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ORIGINAL RESEARCH

Adjuvant immunotherapy of dendritic cells and cytokine-induced killer cells is safe and enhances chemotherapy efficacy for multiple myeloma in China: a meta-analysis of clinical trials

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Objective: The aim of this study was to systematically evaluate the efficacy and safety of the combination of dendritic cells and cytokine-induced killer cells (DC–CIK) adjuvant immunotherapy and chemotherapy in the treatment of multiple myeloma (MM).

Methods: Clinical trials were gathered by searching Web of Science, PubMed, Embase, Cochrane Library, Wanfang, and CNKI database. Outcome measurements including therapeutic efficacy, prognosis, immune function, and adverse events were extracted and evaluated.

Results: A total of 12 trials including 594 MM patients were involved in this study for statistical analysis. Results indicated that compared to chemotherapy alone, the combination of DC–CIK immunotherapy with chemotherapy significantly improved patients' overall response rate (ORR, odds ratio [OR] =2.77, 95% confidence interval [CI] =1.88–4.10, $P<0.00001$), disease control rate (DCR, OR =2.90, CI =1.72–4.90, $P<0.0001$), and life quality ($P<0.00001$). The combined therapy showed advantages over chemotherapy alone in prognostic indicators including percentage of tumor cells ($P=0.04$), serum levels of β2-microglobin ($P<0.0001$), M protein ($P<0.00001$), and creatinine ($P<0.0001$), and 24 h urine light chains ($P<0.00001$). After combined treatment, $CD4^+$ lymphocyte subsets' percentages, $CD4^+/CD8^+$ ratio, and cytokines levels of AgNOR, IFN-γ, IL-2, and IL-12 were significantly increased ($P<0.05$), whereas $CD8^+$ and $CD4^+CD25^+$ percentages and IL-4, IL-6, IL-10, and TGF-β levels were obviously decreased ($P<0.01$), indicating a recovered immune condition.

Conclusion: Adjuvant DC–CIK immunotherapy enhances the efficacy of chemotherapy for MM and improves prognosis probably by reconstructing immune function.

Keywords: cytokine-induced killer cells, dendritic cells, multiple myeloma, adjuvant immunotherapy, meta-analysis

Introduction

Multiple myeloma (MM) is the second most common hematological malignancy.[1] It is characterized by aberrant bone marrow plasma cells proliferation, accompanied with excessive monoclonal protein production, which eventually causes hypercalcemia, renal failure, anemia, or lytic bone lesions.[2] The incidence of MM has been increased in the past years, and it accounts for ~1.8% of all cancers and 15% of all hematological malignancies, with an annual incidence of 6.5 per 100,000 people in western countries.[1,3] Chemotherapy is one of the standard treatment regimens for MM, and the common chemotherapeutic drugs include thalidomide, velcade (bortezomib), lenalidomide, and other traditional drugs such as vincristine and cytoxan.[4,5] These

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Table 1 Information of DC–CIK immunotherapy

Included studies	Therapeutic regimen	Administration route	Culture conditions	Cell dose (cycles)
Ding (2013)[14]	CT (VD), CIK	Intravenous infusion	IFN-γ, IL-1α, IL-2, CD3	$>1\times10^{10}$ (ND)
Gao (2015)[15]	CT (VDP/DEV/DCEV), DC–CIK	Intravenous infusion	ND	$2.5–5\times10^{9}$ (>3 cycles)
Lu (2015)[16]	CT (VCD), DC–CIK	Intravenous infusion	IFN-γ, IL-1α, IL-2, CD3, GM-CSF, IL-4	$1.2–2.4\times10^{10}$ (≧2 cycles)
Sun (2012)[17]	CT (Thd), DC–CIK	Intravenous infusion	IFN-γ, IL-1α, IL-2, CD3, GM-CSF, IL-4	$1\times10^{9–10}$ (2 cycles)
Tan (2016)[18]	CT (DEV), CIK	Intravenous infusion	IFN-γ, IL-12, CD3	ND (3 cycles)
Wang (2015)[19]	CT (VD/VDT/VCD/MVP), DC–CIK	Intravenous infusion	IFN-γ, IL-1α, IL-12, CD3, GM-CSF, IL-4	$1.5–3\times10^{10}$ (>3 cycles)
Yan (2014)[20]	CT (DEV/PM/DCEV/VDP), DC–CIK	Intravenous infusion	ND	$2–5\times10^{9}$ (>3 cycles)
Ye (2016)[21]	CT (ND), DC–CIK	Intravenous infusion	ND	ND
Yu (2016)[22]	CT (VD), DC–CIK	Intravenous infusion	ND	$1.2–3\times10^{10}$ (≧3 cycles)
Zhao (2015)[23]	CT (VD), DC–CIK	Intravenous infusion	IFN-γ, IL-1α, IL-12, CD3, GM-CSF, IL-4	$1.2–3\times10^{10}$ (>3 cycles)
Zhao (2016)[24]	CT (VD), DC–CIK	Intravenous infusion	IFN-γ, IL-1α, IL-12, CD3, GM-CSF, IL-4	$1.2–3\times10^{10}$ (>3 cycles)
Zhong (2012)[25]	CT (MP/DEV/DCEV/VEDM/VD/VED), DC–CIK	Intravenous infusion	IFN-γ, IL-1α, IL-12, CD3, GM-CSF, IL-4	$1.5–3\times10^{10}$ (>3 cycles)

Abbreviations: CIK, cytokine-induced killer cells; CT, chemotherapy; DC–CIK, dendritic cells and CIK; DCEV, dexamethasone + cytoxan + epirubicin + vindesine; DEV, dexamethasone + epirubicin + vindesine; MP, melphalan + prednisone; MVP, melphalan + velcade + prednisone; ND, nondetermined; Thd, thalidomide; VCD, velcade + cytoxan + dexamethasone; VD, velcade + dexamethasone; VDP, velcade + dexamethasone + pirarubicin; VDT, velcade + dexamethasone + thalidomide; VED, velcade + epirubicin + dexamethasone; VEDM, vincristine + epirubicin + dexamethasone + melphalan.

