–1.72, 95% CI =–2.15 to −1.28, $P<0.00001$), whereas the proportions of $CD4^+$, $CD8^+$ and $CD3^+CD56^+$ and the $CD4^+/CD8^+$ ratio did not show much differences ($CD4^+$: OR =2.93, 95% CI =–2.42 to 8.29, P=0.28; $CD8^+$: OR =2.00, 95% CI =–4.11 to 8.11, P=0.52; $CD4^+/CD8^+$: OR =–0.01, 95% CI =–0.53 to 0.51, P=0.97; $CD3^+CD56^+$: OR =6.24, 95% CI =–2.48 to 14.97, P=0.16).

On the other hand, patients' cytokines levels were also significantly increased after CIK/DC-CIK therapy

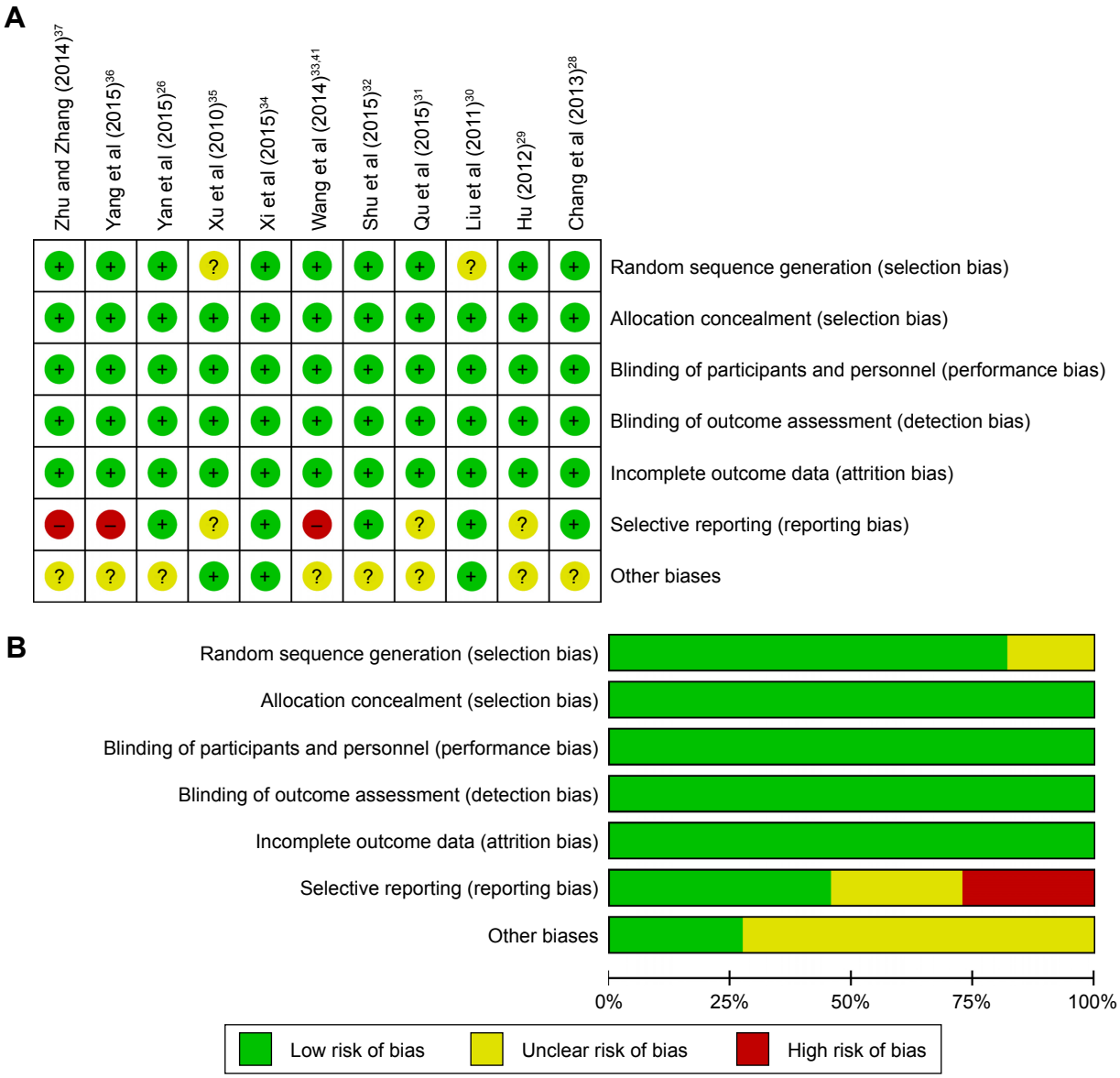

Figure 2 Review of authors' judgments about each risk of bias item for included studies.
Notes: (**A**) Risk of bias summary. (**B**) Risk of bias graph: review of authors' judgments about each risk of bias item presented as percentages across all included studies.

