Chinese Medical Journal®

Efficacy and safety of chimeric antigen receptor-T cells in the treatment of B cell lymphoma: a systematic review and meta-analysis

Xiao-Hong Zheng[1], Xi-You Zhang[2], Qian-Qian Dong[3], Feng Chen[1], Shou-Bo Yang[1], Wen-Bin Li[1]

[1]Department of Neuro-Oncology, Neurosurgery Center, Beijing Tiantan Hospital, Capital Medical University, Beijing 100070, China;
[2]Department of Nephrology, Beijing Shijitan Hospital, Capital Medical University, Beijing 100038, China;
[3]Department of General Medicine, Ningbo Medical Center, Li Huili Hospital, Ningbo, Zhejiang 315000, China.

Abstract

Background: Conventional treatment has limited efficacy in relapsed/refractory B-cell lymphoma. Since chimeric antigen receptor T-cell (CAR-T) technology has shown high safety and results in high remission rates, we investigated its efficacy and safety in B-cell lymphoma treatment and analyzed potential affecting factors to provide evidence for therapeutic strategies and applications.

Methods: We searched databases including PubMed, Embase, and Cochrane up to July 2019. Meta-analysis 1 was conducted to study the efficacy of CAR-T cell for treating B-cell lymphoma, measuring the response rate and complete remission rate as outcomes. Sub-group analysis was performed for age, pathological type, target antigen, co-stimulatory molecule, and conditioning chemotherapy. Meta-analysis 2 was undertaken on the safety of the treatment with the incidence rate of toxicity (cytokine-releasing syndrome [CRS], neurotoxicity) as an outcome.

Results: Seventeen studies were included in the systematic review and meta-analysis. It was found that CAR-T cells had good therapeutic effects in the following cases: B-cell lymphoma (patients ≥65 years old); diffuse large B-cell lymphoma pathological type; patients with treatment target antigen other than CD19; patients treated with co-stimulatory molecules other than CD28, including 4-1BB+CD28 or 4-1BB; and patients treated with cyclophosphamide/fludarabine pre-treatment protocol conditioning chemotherapy. Although the CRS and neurotoxicity incidences were high, most were reversible with minimal risk of death.

Conclusion: CAR-T cell treatment is safe for clinical application; however, toxicity effects should be monitored.

Keywords: Lymphoma; B-cell; Meta-analysis; Chimeric antigen receptor T-cell

Introduction

Non-Hodgkin lymphoma (NHL) comprises a group of malignant tumors originating from B lymphocytes, T lymphocytes, and natural killer cells. According to the American Cancer Society reports, approximately 71,000 new NHL cases occurred in the United States in 2015, ranking seventh among all malignant tumors and accounting for 3% (19,700) of cancer-related deaths.[1] NHL sub-types include diffuse large B-cell lymphoma (DLBCL; 32.5%), chronic lymphocytic leukemia/small lymphocytic (18.6%), and follicular lymphomas (FL; 17.1%).[2] In 2016, the largest proportion of new B-cell lymphomas cases comprised DLBCL (26%) in the United States, followed by FL (13%), marginal zone (7%), and mantle cell lymphomas (3%).[3]

The standard first-line treatment for DLBCL is rituximab, cyclophosphamide (Cy), doxorubicin, vincristine, and prednisone. Rituximab significantly improves the overall survival (OS) of patients with DLBCL, although 30% to 40% will show relapse and lose sensitivity to the original chemotherapy regimen.[4] Lymphoma recurrence generally occurs within 3 years of initial diagnosis.[5] The prognosis of patients with refractory DLBCL (stable or progression after initial treatment), early relapse (recurrence within 1 year of diagnosis or 6 months after treatment), or progression within 2 years is poor. In these cases, the current standard of treatment is salvage chemotherapy, followed by autologous stem cell transplantation (ASCT). Approximately 30% to 40% of patients with primary refractory disease or early relapse respond to salvage chemotherapy and can be administered ASCT. However,

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000000568

Xiao-Hong Zheng and Xi-You Zhang contributed equally to this work.

Correspondence to: Prof. Feng Chen, Department of Neuro-Oncology, Neurosurgery Center, Beijing Tiantan Hospital, Capital Medical University, Beijing 100070, China
E-Mail: chenfeng406@sina.com

Chinese Medical Journal 2020;133(1)

Received: 19-08-2019 **Edited by:** Peng Lyu

approximately 50% of the cases eventually relapse,[6] exhibiting poor prognosis; particularly, those who show relapse within 6 months of ASCT treatment, have an OS of 5.7 months.[7]

Recent advances in immunotherapy for hematological tumors include chimeric antigen receptor T cell (CAR-T) technology in B cell lymphoma. The basic structure of the artificial fusion CAR protein includes antigen recognition, transmembrane, and intracellular domains.[8] CAR-T cells comprise autologous T cells genetically engineered to express a tumor-targeting CAR. An ideal target for CAR-T treatment of B-cell lymphoma is CD19, a transmembrane glycoprotein that regulates B cell activation in an antigen receptor-dependent manner, as CD19 is expressed throughout B cell differentiation and often during B cell malignant transformation.[9] CAR-T cell treatment targeting CD19 is the most comprehensive and widely used clinical treatment strategy,[10] although CD20,[11] CD22,[12] κ light chain,[13] and receptor-tyrosine-kinase-like orphan receptor 1[14] are also considered as potential targets.