medications were either singly used or more often used as combinations for newly diagnosed or relapsed/refractory MM, and their usage usually varies across different countries depending on their availability (Table 1).[4–7] Although chemotherapy usually relieves the gravity and improves survival, most patients eventually relapse and develop resistance to treatment, which were not able to completely eradicate small lesions and metastatic tumor cells.[2]

Adoptive cellular immunotherapy has been clinically used alongside chemotherapy for MM. It is achieved upon in vitro expansion of autologous immune effector cells and transfusion back to patients.[8] Many studies have reported that adoptive immunotherapy can increase the efficacy of chemotherapy for various malignant tumors by reconstructing patients' immune function.[9,10] Dendritic cells and cytokine-induced killer cells (DC–CIK)-based adoptive immunotherapy have been widely applied to treat various malignancies.[11] Dendritic cells (DC) are the most potent antigen-presenting cells. Cytokine-induced killer cells (CIK) compose a heterogeneous subset of T lymphocytes presenting phenotypes of both T cells and natural killer (NK) cells ($CD3^+CD56^+$) and can be induced by IFN-γ, anti-CD3 monoclonal antibodies, and IL-2 in vitro.[8,11] Studies have shown that co-culture with DC enhanced the cytotoxicity of CIK indicated by increased proportion of $CD3^+CD56^+$ cells and improved levels of cytokines such as IFN-γ and IL-2.[12,13] Compared to other immune cells such as cytotoxic T lymphocytes, NK cells, and tumor-infiltrating lymphocytes, DC–CIK have higher proliferative capability, broader anti-tumor spectrum, and stronger antitumor ability.[12]

In several clinic trials, DC–CIK immunotherapy combined with chemotherapy exhibited more prominent therapeutic effects for MM compared to chemotherapy alone,[14–25] but systematic analysis assessing the therapeutic efficacy of this combined therapeutic strategy remains scant for now. In this study, we conducted a meta-analysis to investigate the treatment effects and safety of adjuvant DC–CIK immunotherapy combined with chemotherapy in comparison with chemotherapy alone for MM in order to provide scientific reference for the design of future clinical trials.

Methods

Search strategy and selection criteria

Literature was searched across Web of Science, PubMed, EMBASE, Cochrane Library, Wanfang, and CNKI database, with key terms "dendritic cells", "cytokine-induced killer cells" combined with "multiple myeloma". No language limits were applied. The initial search was performed in October 2016 and updated in March 2017.

Studies concerning MM patients were involved in our analysis. Patients in the experimental group received adjuvant DC–CIK immunotherapy combined with chemotherapy, and patients in the control group were treated by chemotherapy alone.

Data extraction and quality assessment

Data were extracted by two investigators independently (YW and BL); disagreements were resolved upon discussing with the third researcher (AZ). All involved studies were summarized as follows: first author's names, years of

publication, study locations, tumor stages, number of cases, patient ages, therapeutic regimens, administration route, in vitro cell culture conditions, and dosages of utilized immune cells. Quality of all involved trials was evaluated based on *Cochrane Handbook*.[26]

Outcome definition

Clinical responses include treatment efficacy, prognosis, and immune function. Treatment efficacy was assessed in terms of the complete response (CR) rates, near CR (nCR), partial response (PR) rates, minuteness response (MR), stable disease (SD) rates, progressive disease (PD) rates, nonresponse (NR) rate, overall response rate (ORR) (ORR = CR + nCR + PR), and disease control rate (DCR) (DCR = CR + nCR + PR + SD). Patients' quality of life (QoL) was evaluated using performance status (Ps) score and Karnofsky performance score (Kps). Prognosis was analyzed based on the following indicators: tumor cells' percentage, serum levels of β2-microglobin (β2-M), M protein, 24 h urine light chains, and serum creatinine (Scr). The immune function of MM patients before and after treatment was evaluated by lymphocyte subsets ($CD4^+$, $CD8^+$, $CD4^+/CD8^+$, and $CD4^+CD25^+$) and cytokines' secretion (silver-staining nucleolar organizer region [AgNOR], IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-12, and TGF-β).

Statistical analysis

The analysis was performed using Review Manager 5.2 (Cochrane Collaboration). $P<0.05$ indicated statistical significance. Heterogeneity among studies was assessed by Cochran's Q test to determine the most suitable analysis model,[27] and funnel plots were used to assess the publication bias of the involved studies. $I^2<50\%$ or $P>0.1$ indicated that the studies were homogenous. Odds ratio (OR) was the principal measurement for therapeutic effects and was presented with a 95% confidence interval (CI). Sensitivity analysis was conducted to evaluate the consistency of the results and the influence of single study on overall risk estimate.

Results

Search results

A total of 2,953 articles were identified upon initial retrieve. After title and abstract review, 2,899 articles were excluded because they did not include clinical trials (n=2,683), were duplication or repetition (n=182), or unrelated studies (n=34), leaving 54 studies as potentially relevant. After detailed assessment of full texts, articles with insufficient data (n=12) or without control group (n=14), and reviews or meta-analyses (n=16) were excluded. Finally, 12 trials involving 594 MM patients were included in this meta-analysis (Figure 1).

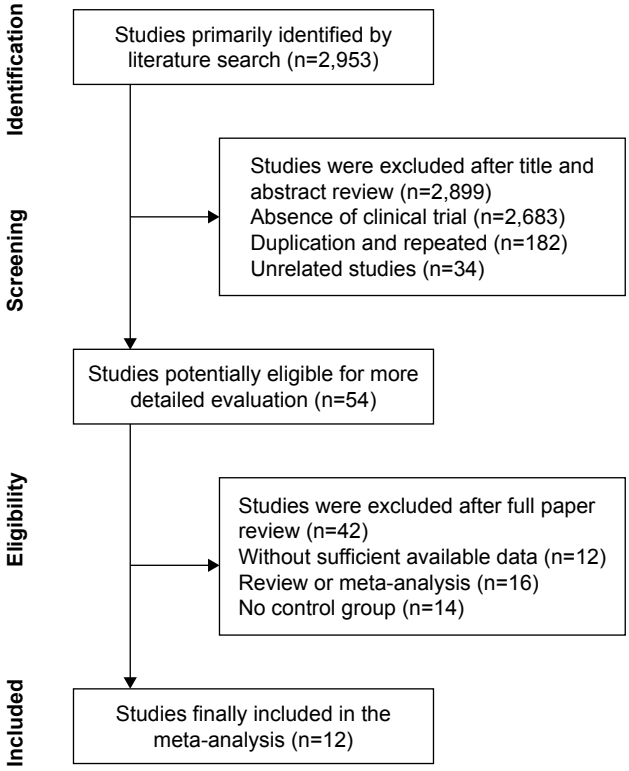

Figure 1 Flow diagram of the selection process.