A

Study or subgroup	Cont + CIK/DC-CIK Events	Total	Cont Events	Total	Weight (%)	Odds ratio M–H, fixed, 95% CI
1-year OS						
Liu et al (2011)[30]	15	20	2	20	2.9	27.00 (4.57, 159.66)
Yang et al (2015)[36]	71	100	57	100	97.1	1.85 (1.03, 3.32)
Subtotal (95% CI)		**120**		**120**	**100**	**2.59 (1.52, 4.40)**
Total events	86		59			
Heterogeneity: χ^2=7.96, *df*=1 (*P*=0.005); I^2=87%						
Test for overall effect: *Z*=3.51 (*P*=0.0005)						
Total (95% CI)		**120**		**120**	**100**	**2.59 (1.52, 4.40)**
Total events	86		59			
Heterogeneity: χ^2=7.96, *df*=1 (*P*=0.005); I^2=87%						
Test for overall effect: *Z*=3.51 (*P*=0.0005)						
Test for subgroup differences: not applicable						

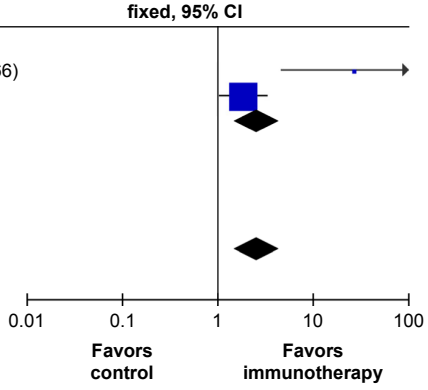

Figure 3 (*Continued*)