Nevertheless, CAR-T cell therapy-associated toxicity, including cytokine-release syndrome (CRS), neurotoxicity, and tumor lysis syndrome (TLS), cannot be neglected.[15] Accordingly, CAR-T cell immunotherapy efficacy and safety constitute research hotspots. A systematic review and meta-analysis of all published clinical trials on CD19 and CD20 CAR-T therapy efficacy and safety for B-cell hematologic malignancies indicated encouraging response rates (RRs) in B cell lymphoma and leukemia, particularly in patients with acute lymphoblastic leukemia.[16] Additionally, a recent systematic review regarding anti-CD19 CAR-T cell efficacy for B-cell malignancies showed a high clinical RR.[17] In this meta-analysis study, we have comprehensively searched studies relevant to CAR-T cell treatment for B-cell lymphoma, and quantitatively synthesized and analyzed CAR-T cell efficacy and safety. We further evaluated the efficacy of CAR-T cell therapy by sub-group analyses. We aimed to provide robust proof for more rational and effective application of CAR-T technology.

Methods

Search strategy

We carefully searched article databases (PubMed, EMBASE, and Cochrane Library) to identify relevant studies published up to July 1, 2019, using "lymphoma" or "receptors" combined with free words like "malignant," "B Cell" or "Chimeric."

(1) Subject word: Lymphoma; Free words: Lymphomas; Lymphoma, Malignant; Lymphomas, Malignant; Malignant Lymphoma; Malignant Lymphomas; Lymphoma, B Cell; Lymphoma, B-Cell; Lymphomas, B Cell; Lymphomas, B-Cell; B Cell Lymphoma; B Cell Lymphomas; B-Cell Lymphoma; B-Cell Lymphomas;
(2) Subject word: Receptors, Chimeric Antigen; Free words: Antigen Receptors, Chimeric; Artificial T Cell Receptors; Artificial T-Cell Receptors; Receptors, Chimeric T Cell; Receptors, Chimeric T-Cell; Receptors, Artificial T Cell; Receptors, Artificial T-Cell; T Cell Receptors, Artificial; T-Cell Receptors, Artificial; T Cell Receptors, Chimeric; T-Cell Receptors, Chimeric; CAR; CAR-T; CAR-T Cell; Chimeric Antigen Receptors; Chimeric Antigen Receptors T Cell; Chimeric Antigen Receptors T-Cell; Chimeric Antigen Receptors-modified T Cell; Chimeric Antigen Receptors-modified T-Cell; Chimeric Antigen Receptors-transduced T Cell; Chimeric Antigen Receptors-transduced T-Cell; Chimeric T Cell Receptors; Chimeric T-Cell Receptors; Chimeric Immunoreceptors; Immunoreceptors, Chimeric.

To guarantee a comprehensive search and appraise all potentially relevant studies, we also examined the reference lists of identified articles and previous meta-analysis.

Inclusion and exclusion criteria

A study needed to have met the following criteria to be considered eligible: (1) related to CAR-T cell therapy for B cell lymphoma; (2) involved patient(s) diagnosed with histopathologically confirmed B-cell lymphoma; (3) reported necessary information completely or partially, such as total patient number, age, pathological type, target antigen, co-stimulatory molecules, and conditioning chemotherapy; (4) provided efficacy evaluation, number of CRS and neurotoxicity cases following CAR-T cell treatment; and (5) related to human clinical trial, published in English.

Studies were excluded if they fell into any of the following categories: (1) from incomplete data or inconsistent research, for example, with unclear sample size; (2) repeated publication or similar research; (3) reviews, letters, reports, conference abstract or paper, mail articles, and editorials; (4) had obvious flaws in statistical methods or experimental design.

Data extraction

Based on the inclusion and exclusion criteria, independent duplicate data extraction was performed by two reviewers (ZXY, ZXH) using a pre-designed data collection form. Any discrepancies were resolved by seeking an opinion from a third reviewer (CF) and discussions between reviewers. The quality of all eligible studies was independently evaluated using the Cochrane Collaboration risk of bias tool (GRADEprofiler software). Information collected included the first author, publication time, total number of patients, age, pathological type, target antigen, co-stimulatory molecule(s), conditioning chemotherapy, efficacy evaluation, and the number of CRS and neurotoxicity cases. Missing data were obtained by emailing the corresponding authors.

Outcome indicators

We included three outcome indicators: RR (the percentage of patients with complete or partial remission out of all patients), complete remission rate (CRR; the percentage of complete remission [CR] patients out of all patients), and grade 3/4 side effects rate (percentage toxic side effect out of the total number of patients. Efficacy was evaluated by the best results achieved following CAR-T cell treatment.