Patient characteristics

After selection, all 12 trials that met our inclusion criteria were conducted in China. In total, 300 MM patients were treated by DC–CIK in combination with chemotherapy, while 294 patients were treated by chemotherapy alone. Detailed information of the involved trials and patients is presented in Table 2. DC and CIK were obtained from autologous peripheral blood and cultured in vitro for amplification. Most patients received intravenous transfusion for more than three time over 1×10^{10} DC–CIK each cycle (Table 1).

Quality assessment

The assessment of bias risk is shown in Figure S1. Five studies were determined as low risk, two studies were not truly randomized controlled trials and the remaining five studies lacked clear description of randomization process. All included trials did not provide clear description of allocation, performance, and detection risks. The attrition risks of involved trials were low; four trials were considered as unclear risk owing to

Table 2 Clinical information from the eligible trials in the meta-analysis

Included studies	Nation	D-S stage	Patients, Con/Exp	Age (years)		Parameter types
				Con	Exp	
Ding (2013)[14]	China	II–III	16/14	71.3±4.0 (mean)	70.5±3.4 (mean)	ORR
Gao (2015)[15]	China	III	60/60	48.2±3.4 (mean)	47.8±3.6 (mean)	ORR, DCR, cytokines, Kps
Lu (2015)[16]	China	I–III (ISS)	12/10	60 (median)	61 (median)	Treg, ORR, DCR
Sun (2012)[17]	China	II–III	24/24	58.2±15.4 (mean)	59.8±17.8 (mean)	ORR, DCR, TC, β2-M, M, ULC, Scr, AgNOR, cytokines
Tan (2016)[18]	China	ND	30/30	58.6±8.8 (mean)	59.6±9.2 (mean)	LYM subsets, ORR, cytokines, ADE
Wang (2015)[19]	China	II–III	14/15	61.4±3.5 (mean)	60.7±4.0 (mean)	ORR, DCR
Yan (2014)[20]	China	ND	18/22	ND	ND	ORR, DCR, TC, β2-M, Scr
Ye (2016)[21]	China	ND	16/17	ND	ND	ORR
Yu (2016)[22]	China	I–III	30/30	57.6±5.3 (mean)	59.3±4.3 (mean)	LYM subsets, ORR, DCR
Zhao (2015)[23]	China	I–III (ISS)	24/26	56.3±10.6 (mean)	57.1±11.8 (mean)	LYM subsets, cytokines, TC, β2-M, M, ULC, Scr, Ps
Zhao (2016)[24]	China	I–III (ISS)	20/22	56.5±12.7 (mean)	57.6±13.5 (mean)	Treg, TC, β2-M, M, ULC, Scr, Ps
Zhong (2012)[25]	China	I–III (ISS)	30/30	58.2±15.4 (mean)	61.8±17.8 (mean)	ORR, DCR, TC, β2-M, ULC, Scr, Ps

Notes: Con, control group (chemotherapy alone group); Exp, experimental group (chemotherapy plus DC–CIK immunotherapy); M, serum level of M protein; β2-M, serum level of β2-microglobin; ULC, 24 h urine light chain content.

Abbreviations: ADE, adverse events; AgNOR, silver-staining nucleolar organizer region; DC–CIK, dendritic cells and cytokine-induced killer cells; DCR, disease control rate; D–S, Durie–Salmon; ISS, International Staging System; Kps, Karnofsky performance score; LYM, lymphocyte; ND, nondetermined; ORR, overall response rate; Ps, performance status; Scr, serum creatinine; TC, tumor cell.

selective reporting, and the other two studies were considered as high risk for missing primary outcome data.

Therapeutic efficacy assessments

As shown in Figures 2A and S2 and Table 3, the pooled analysis showed that patients who underwent combined therapy had significantly improved CR, PR, ORR, and DCR (CR: OR =2.71, CI =1.60–4.58, P=0.0002; PR: OR =1.49, CI =1.01–2.20, P=0.04; ORR: OR =2.77, CI =1.88–4.10, P<0.00001; DCR: OR =2.90, CI =1.72–4.90, P<0.0001) and significantly decreased PD and NR (PD: OR =0.34, CI =0.20–0.58, P<0.0001; NR: OR =0.24, CI =0.07–0.80, P=0.02), whereas the nCR, MR, and SD did not show significant difference from patients who received chemotherapy alone (nCR: OR =1.35, CI =0.58–3.11, P=0.48; MR: OR =0.91, CI =0.52–1.57, P=0.73; SD: OR =0.66, CI =0.35–1.25, P=0.21). Fixed-effect models were used to analyze OR rate because of low heterogeneity. Funnel plots of ORR and DCR showed no substantial publication bias in our analysis (Figure 2B and C).

Prognosis evaluation

As shown in Figure 3, the analysis confirmed that the percentage of tumor cells and the levels of β2-M, M protein, 24 h urine light chains, and Scr were significantly decreased after combined treatment (percentage of tumor cells: OR =–35.15, CI =–43.58 to –26.71, P<0.00001; β2-M: OR =–13.80, CI =–17.49 to –10.10, P<0.00001; M protein: OR =–17.77, CI =–21.68 to –13.87, P<0.00001; urinary light chain: OR =–11.39, CI =–13.00 to –9.78, P<0.00001; Scr: OR =–215.79, CI =–246.51 to –185.06, P<0.00001). Compared to patients treated by chemotherapy alone, those who received combined therapy showed better prognosis (Figure S3, percentage of tumor cells: OR =–7.15, CI =–13.92 to –0.38, P=0.04; β2-M: OR =–4.59, CI =–6.88 to –2.29, P<0.0001; M protein: OR =–9.55, CI =–12.61 to –6.49, P<0.00001; urinary light chain: OR =–4.95, CI =–6.20 to –3.69, P=0.006; Scr: OR =–58.52, CI =–85.30 to –31.72, P<0.0001). No significant publication bias was found (Figure 4), and the random effects model was applied for significant heterogeneity.