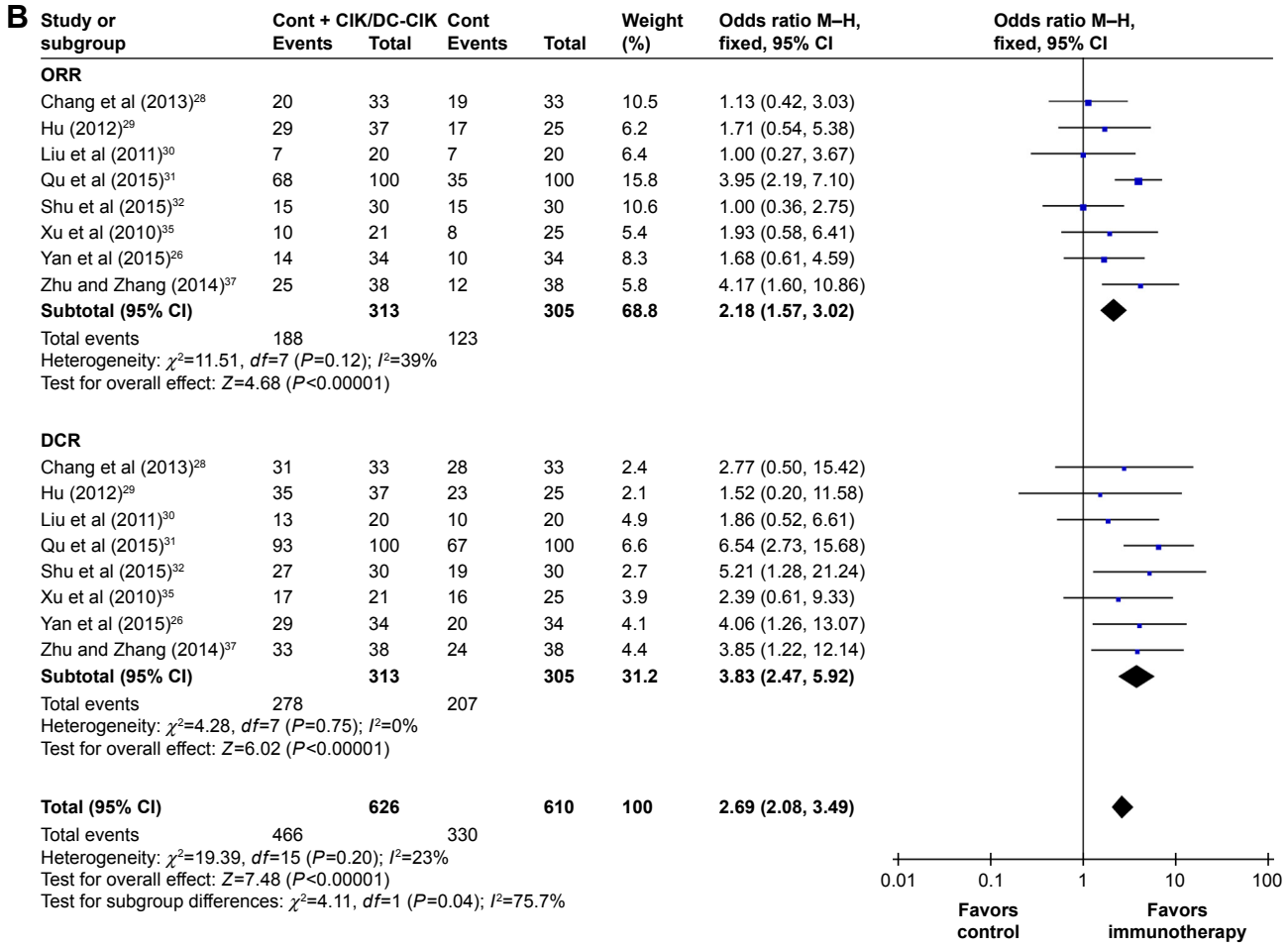

Figure 3 Forest plots of the comparisons of (**A**) OS and (**B**) ORR and DCR.
Note: The fixed-effects meta-analysis model (Mantel–Haenszel method) was used.
Abbreviations: CI, confidence interval; CIK/DC-CIK, immunotherapy with cytokine-induced killer cells or combination of dendritic cells and cytokine-induced killer cells; Cont, conventional therapy; DCR, disease control rate; M–H, Mantel–Haenszel method; ORR, overall response rate; OS, overall survival.

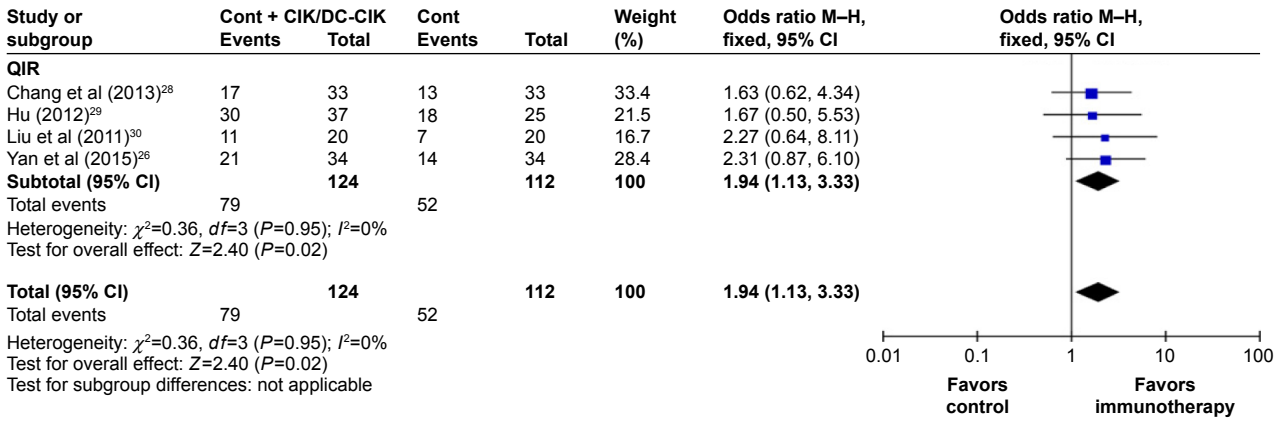

Figure 4 Forest plot for the comparison of QIR.
Note: The fixed-effects meta-analysis model (Mantel–Haenszel method) was used.
Abbreviations: CI, confidence interval; CIK/DC-CIK, immunotherapy with cytokine-induced killer cells or combination of dendritic cells and cytokine-induced killer cells; Cont, conventional therapy; M–H, Mantel–Haenszel method; QIR, quality-of-life improved rate.