Statistical analysis

The statistical analyses were performed using Stata 12.0 (StataCorp, College Station, TX, USA). For CAR-T cell therapeutic regime efficacy, we calculated the RRs and CRRs, with 95% confidence intervals. We assessed between-study heterogeneity using the Chi-square test (Q statistic) and the I^2 statistic. If $P \geq 0.10$ and/or $I^2 < 50\%$, a fixed effect (Mantel-Haenszel method) model was used because heterogeneity was regarded as low; otherwise, a random-effects (Mantel-Haenszel method) model was employed. Sensitivity analysis was conducted by eliminating individual studies one at a time. Sub-group analysis for the following variables were undertaken to explore the potential effects of different factors on the outcome measures: age (<65 *vs.* ≥65 years), pathological type (DLBCL *vs.* non-DLBCL), target antigen (CD19 *vs.* non-CD19), co-stimulatory molecule (CD28 *vs.* non-CD28), and pre-treatment protocol (Cy/fludarabine [Flu] *vs.* non-Cy/Flu). We assessed publication bias using Begg and Egger tests and defined significant publication bias as a P value <0.05.

Results

Study characteristics and quality assessment

We first identified 2210 studies, of which 17 (with data for 280 patients) were included in the analysis [Figure 1]. The classification and features of all studies included are shown in Table 1. Only one large multi-center research sample was found because CAR-T cell immunotherapy, as an emerging anti-tumor treatment, has not been widely utilized in the clinic. All included studies represented one-arm trials because control groups could not be ethically established. All studies were independently evaluated for quality using the Cochrane Collaboration risk of bias tool. The clinical studies of CAR-T cell therapy conducted were single-arm studies with small sample size. Therefore, we performed a quality evaluation of the included single-arm clinical research, and the results revealed were extremely low evidence.

Meta-analysis

Overall RR and CRR outcomes

All 17 studies assessed the overall RR and CRR during CAR-T cell treatment. Pooling of the data revealed an overall RR of 63% (95% confidence interval [CI]: 0.41–0.85) [Figure 2] and CRR of 39% (95% CI: 0.25–0.54) [Figure 3], each showing significant heterogeneity ($I^2 = 97.3\%$, $P < 0.001$) and ($I^2 = 92.8\%$, $P < 0.001$), respectively. Analysis using the random effect version of the Mantel-Haenszel method confirmed the considerable efficacy of CAR-T cell treatment in B cell lymphoma.

Sub-group analysis on age

The RR and CRR for age group were evaluated in 14 studies. The older patients (≥65 years old) appeared to have higher RRs (79%, 95% CI: 0.55–1.04) than younger

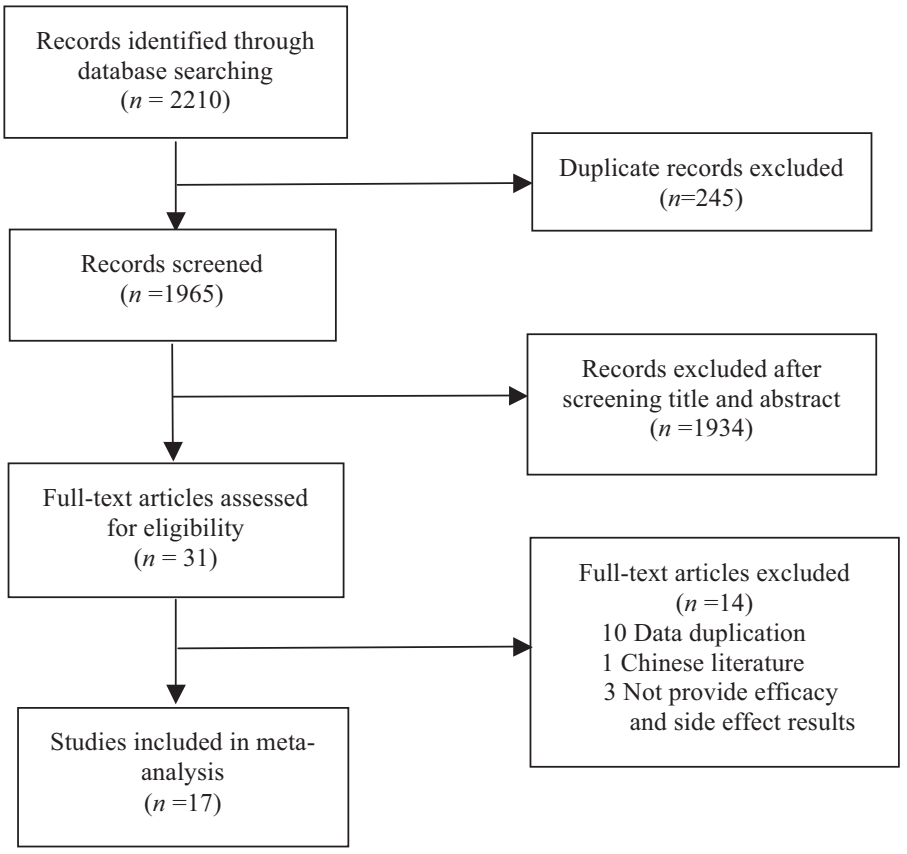

Figure 1: Flow diagram of the study selection process. A total of 280 patients were enrolled.