QoL assessment

QoL was evaluated in this analysis. Result showed that patients' QoL was significantly improved, indicated by Ps and Kps after treatment (Figure 5, Ps: OR =–1.27, CI =–1.83 to –0.71, P<0.00001; Kps: OR =28.90, CI =27.51–30.29, P<0.00001). QoL of patients in the combined group was more superior with statistical significance than that of the control group (Figure S4, Ps: OR =–0.44, CI =–0.60 to –0.28, P<0.00001; Kps: OR =6.00, CI =4.52–7.48, P<0.00001).

Immune function evaluation

The immune status of MM patients was examined before and after treatment. The percentages of $CD4^+$ cells and $CD4^+/CD8^+$ ratio were significantly increased after DC–CIK treatment (Figure S5 and Table 4, $CD4^+$: OR =3.14, CI =1.65–4.64, P<0.0001; $CD4^+/CD8^+$: OR =0.38,

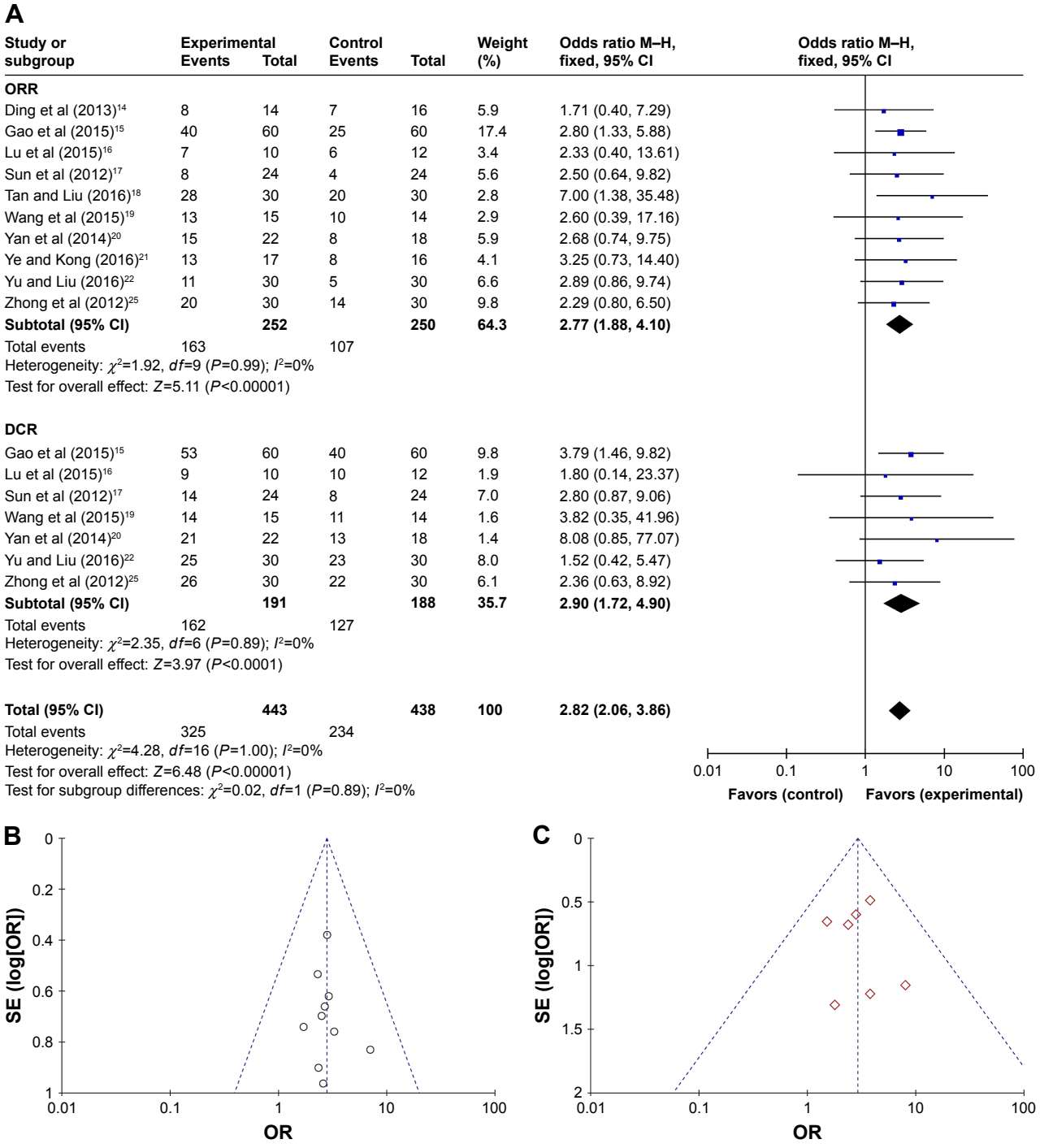

Figure 2 (**A**) Forest plot of the comparison of ORR and DCR between the experimental and control groups. (**B**) Funnel plot of ORR. (**C**) Funnel plot of DCR. Control group, chemotherapy alone group; experimental group, chemotherapy plus DC–CIK immunotherapy. The fixed-effects meta-analysis model (Mantel–Haenszel method) was used.
Abbreviations: CI, confidence interval; DC–CIK, dendritic cells and cytokine-induced killer cells; DCR, disease control rate; IV, inverse variance; M–H, Mantel–Haenszel test; OR, odds ratio; ORR, overall response rate; SE, standard error.