Study or subgroup	DC-CIK after treatment Mean	SD	Total	DC-CIK before treatment Mean	SD	Total	Weight (%)	Mean difference IV, random, 95% CI
$CD3^+$								
Liu et al (2011)[30]	71.4	4.4	20	63.7	5.3	20	3.5	7.70 (4.68, 10.72)
Shu et al (2015)[32]	73.5	5.6	30	66.8	5.3	30	3.5	6.70 (3.94, 9.46)
Wang et al (2014)[33,41]	67.2	4.3	62	53.7	4.8	62	3.9	13.50 (11.90, 15.10)
Xi et al (2015)[34]	65.3	8.2	26	57.7	3.5	26	3.3	7.60 (4.17, 11.03)
Xu et al (2010)[35]	70.9	9.2	21	66.3	10.2	21	2.5	4.60 (–1.27, 10.47)
Yan et al (2015)[26]	69.2	6.1	34	54.2	7.3	34	3.4	15.00 (11.80, 18.20)
Subtotal (95% CI)			**193**			**193**	**20.1**	**9.48 (6.19, 12.77)**
Heterogeneity: τ^2=13.99; χ^2=37.60, *df*=5 (*P*<0.00001); I^2=87%								
Test for overall effect: *Z*=5.64 (*P*<0.00001)								
$CD4^+$								
Liu et al (2011)[30]	39.4	8.2	20	34.6	1.5	20	3.2	4.80 (1.15, 8.45)
Shu et al (2015)[32]	36.7	1.6	30	32.9	3.4	30	3.9	3.80 (2.46, 5.14)
Wang et al (2014)[33,41]	28.6	6.9	62	38.3	7.3	62	3.6	–9.70 (–12.20, –7.20)
Xi et al (2015)[34]	40.1	10.9	26	31.2	9.7	26	2.6	8.90 (3.29, 14.51)
Xu et al (2010)[35]	33.1	6.4	21	29.3	7.8	21	3.0	3.80 (–0.52, 8.12)
Yan et al (2015)[26]	38.8	4.2	34	31.9	6	34	3.6	6.90 (4.44, 9.36)
Subtotal (95% CI)			**193**			**193**	**20.0**	**2.93 (–2.42, 8.29)**
Heterogeneity: τ^2=41.56; χ^2=114.18, *df*=5 (*P*<0.00001); I^2=96%								
Test for overall effect: *Z*=1.07 (*P*=0.28)								
$CD8^+$								
Liu et al (2011)[30]	31.9	4.2	20	30.5	11.1	20	2.7	1.40 (–3.80, 6.60)
Shu et al (2015)[32]	25.8	3.2	30	26.8	2	30	3.9	–1.00 (–2.35, 0.35)
Wang et al (2014)[33,41]	32.7	0.5	62	22.5	0.4	62	4.0	10.20 (10.04, 10.36)
Xi et al (2015)[34]	27.8	5.3	26	33.2	6.7	26	3.4	–5.40 (–8.68, –2.12)
Xu et al (2010)[35]	27.9	6.1	21	30.2	7.1	21	3.1	–2.30 (–6.30, 1.70)
Yan et al (2015)[26]	36.1	4.3	34	27.6	5.5	34	3.7	8.50 (6.15, 10.85)
Subtotal (95% CI)			**193**			**193**	**20.8**	**2.00 (–4.11, 8.11)**
Heterogeneity: τ^2=55.77; χ^2=393.70, *df*=5 (*P*<0.00001); I^2=99%								
Test for overall effect: *Z*=0.64 (*P*=0.52)								
$CD4^+/CD8^+$								
Liu et al (2011)[30]	1.2	0.2	20	1.1	0.1	20	4.0	0.10 (0.00, 0.20)
Wang et al (2014)[33,41]	0.8	0.1	62	1.3	0.1	62	4.0	–0.50 (–0.54, –0.46)
Xi et al (2015)[34]	1.4	0.5	26	1	0.4	26	4.0	0.40 (0.15, 0.65)
Subtotal (95% CI)			**108**			**108**	**12.1**	**–0.01 (–0.53, 0.51)**
Heterogeneity: τ^2=0.21; χ^2=170.60, *df*=2 (*P*<0.00001); I^2=99%								
Test for overall effect: *Z*=0.04 (*P*=0.97)								
$CD3^-CD56^+$								
Wang et al (2014)[33,41]	28.5	4	62	21.9	3.6	62	3.9	6.60 (5.26, 7.94)
Xi et al (2015)[34]	28	6.1	26	16.3	5.4	26	3.4	11.70 (8.57, 14.83)
Yan et al (2015)[26]	12.1	5.2	34	10.3	3.7	34	3.7	1.80 (–0.35, 3.95)
Subtotal (95% CI)			**122**			**122**	**11.1**	**6.57 (2.00, 11.14)**
Heterogeneity: τ^2=14.95; χ^2=28.22, *df*=2 (*P*<0.00001); I^2=93%								
Test for overall effect: *Z*=2.82 (*P*=0.005)								
$CD3^+CD56^+$								
Liu et al (2011)[30]	2.4	0.3	20	0.6	0.3	20	4.0	1.80 (1.61, 1.99)
Shu et al (2015)[32]	16.1	1.6	30	5.4	0.9	30	4.0	10.70 (10.04, 11.36)
Subtotal (95% CI)			**50**			**50**	**8.1**	**6.24 (–2.48, 14.97)**
Heterogeneity: τ^2=39.54; χ^2=652.83, *df*=1 (*P*<0.00001); I^2=100%								
Test for overall effect: *Z*=1.40 (*P*=0.16)								
$CD4^+CD25^+CD127^-$								
Wang et al (2014)[33,41]	4.8	1.3	62	6.5	1.3	62	4.0	–1.70 (–2.16, –1.24)
Xu et al (2010)[35]	4.2	1.8	21	6.1	3.1	21	3.9	–1.90 (–3.43, –0.37)
Subtotal (95% CI)			**83**			**83**	**7.9**	**–1.72 (–2.15, –1.28)**
Heterogeneity: τ^2=0.00; χ^2=0.06, *df*=1 (*P*=0.81); I^2=0%								
Test for overall effect: *Z*=7.67 (*P*<0.00001)								
Total (95% CI)			**942**			**942**	**100**	**4.00 (2.53, 5.48)**
Heterogeneity: τ^2=14.01; χ^2=18,699.17, *df*=27 (*P*<0.00001); I^2=100%								
Test for overall effect: *Z*=5.32 (*P*<0.00001)								
Test for subgroup differences: χ^2=78.19, *df*=6 (*P*<0.00001); I^2=92.3%								