Table 1: Basic characteristics of the included studies.

Studies	Age (years)	Sample size, *n*	Pathological type	Target antigen	Co-stimulatory molecule	Conditioning chemotherapy	Efficacy evaluation	Number of CRS	Number of neuro-toxicity cases
Jensen *et al*[11] 2010	–	4	FL/DLBCL	CD19/CD20	None	Flu/Auto-HSCT	RR: 2/4 CRR: 2/4	0	0
Savoldo *et al*[18] 2011	46–59	6	SLL/TFL/ DLBCL/PCNSL	CD19	CD28	None	RR: 0/6 CRR: 0/6	0	0
Kochenderfer *et al*[19] 2012	48–63	5	FL/SMZL	CD19	CD28	Cy + Flu	RR: 4/5 CRR: 0/5	4	1
Till *et al*[20] 2012	28–80	3	MCL/FL	CD20	CD28 + 4-1BB	Cy	RR: 3/3 CRR: 2/3	1	0
Wang *et al*[21] 2014	37–85	7	DLBCL	CD20	4-1BB	None/COD/COED/ CHODE/ESHAP	RR: 5/7 CRR: 1/7	1	0
Kochenderfer *et al*[22] 2015	30–64	11	SMZL/PMBCL/ DLBCL/ low-grade NHL	CD19	CD28	Cy + Flu	RR: 8/11 CRR: 5/11	11	5
Brudno *et al*[23] 2016	44–63	10	DLBCL/MCL/TFL	CD19	CD28	None	RR: 2/10 CRR: 1/10	3	0
Ramos *et al*[13] 2016	53–75	7	DLBCL/TFL/ MCL/LPL	κ light chain	CD28	None/Cy	RR: 3/7 CRR: 2/7	0	0
Turtle *et al*[24] 2016	36–70	30	LBCL/TFL/ MCL/FL	CD19	CD28 + 4-1BB	Cy/Cy + E/Cy + Flu	RR: 19/30 CRR: 10/30	4	9
Wang *et al*[25] 2016	23–75	16	DLBCL/MCL	CD19	None/CD28	Auto-HSCT	RR: 15/16 CRR: 13/16	0	0
Zhang *et al*[26] 2016	25–70	11	DLBCL/FL/ MCL/PCMZL	CD20	4-1BB	None/CHOP/MACH/ Cy + Flu/EOCH/ CHOD/CHODE	RR: 9/11 CRR: 6/11	0	0
Kochenderfer *et al*[27] 2017	26–67	22	DLBCL/FL/ PMBCL/MCL	CD19	CD28	Cy + Flu	RR: 16/22 CRR: 12/22	10	12
Locke *et al*[28] 2017	29–69	7	DLBCL	CD19	CD28	Cy + Flu	RR: 5/7 CRR: 4/7	1	4
Neelapu *et al*[29] 2017	23–76	101	DLBCL/FL/PMBCL	CD19	CD28	Cy + Flu	RR: 83/101 CRR: 55/101	13	28
Schuster *et al*[30] 2017	25–77	28	DLBCL/FL	CD19	CD28	Cy + Flu	RR: 18/28 CRR: 16/28	5	3
Enblad *et al*[31] 2018	39–70	9	DLBCL/MCL/FL	CD19	CD28 + 4-1BB	None/Cy + Flu	RR: 3/9 CRR: 3/9	1	1
Ramos *et al*[32] 2018	46–75	13	DLBCL/SLL/BCLU	CD19	CD28 + 4-1BB	Cy + Flu	RR: 9/13 CRR: 7/13	0	–

FL: Follicular lymphoma; DLBCL: Diffuse large B-cell lymphoma; SLL: Small lymphocytic lymphoma; TFL: Transformed follicular lymphoma; PCNSL: Primary central nervous system lymphoma; SMZL: Splenic marginal lymphoma; MCL: Mantle cell lymphoma; PMBCL: Primary mediastinal large B-cell lymphoma; NHL: Non-Hodgkin's lymphoma; LPL: Lymphocyte lymphoma; LBCL: Large B cell lymphoma; PCMZL: Primary skin marginal zone B-cell lymphoma; BCLU: B-cell lymphoma characterized by DLBCL and Burkitt lymphoma; Flu: Fludarabine; Cy: Cyclophosphamide; Auto-HSCT: Autologous hematopoietic stem cell transplantation; COD: Cyclophosphamide + vincristine + dexamethasone; COED: Cyclophosphamide + vincristine + etoposide + dexamethasone; CHODE: Cyclophosphamide + doxorubicin + vincristine + dexamethasone + etoposide; ESHAP: Methylprednisolone + etoposide + carboplatin + high dose cytarabine; CHOP: Cyclophosphamide + doxorubicin + vincristine + prednisone; MACH: Mitoxantrone + cytarabine + cyclophosphamide + doxorubicin; EOCH: Etoposide + vincristine + cyclophosphamide + doxorubicin; CHOD: Cyclophosphamide + doxorubicin + vincristine + dexamethasone; Cy + E: Cyclophosphamide + etoposide; RR: Response rate; CRR: Complete remission rate; CRS: Cytokine release syndrome; –: No data.