CI =0.18–0.59, P=0.0002), whereas proportions of $CD8^+$ and $CD4^+CD25^+$ were significantly decreased (Figure S5 and Table 4, $CD8^+$: OR =–9.26, CI =–11.58 to –6.95, P<0.00001; $CD4^+CD25^+$: OR =–2.97, CI =–4.44 to –1.50, P<0.0001). Levels of AgNOR, IFN-γ, IL-2, and IL-12 were significantly increased after DC–CIK therapy (Figure S6 and Table 5, AgNOR: OR =1.05, CI =0.40–1.70, P=0.002; IFN-γ: OR =33.91, CI =30.68–37.14, P<0.00001; IL-2: OR =19.56, CI =9.74–29.39, P<0.0001; IL-12: OR =12.25, CI =1.53–22.97, P=0.03), whereas levels of IL-4, IL-6, IL-10,

Table 3 Comparison of CR, nCR, PR, MR, SD, PD, NR, ORR, and DCR between the experimental and control groups

Parameter	Number of patients (n)		Analysis method	Heterogeneity		OR	95% CI	*P*-value
	Experimental group	Control group		I^2 (%)	*P*-value			
CR	252	250	Fixed	0	0.98	2.71	1.60–4.58	0.0002
nCR	105	102	Fixed	0	0.88	1.35	0.58–3.11	0.48
PR	252	250	Fixed	0	1.00	1.49	1.01–2.20	0.04
MR	162	160	Fixed	0	0.79	0.91	0.52–1.57	0.73
SD	100	102	Fixed	0	0.58	0.66	0.35–1.25	0.21
PD	191	188	Fixed	0	0.89	0.34	0.20–0.58	<0.0001
NR	47	46	Fixed	15	0.28	0.24	0.07–0.80	0.02
ORR	252	250	Fixed	0	0.99	2.77	1.88–4.10	<0.00001
DCR	191	188	Fixed	0	0.89	2.90	1.72–4.90	<0.0001

Abbreviations: CI, confidence interval; CR, complete response; DCR, disease control rate; MR, minuteness response; nCR, near CR; NR, nonresponse; OR, odds ratio; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

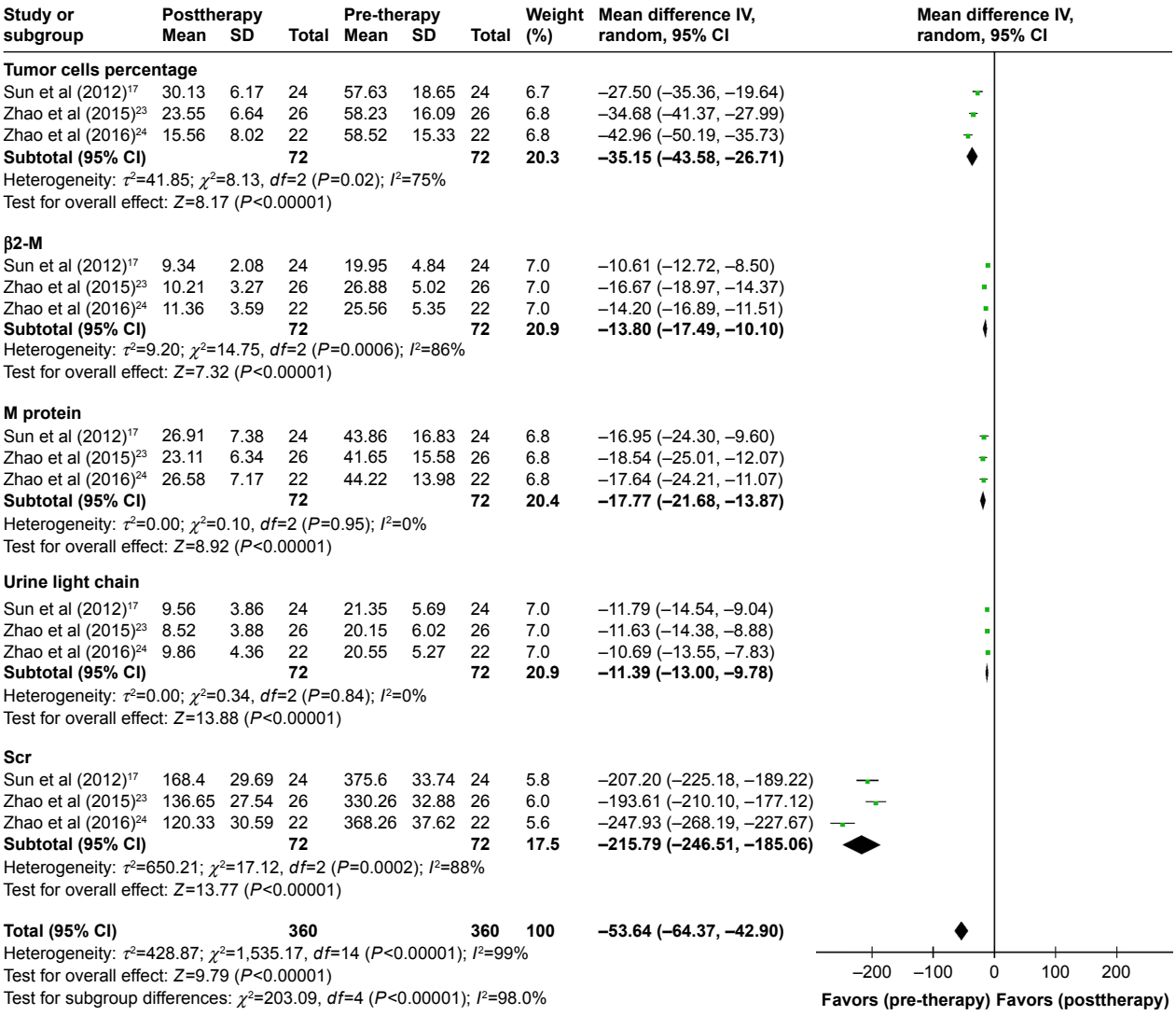

Figure 3 Forest plot of the comparison of percentage of tumor cells, serum level of β2-M, serum level of M protein, 24 h urine light chain content, and Scr level between the experimental and control groups. Control group, chemotherapy alone group; experimental group, chemotherapy plus DC–CIK immunotherapy. The random effects meta-analysis model (inverse variance method) was used.