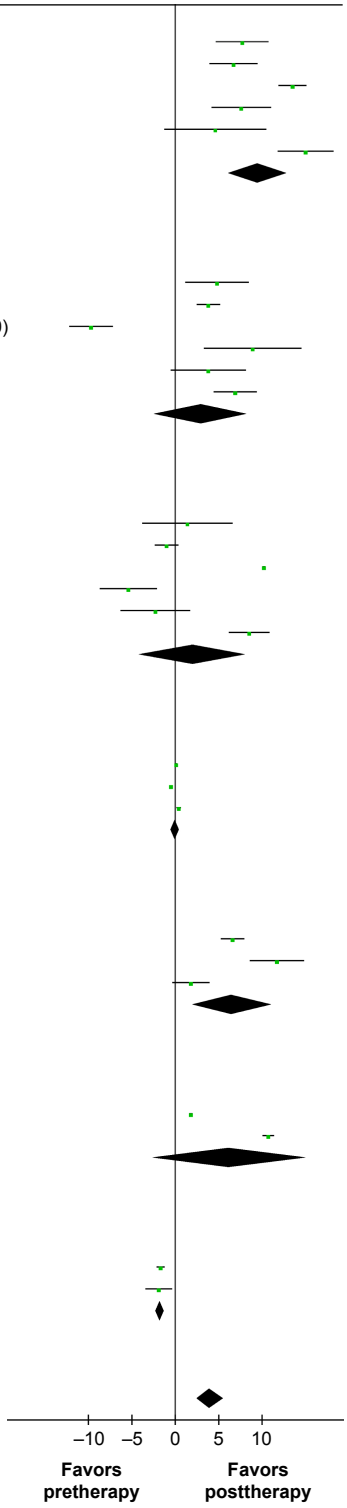

Figure 5 Forest plot of immunophenotype assessment before and after treatment with CIK/DC-CIK.
Note: The random-effects meta-analysis model (Mantel–Haenszel method) was used in this analysis.
Abbreviations: CD, cluster of differentiation; CI, confidence interval; CIK/DC-CIK, immunotherapy with cytokine-induced killer cells or combination of dendritic cells and cytokine-induced killer cells; SD, standard deviation.