patients (<65 years old) (62%, 95% CI: 0.35–0.89) [Supplementary Figure S1, http://links.lww.com/CM9/A139]. Both groups exhibited heterogeneity ($I^2 = 97.2\%$, $P < 0.001$ for the older, and $I^2 = 98.2\%$, $P < 0.001$ for the younger); therefore, the Mantel-Haenszel random-effect model was used. The respective CRRs for the older and younger groups were 29% (95% CI: 0.17–0.41) and 61% (95% CI: 0.24–0.98) with significant heterogeneity observed in both groups ($I^2 = 84.9\%$, $P < 0.001$; $I^2 = 98.2\%$, $P < 0.001$, respectively) [Supplementary Figure S2, http://links.lww.com/CM9/A140]. The pooled results showed better efficacy in the senior group.

Sub-group analysis outcome on pathological type

All 17 studies included the RR and CRR of CAR-T cell therapy by pathological type (DLBCL *vs*. non-DLBCL). The RR from pooled data for DLBCL cases was 61% (95% CI: 0.37–0.86) and this was higher than that for non-DLBCL cases (55%, 95% CI: 0.28–0.82) [Supplementary Figure S3, http://links.lww.com/CM9/A141]. Again, the Mantel-Haenszel random-effect model was used because of the heterogeneity in both groups ($I^2 = 96.7\%$, $P < 0.001$ and $I^2 = 97.8\%$, $P < 0.001$, respectively). The respective CRRs were 43% (95% CI: 0.21–0.66) and 39% (95% CI: 0.16–0.62), with significant heterogeneity observed in both groups ($I^2 = 95.4\%$, $P < 0.001$; $I^2 = 98.3\%$, $P < 0.001$) [Supplementary Figure S4, http://links.lww.com/CM9/A142]. The pooled outcomes indicated better efficacy in the DLBCL group.

Sub-group analysis outcome on target antigen

All 17 trials presented the RR and CRR of CAR-T cell therapy on target antigen sub-group (CD19 *vs*. non-CD19). Pooled analyses revealed a higher RR of 88% (95% CI: 0.74–1.01) for non-CD19 cases compared with 55% (95% CI: 0.31–0.80) for CD19 cases [Supplementary