Abbreviations: CI, confidence interval; DC–CIK, dendritic cells and cytokine-induced killer cells; β2-M, β2-microglobin; IV, inverse variance; Scr, serum creatinine.

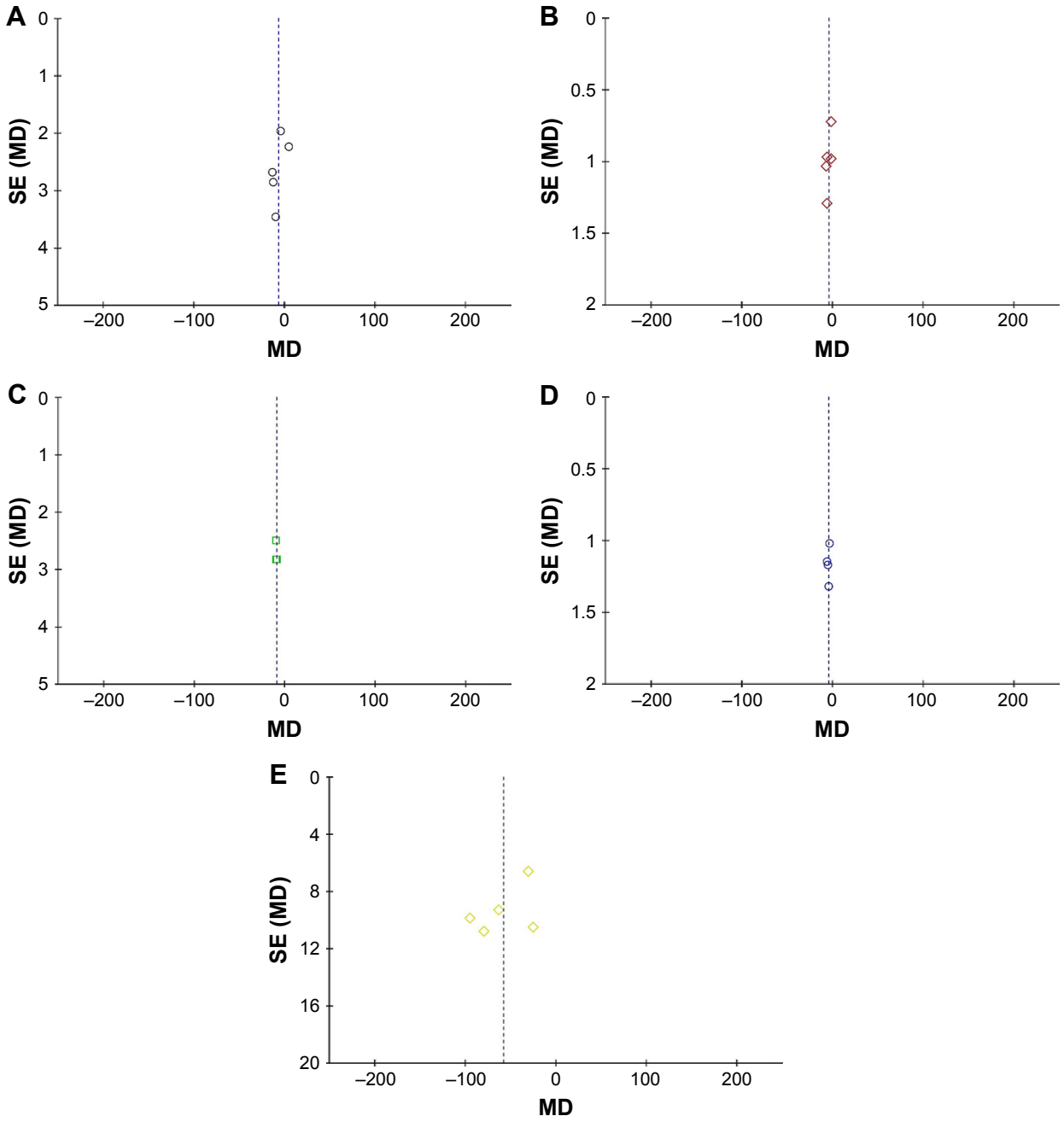

Figure 4 Funnel plot of percentage of tumor cells (**A**), serum level of β2-M (**B**), serum level of M protein (**C**), 24 h urine light chain content (**D**), and Scr level (**E**).
Abbreviations: β2-M, β2-microglobin; MD, mean difference; Scr, serum creatinine; SE, standard error.

and TGF-β were significantly decreased (Figure S6 and Table 5, IL-4: OR =–8.34, CI =–10.06 to –6.62, $P<0.00001$; IL-6: OR =–35.01, CI =–59.02 to –11.00, $P=0.004$; IL-10: OR =–11.10, CI =–13.13 to –9.07, $P<0.00001$; TGF-β: OR =–0.35, CI =–0.58 to –0.13, $P=0.002$).

Adverse events assessment

Safety of DC–CIK therapy was evaluated in this meta-analysis. The most common side effects include chills and fever, which subsided naturally within 24 h without any medical treatment. No serious adverse events or death was reported after DC–CIK therapy (Table 6). However, incidence of side effects in the experimental and control groups was not compared in most included trials. As shown in Figure S7, adverse events including nausea and vomiting, palpitation and chest tightness, myocardial enzyme increased, aminotransferase increased, and gastrointestinal adverse reaction were compared in Tan et al's[18] and Yu et al's[22] studies