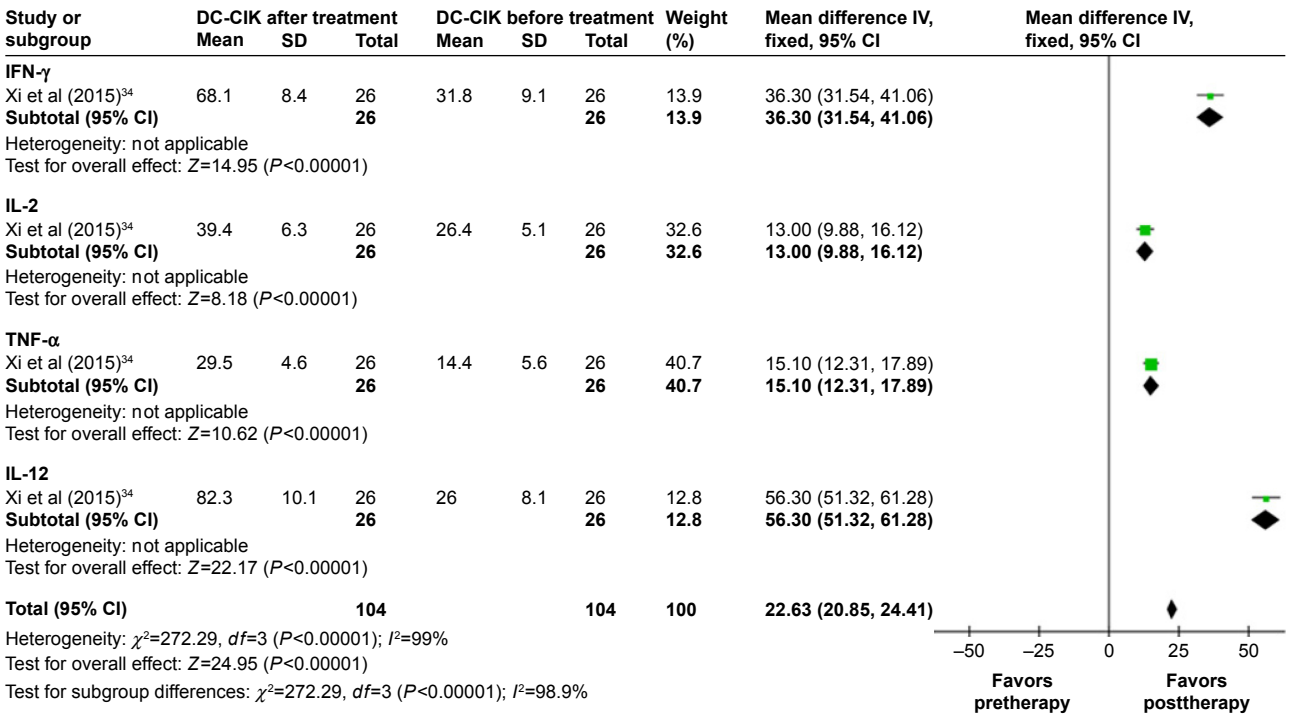

Figure 6 Forest plot of cytokines before and after treatment with CIK/DC-CIK.
Note: The fixed-effects meta-analysis model (Mantel–Haenszel method) was used in this analysis.
Abbreviations: CI, confidence interval; CIK/DC-CIK, immunotherapy with cytokine-induced killer cells or combination of dendritic cells and cytokine-induced killer cells; IFN, interferon; IL, interleukin; SD, standard deviation; TNF, tumor necrosis factor.

(Figure 6, IFN-γ: OR =36.30, 95% CI =31.54–41.06, $P<0.00001$; IL-2: OR =13.00, 95% CI =9.88–16.12, $P<0.00001$; TNF-α: OR =15.10, 95% CI =12.31–17.89, $P<0.00001$; IL-12: OR =56.30, 95% CI =51.32–61.28, $P<0.00001$).

Assessment of adverse events

The safety of CIK/DC-CIK therapy in the treatment of EC was evaluated in this meta-analysis. As shown in Figure 7, no serious adverse events or death occurrence was reported in the involved literature. The most common side effect was fever, which subsided naturally within 24 hours. Except the higher incidence of fever in the combination therapy group than in the conventional therapy group (fever: OR =6.46, 95% CI =2.42–17.21, $P=0.0002$), no significant difference was observed in terms of leukopenia, gastrointestinal adverse reaction and peripheral neurotoxicity (leukopenia: OR =0.91, 95% CI =0.39–2.12, $P=0.83$; gastrointestinal adverse reaction: OR =0.51, 95% CI =0.22–1.22, $P=0.13$; peripheral neurotoxicity: OR =0.75, 95% CI =0.26–2.15, $P=0.59$).