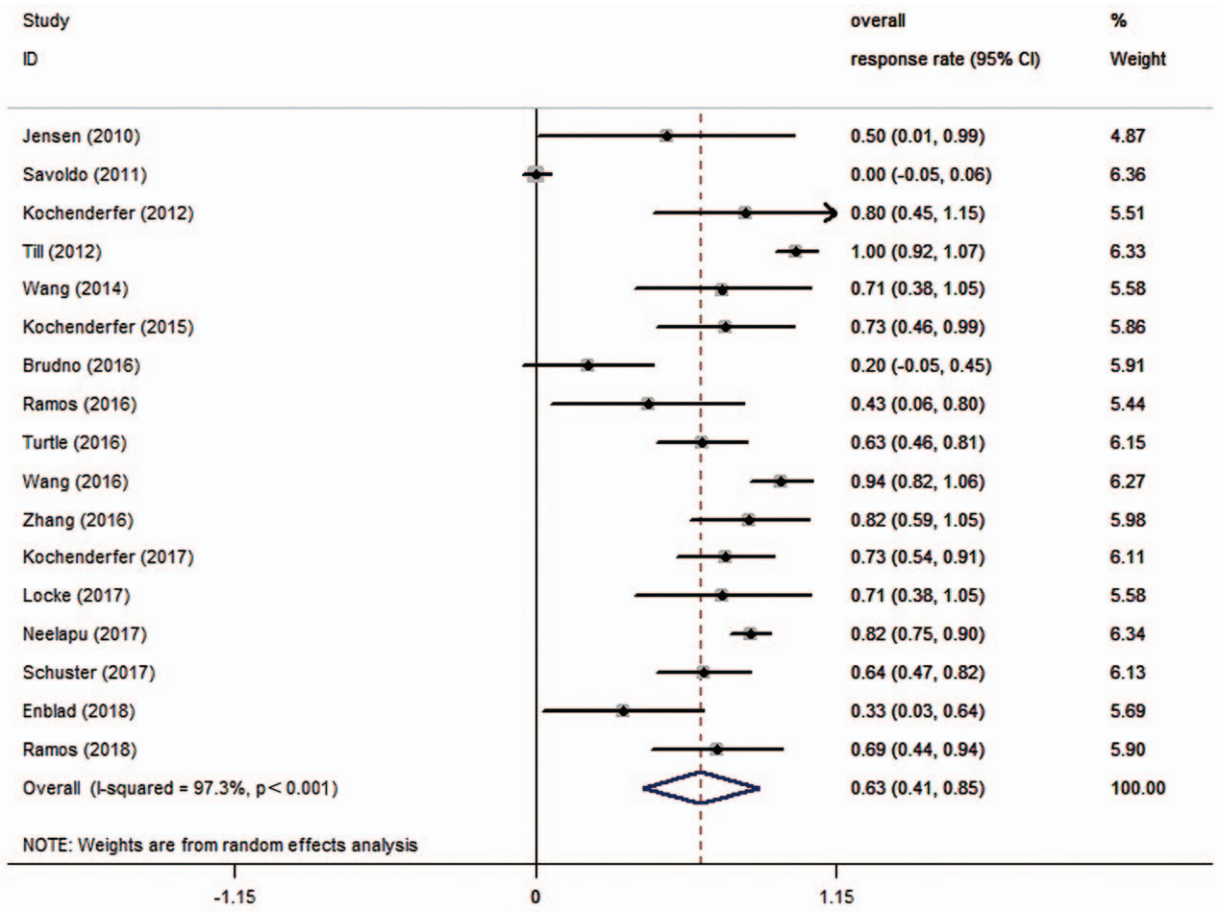

Figure 2: Forest plot of the overall response rate (random effect model). The analysis showed that the overall response rate was 63% (95% CI: 0.41–0.85). CI: Confidence interval; Effect size: Overall response rate.

Figure S5, http://links.lww.com/CM9/A143]. The random-effect model was chosen as both groups exhibited high heterogeneity ($I^2 = 97.6\%$, $P < 0.001$ for CD19 cases and $I^2 = 68.9\%$, $P < 0.001$ for non-CD19 cases). The respective CRRs were 36% (95% CI: 0.20–0.51) and 53% (95% CI: 0.12–0.94), with significant heterogeneity observed in both groups ($I^2 = 94.6\%$, $P < 0.001$; $I^2 = 92.3\%$, $P < 0.001$) [Supplementary Figure S6, http://links.lww.com/CM9/A144]. Analysis of pooled data indicated greater CAR-T cell treatment efficacy in non-CD19 patients.

Sub-group analysis outcomes on co-stimulatory molecule

All 17 studies assessed the RR and CRR after CAR-T cell treatment for the co-stimulatory molecule sub-group, representing CD28 (alone) and non-CD28 (4-1BB domain alone or CD28 + 4-1BB) groups. Non-CD28 cases had a higher RR of 72% (95% CI: 0.51–0.92) in comparison to CD28 cases with a RR of 61% (95% CI: 0.28–0.93) [Supplementary Figure S7, http://links.lww.com/CM9/A145]. The random-effect model was used as both groups exhibited heterogeneity ($I^2 = 98.8\%$, $P < 0.001$ for CD28 cases and $I^2 = 84.0\%$, $P < 0.001$ for non-CD28 cases). The respective CRRs were 41% (95% CI: 0.08–0.73) and 39% (95% CI: 0.25–0.52), with significant heterogeneity observed in both groups ($I^2 = 99.0\%$, $P < 0.001$; $I^2 = 30.7\%$, $P = 0.205$) [Supplementary Figure S8, http://links.lww.com/CM9/A146]. Findings from pooled data suggested greater CAR-T cell efficacy with non-CD28 co-stimulatory molecule(s).

Sub-group analysis outcome on conditioning chemotherapy

All 17 studies assessed the RR and CRR after CAR-T cell treatment for the conditioning chemotherapy sub-group, representing the Cy/Flu (conditioning with Cy/Flu) and non-Cy/Flu groups (no or other conditioning regimens). The Cy/Flu group had a higher RR (74%, 95% CI: 0.66–0.81) than the non-Cy/Flu group (55%, 95% CI: 0.19–0.91) [Supplementary Figure S9, http://links.lww.com/CM9/A147]. The Mantel-Haenszel random-effect model was used as both groups exhibited heterogeneity ($I^2 = 19.3\%$, $P = 0.271$ and $I^2 = 98.2\%$, $P < 0.001$, respectively). The respective CRRs were 45% (95% CI: 0.22–0.67) and 32% (95% CI: 0.12–0.52) for the Cy/Flu and non-Cy/Flu groups and both groups showed significant heterogeneity ($I^2 = 93.8\%$, $P < 0.001$; $I^2 = 89.0\%$, $P < 0.001$, respectively) [Supplementary Figure S10, http://links.lww.com/CM9/A148]. The results confirmed a better efficacy in cases with Cy/Flu conditioning chemotherapy.