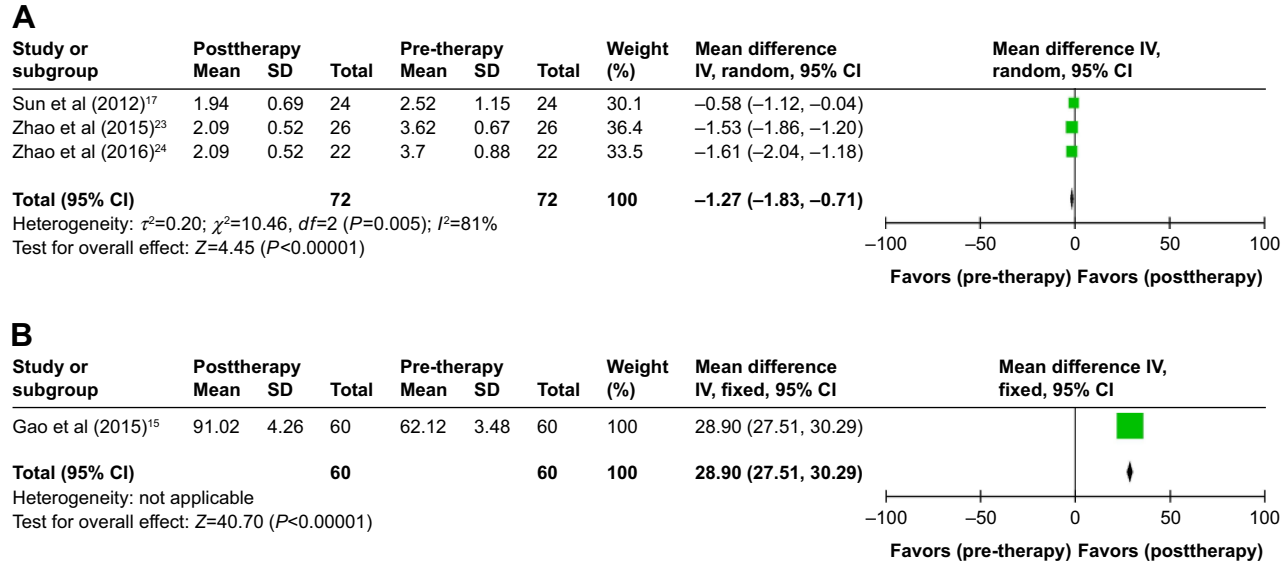

Figure 5 Forest plot of the comparison of Ps (**A**) score and Kps (**B**) in pre- and posttherapy.
Abbreviations: CI, confidence interval; IV, inverse variance; Kps, Karnofsky performance score; Ps, performance status.

and the result showed no significant difference between the two groups (nausea and vomit: OR =0.56, CI =0.12–2.57, *P*=0.45; palpitation and chest tightness: OR =0.73, CI =0.16–3.46, *P*=0.70; myocardial enzyme increased: OR =1.38, CI =0.28–6.80, *P*=0.69; aminotransferase increased: OR =1.00, CI =0.13–7.60, *P*=1.00; gastrointestinal adverse reaction: OR =2.07, CI =0.18–24.15, *P*=0.56).

Sensitivity analysis

Sensitivity analysis was conducted, and two trials[14,18] were excluded because DC was not applied to patients. Moreover, by eliminating two low-quality trials,[20,21] the stability of primary endpoints (ORR and DCR) was numerically verified (Table 7). The results of this analysis were consistent with our overall analysis of the pooled trials.

Discussion

DC–CIK immunotherapy has been considered as a promising option to treat malignancies due to its unique biological characteristics.[28–31] DC–CIK kill tumor cells through FasL- and perforin-mediated pathways upon direct contact and secretion of cytokines such as IL-2, TNF-α, and IFN-γ.[11,29] Furthermore, CIK lyse cancer cells in an MHC-unrestricted manner through activating NK cell receptors such as DNAX accessory molecule-1, NKp46, NKG2D, and NKp30.[28,30,31] In recent years, several studies reported that DC–CIK immunotherapy is a safe and feasible treatment option for MM patients.[23,25] However, the diverse clinical protocols may lead to different therapeutic effects and immune responses. In this study, we investigated a great number of clinical trials to achieve a high statistical reliability.

Our meta-analysis revealed that patients who received adjuvant immunotherapy of DC–CIK combined with chemotherapy presented a more favorable efficacy and prognosis compared to MM patients who received chemotherapy alone. Compared to patients treated by chemotherapy alone, patients treated with combined therapy showed markedly increased ORR (*P*<0.00001) and DCR (*P*<0.0001). As the main evaluation for curative effects and prognosis, percentage of tumor cells and levels of β2-M, M protein, 24 h urine light chains, and Scr also showed promising results in combined therapy.[32–36] Patients' QoL was also significantly improved after combined

Table 4 Comparison of lymphocyte subsets before and after DC–CIK therapy

Parameter	DC–CIK		Analysis method	Heterogeneity		OR	95% CI	*P*-value
	After treatment (n)	Before treatment (n)		I^2 (%)	*P*-value			
$CD4^+$	86	86	Fixed	43	0.17	3.14	1.65 to 4.64	<0.0001
$CD8^+$	86	86	Fixed	55	0.11	–9.26	–11.58 to –6.95	<0.00001
$CD4^+/CD8^+$	86	86	Fixed	72	0.03	0.38	0.18 to 0.59	0.0002
$CD4^+CD25^+$	88	88	Fixed	80	0.002	–2.97	–4.44 to –1.50	<0.0001

Abbreviations: CI, confidence interval; DC–CIK, dendritic cells and cytokine-induced killer cells; OR, odds ratio.

Table 5 Comparison of cytokines before and after DC–CIK therapy

Parameter	CIK/DC–CIK	CIK/DC–CIK	Analysis method	Heterogeneity		OR	95% CI	*P*-value
	After treatment (n)	Before treatment (n)		I^2 (%)	*P*-value			
AgNOR	50	50	Random	67	0.08	1.05	0.40 to 1.70	0.002
IFN-γ	26	26	Random			33.91	30.68 to 37.14	<0.00001
IL-2	86	86	Random	97	<0.00001	19.56	9.74 to 29.39	<0.0001
IL-4	26	26	Random			−8.34	−10.06 to −6.62	<0.00001
IL-6	30	30	Random			−35.01	−59.02 to −11.00	0.004
IL-10	26	26	Random			−11.10	−13.13 to −9.07	<0.00001
IL-12	24	24	Random			12.25	1.53 to 22.97	0.03
TGF-β	56	56	Random	0	0.34	−0.35	−0.58 to −0.13	0.002

Abbreviations: AgNOR, silver-staining nucleolar organizer region; CI, confidence interval; CIK, cytokine-induced killer cells; DC–CIK, dendritic cells and CIK; OR, odds ratio.

therapy ($P<0.00001$). These results indicated that DC–CIK immunotherapy lowered the tumor load possibly through killing tumor cells, by which patients' life quality improved.