Discussion

Clinical trials have been conducted on CIK/DC-CIK immunotherapy for the treatment of EC.[26,31] In this study, we performed an extensive online search, followed by rigorous meta-analysis, in order to evaluate its therapeutic efficacy and safety. Our meta-analysis revealed that the combination of CIK/DC-CIK immunotherapy and conventional therapy was a safe and effective regimen for the treatment of EC. Compared to conventional regimens alone, patients with combination therapy demonstrated higher OS rate, ORR and DCR, as well as improved immune function and QoL.

This study confirmed the safety of CIK/DC-CIK immunotherapy for EC patients, and the adverse events caused were tolerated by all patients. Fever was the most common side effect when patients were treated with combination conventional-plus-CIK/DC-CIK therapy, and its incidence was higher than when treated by conventional therapy alone ($P<0.05$). No significant difference was observed in terms of other adverse events, such as leukopenia, gastrointestinal adverse reaction and peripheral neurotoxicity between the 2 groups ($P>0.05$). CIK/DC-CIK immunotherapy enhanced the efficiency of conventional therapy in the treatment of EC. Compared with the conventional therapy-alone group, 1-year OS, ORR and DCR of patients in the combination therapy group were improved remarkably ($P<0.01$). Moreover, the combination therapy improved patients' QoL ($P<0.05$) by relieving pain, reducing fatigue and insomnia as well as improving appetite.