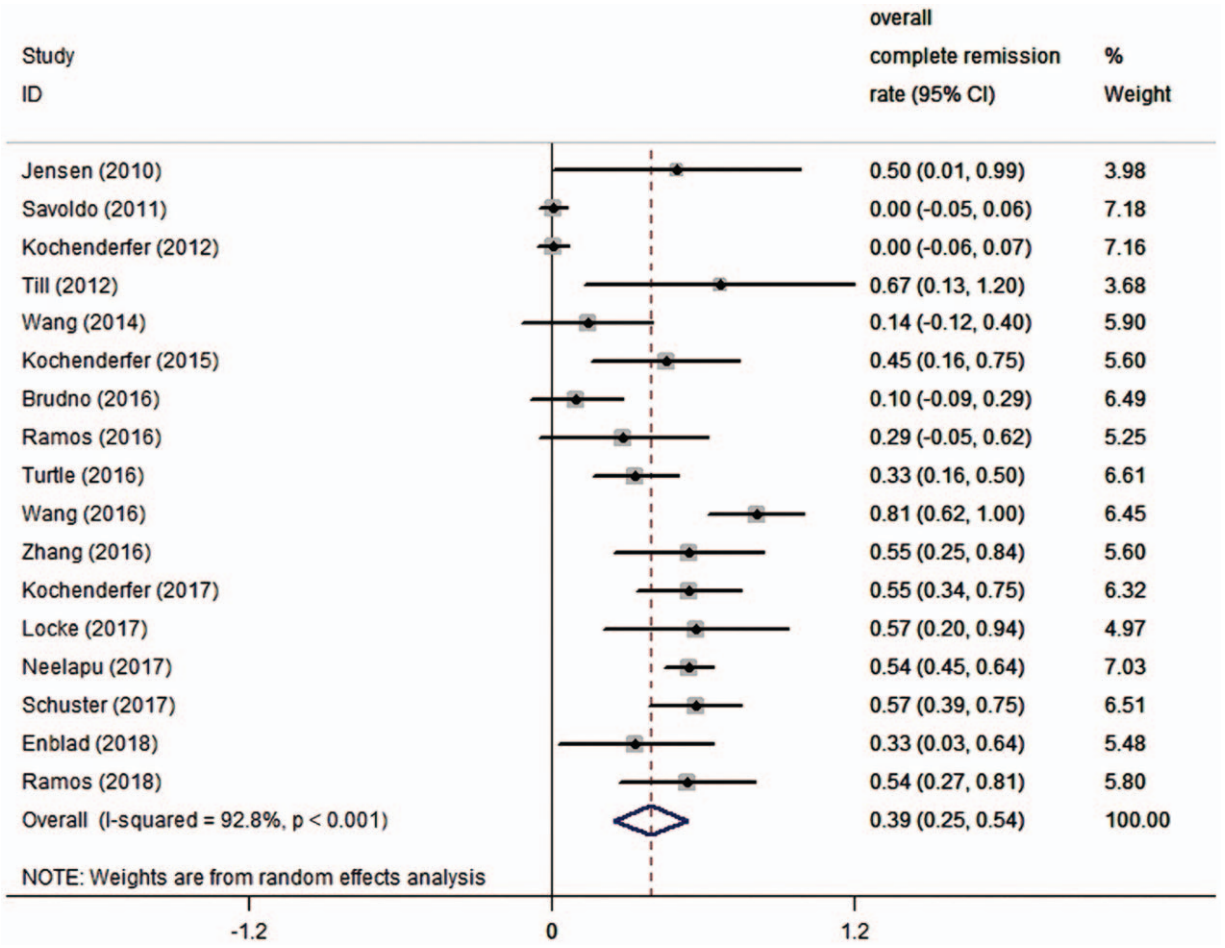

Figure 3: Forest plot of the overall complete remission rate (random effect model). The analysis showed that the overall complete remission rate was 39% (95% CI: 0.25–0.54). CI: Confidence interval; Effect size: Overall complete remission rate.

Total CRS incidence rate

All 17 studies assessed the CRS incidence rate during CAR-T cell treatment. Pooling of the data indicated an incidence rate of 21% (95% CI: 0.03–0.39) [Figure 4], with significant heterogeneity ($I^2 = 99.1\%$, $P < 0.001$). The pooled outcome analysis employing the Mantel-Haenszel random-effect model showed an increased likelihood of grade 3/4 CRS during CAR-T cell therapy.

Total neurotoxicity incidence rate

Pooled analysis of the 16 studies that assessed the neurotoxicity incidence rate during CAR-T cell treatment indicated a total incidence rate of 9% (95% CI: 0.04–0.14) [Figure 5], with significant between-study heterogeneity ($I^2 = 83.7\%$, $P < 0.001$). Again, an increased likelihood of grade 3/4 neurotoxicity during CAR-T cell therapy was observed from the pooled analyses of the studies.

Analysis of publication bias

We performed a publication bias analysis using the Begg and Egger method [Table 2]. The funnel chart of Begg and Egger tests is shown included in [Figure 6]. If P value >0.05 was met in both methods, it was regarded as no publication bias. Publication bias was identified for RR, in the older, non-CD19, non-CD28, and Cy/Flu groups from the pooled data of all studies included. For CRR, publication bias occurred in the younger, CD19, and non-Cy/Flu groups. Publication bias was also evident in the CRS and neurotoxicity incidence rates.

Sensitivity analysis

One study[18] was found to have the most significant heterogeneity in both overall RR and CRR [Figure 7A and 7B]. There are three studies[22,27,29]with significant heterogeneity in CRS and neurotoxicity incidence rate [Figure 7C and 7D].