Immune reconstitution is important for treating malignancies, and the immunosuppressed status of cancer patients has been reported previously.[8,37] The exercise of immune function depends on a proper $CD4^+/CD8^+$ ratio. Moreover, $CD4^+CD25^+$ regulatory T cells negatively regulated the anti-tumor activity of DC–CIK immune cells.[38] Our analysis showed significantly increased percentages of $CD4^+$ cells and $CD4^+/CD8^+$ ratio and significantly decreased percentages of $CD8^+$ cells and $CD4^+CD25^+$ cells upon DC–CIK immunotherapy ($P<0.00001$). AgNOR is an indicator of rDNA transcriptional activity in the nucleus. T-lymphocyte AgNOR content further reflects the T lymphocyte and extended cellular immune function status.[39] In our analysis, AgNOR was significantly increased after DC–CIK treatment. All these results indicated that the immune function of MM patients was improved upon DC–CIK immunotherapy.

The balance between Th1 and Th2 cells is crucial in DC–CIK immunotherapy. Th1 cytokines, including IFN-γ, IL-2, and IL-12, enhance the cytotoxicity of DC–CIK, whereas Th2 cytokines, such as IL-4, IL-6, IL-10, and TGF-β, are associated with tumor immune escape.[11,12] Our analysis showed that DC–CIK immunotherapy was associated with increased IFN-γ, IL-2, and IL-12 ($P<0.01$) whereas it decreased IL-4, IL-6, IL-10, and TGF-β ($P<0.01$), indicating an important role of Th1/Th2 cytokines in DC–CIK immunotherapy.

Safety is the top priority of the clinical treatment, and it is also key factor for the development of DC–CIK immunotherapy's clinical application. Our analysis showed that DC–CIK was safe for MM therapy. Chills and fever were the most common side effects during DC–CIK therapy but were well tolerated, and no serious adverse events or death occurred during therapy.

There are some limitations in our analysis. First of all, the number of MM patients included in this study is not big enough and the follow-up time was short. Apart from that, the

Table 6 Information of adverse effects during the therapy

Included studies	Control group	Experimental group (events/total)
Ding et al (2013)[14]	Fatigue (7/16), thrombocytopenia (6/16), peripheral neuropathy (II, 5/16)	Fever (2/14)
Gao et al (2015)[15]	ND	ND
Lu et al (2015)[16]	ND	Chills, fever, and skin itching (2/10)
Sun et al (2012)[17]	Dizziness, constipation, fatigue, nausea, rash	Chills and fever (2/24)
Tan and Liu (2016)[18]	Nausea and vomit (5/30), palpitation and chest tightness (3/30), myocardial enzyme increased (3/30), aminotransferase increased (2/30), creatinine increased (4/30)	Nausea and vomit (3/30), palpitation and chest tightness (2/30), myocardial enzyme increased (4/30), aminotransferase increased (2/30), creatinine increased (3/30)
Wang et al (2015)[19]	Herpes zoster virus infection (3/14)	Chills and fever (1/15)
Yan et al (2014)[20]	ND	ND
Ye and Kong (2016)[21]	ND	ND
Yu and Liu (2016)[22]	Gastrointestinal reaction (1/30), palpitation (1/30)	Gastrointestinal reaction (2/30), myocardial enzyme increased (1/30), palpitation (1/30)
Zhao et al (2015)[23]	Nerve terminal injury (5/24)	Chills and fever (2/26)
Zhao et al (2016)[24]	Nerve terminal injury (5/20)	Chills and fever (2/22)
Zhong et al (2012)[25]	ND	Chills and fever (4/30)

Abbreviation: ND, nondetermined.

Table 7 Outcomes of sensitivity analysis

Parameter	Number of patients (n)	Number of patients (n)	Analysis method	Heterogeneity		OR	95% CI	*P*-value
	Exp group	Con group		I^2 (%)	*P*-value			
ORR								
With low quality	252	250	Fixed	0	0.99	2.77	1.88–4.10	<0.00001
Without low quality	213	216	Fixed	0	0.97	2.75	1.79–4.21	<0.00001
With CIK therapy	252	250	Fixed	0	0.99	2.77	1.88–4.10	<0.00001
Without CIK therapy	208	204	Fixed	0	1.00	2.67	1.75–4.08	<0.00001
DCR								
With low quality	191	188	Fixed	0	0.89	2.90	1.72–4.90	<0.0001
Without low quality	169	170	Fixed	0	0.92	2.70	1.57–4.64	0.0003

Notes: Con, control group (chemotherapy alone group); Exp, experimental group (chemotherapy plus DC–CIK immunotherapy).
Abbreviations: CI, confidence interval; CIK, cytokine-induced killer cells; DC–CIK, dendritic cells and CIK; DCR, disease control rate; OR, odds ratio; ORR, overall response rate.

12 trials included in this meta-analysis were conducted based on Chinese population. Although the effectiveness of DC-CIK immunotherapy on hematopoietic malignancies has been widely reported,[40–46] its usage on MM is still rare outside of China. One American trial was included firstly upon literature retrieve, but it was then excluded because of insufficient data.[44] There are three registered ongoing clinical trials in which MMs are treated by DC–CIK immunotherapy accompanied with conventional regimens, among which two are registered in the USA (NCT00477035 and NCT00185757) and one in Singapore (NCT00460694). Up to now, no paper based on these trials and meeting our inclusion criteria has been published. We will keep paying close attention to global studies in our later studies. Finally, the selection of chemotherapy regimen is also important for the determination of optimal therapeutic strategy. However, based on currently available literatures, there are insufficient data to perform a statistical analysis to compare different combination therapies. We will keep following up with upcoming clinical trials to obtain relevant data when available.

Conclusion

Our study verified the therapeutic effects and safety of adjuvant DC–CIK immunotherapy in combination with chemotherapy for MM patients. DC–CIK immunotherapy enhances the treatment efficacy of chemotherapy remarkably and improves prognosis of MM patients by reconstructing the immune system. Therefore, adjuvant immunotherapy of DC–CIK combined with chemotherapy is a promising treatment option for MM patients.

Author contributions

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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