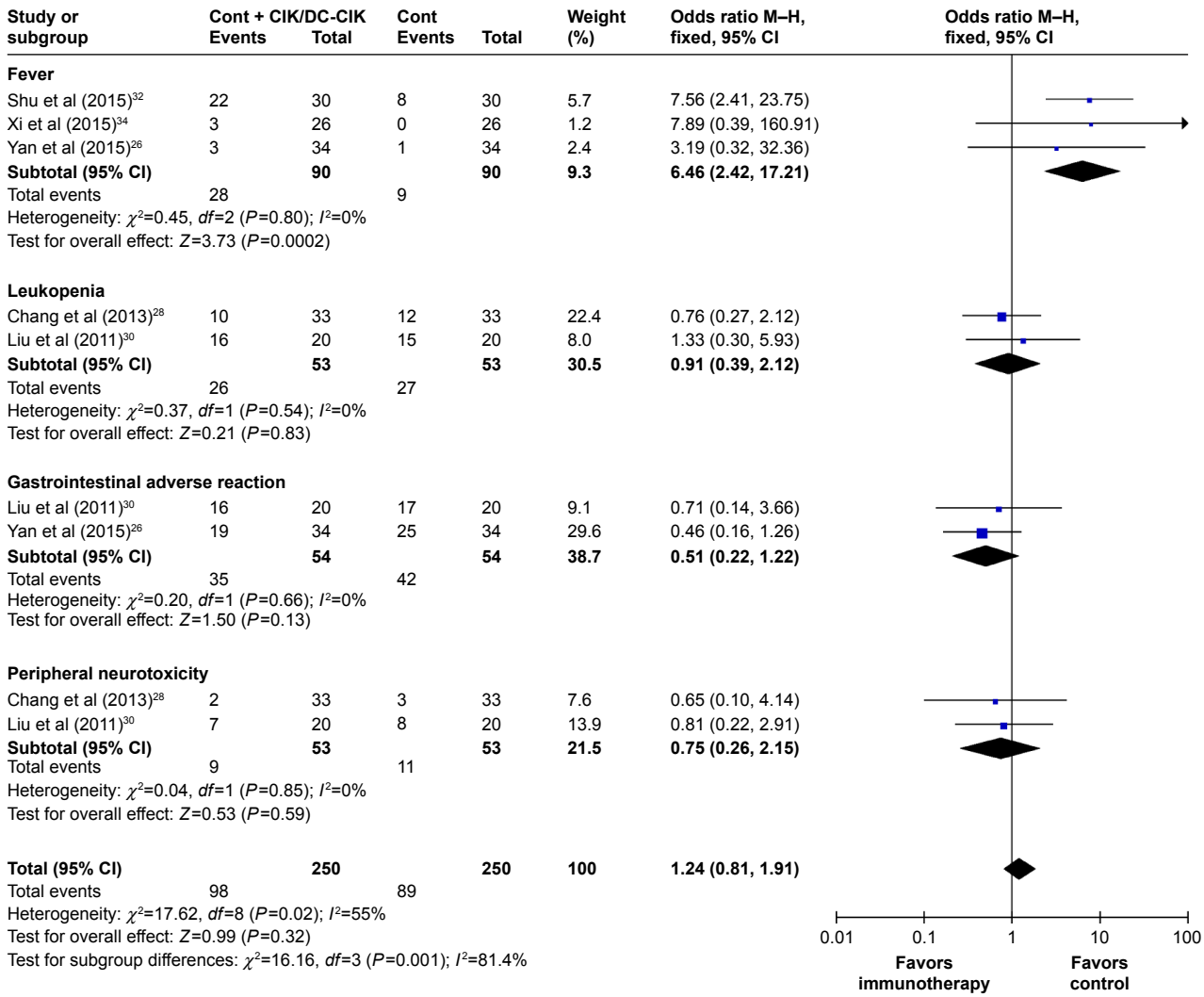

Figure 7 Forest plot of the comparison of adverse effects.
Note: The random-effects meta-analysis model (Mantel–Haenszel method) was used in this analysis.
Abbreviations: CI, confidence interval; CIK/DC-CIK, immunotherapy with cytokine-induced killer cells or combination of dendritic cells and cytokine-induced killer cells; Cont, conventional therapy; M–H, Mantel–Haenszel method.

Health status is closely related to human immune function, and a healthy human body has a robust immune system to detect and kill cancer cells.[5,6] However, the immune function in cancer patients is compromised, and the percentage of T-lymphocyte subsets in the peripheral blood is usually disordered.[5,6] Immune system reconstruction is one of the key factors to effectively treat malignant tumors.[6] The antitumor activity of CIK/DC-CIK is mainly attributed to $CD3^-CD56^+$ and $CD3^+CD56^+$ cells.[41] Our analysis indicated that the proportions of $CD3^+$, $CD3^-CD56^+$ and $CD3^+CD56^+$ T cells were increased after CIK/DC-CIK treatment, although the percentages of $CD3^+CD56^+$ T cells did not reach statistical significance. However, no significant differences were found in the percentages of $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ ratios before and after immunotherapy. This may be caused by the different time points when the T-lymphocyte subsets were tested in these trials.[6,19,42,43] Our analysis revealed a decreased proportion of $CD4^+CD25^+$ $CD127^-$ Tregs. This is consistent with a previous study that illustrated a negative role of Tregs in the implementation of CIK's antitumor activity.[44] Besides, the balance between the 2 helper T-cell (Th1 and Th2 cells) classes is also important in immunotherapy.[5,41] Th1 cells enhance killer cells' cytotoxicity and trigger delayed-type hypersensitivity, whereas Th2 cells are associated with tumor immune escape.[5,45] Our analysis showed that after CIK/DC-CIK immunotherapy, the levels of Th1 cytokines, including IFN-γ, IL-2, TNF-α and IL-12, were significantly increased ($P<0.00001$), indicating a strong association between Th1 cytokines and efficacy of CIK/DC-CIK immunotherapy. Although our results indicated that CIK/DC-CIK

immunotherapy enhanced the immune function in EC patients, the exact underlying mechanism of action of CIK/DC-CIK immunotherapy on hosts' immune system remains unclear, which requires further studies on its mechanism.

This meta-analysis has some limitations. First of all, although CIK/DC-CIK immunotherapy has been applied to treat malignancies worldwide for its outstanding curative effects,[46–48] all of the clinic trials that met our inclusion criteria were carried out in the Chinese population. We will follow updated publications on CIK/DC-CIK immunotherapy for EC conducted both in China and other countries and subsequently perform further systematic research on it. Moreover, the analysis performed in this study was not subjected to an open external evaluation procedure, which may lead to an overestimation of treatment effects. In addition, insufficient information regarding some patients, small sample sizes and other variables may have introduced bias into our conclusions. Besides, the clinical application of adoptive CIK/DC-CIK immunotherapy was limited due to the low specificity, although it is a promising strategy for the treatment of malignant tumors. Many new methods of immunotherapy, such as chimeric antigen receptor-modified T cells and T-cell receptor-modified T cells, have been developed currently,[49–51] limiting the importance of this study.

Conclusion

Taken together, this meta-analysis suggests that the combination of CIK/DC-CIK immunotherapy and conventional regimens is safe and effective in treating patients with EC, with markedly prolonged survival time, enhanced immune function and improved therapeutic efficacy. Considering the limitations of our research, further analysis on studies conducted in countries other than China with larger sample sizes and going through open external evaluation procedure will be valuable to verify the credibility of our conclusions.

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Author contributions

Changhui Zhou and Yingxin Zhang conceived and designed the experiments. Yan Liu, Ying Mu and Shaoda Ren performed the experiments. Weihua Wang and Jiaping Xie analyzed the data. Yan Liu and Ying Mu drafted the manuscript. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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