Discussion

Lymphoma treatments have evolved significantly since the advent of rituximab as the first successful immunotherapy for B-cell NHL in the mid-1990s, resulting in multiple new targeted and immunotherapeutic approaches. Recently, CAR-T cell immunotherapy was introduced as a promising strategy for the remaining 35% of patients with aggressive B-cell NHL who still experience disease progression following standard frontline therapy.[33]

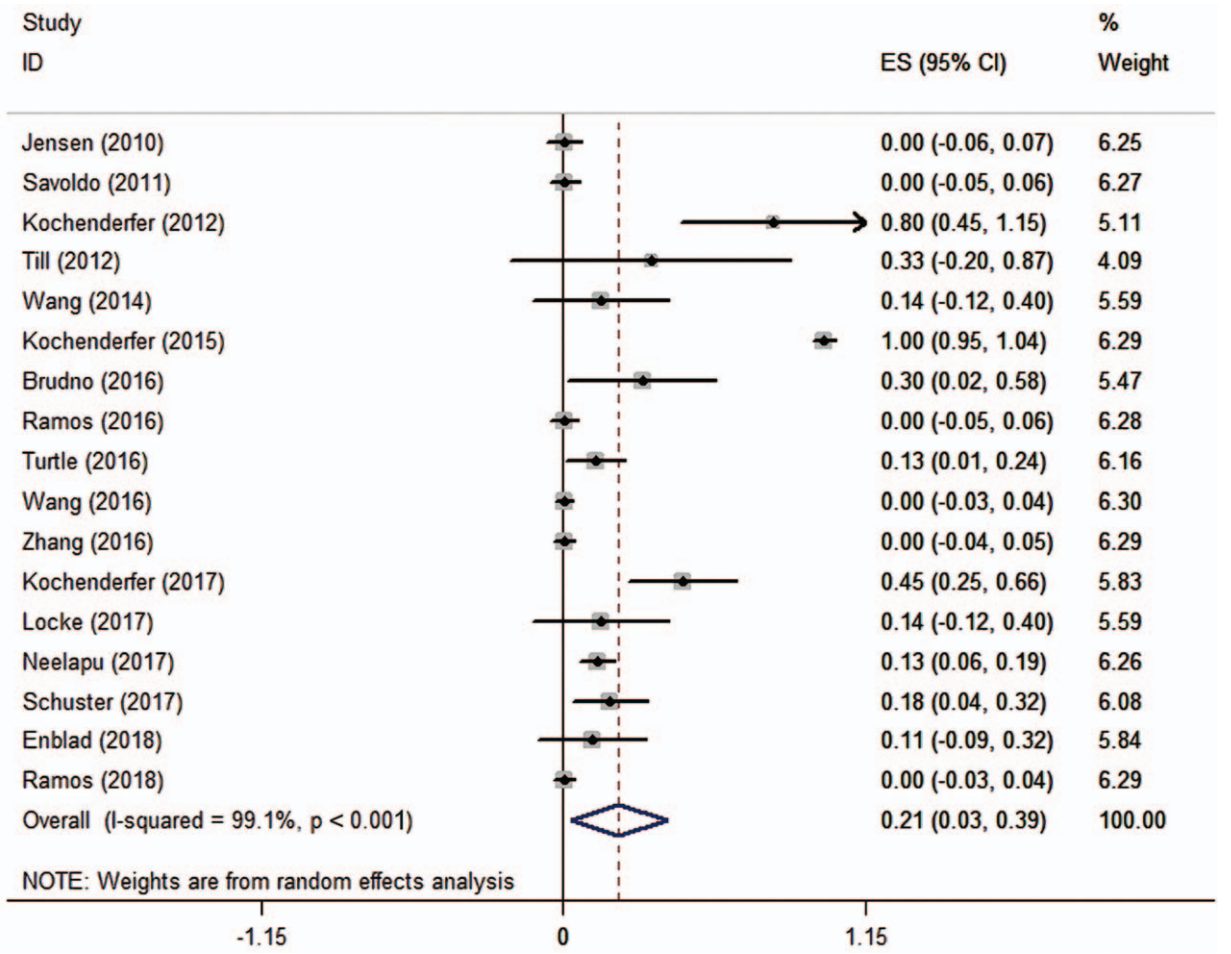

Figure 4: Forest plot of the total incidence rate of cytokine release syndrome (random effect model). The analysis showed that the total incidence rate of cytokine release syndrome 21% (95% CI: 0.03–0.39). CI: Confidence interval; Effect size (ES): Cytokine release syndrome incidence rate.

To date, the vast majority of B-cell lymphomas treated with anti-CD19-CAR-T cells have constituted aggressive B-cell lymphomas, especially DLBCL. For example, the use of first-generation anti-CD19-CAR-T (without a co-stimulatory domain) for treating patients with B-cell lymphoma found no response to the therapy in two patients with FL.[11] Treatment of patients with B-cell lymphoma using second-generation anti-CD19-CAR-T with a CD28 co-stimulatory domain yielded significant proliferation of CAR-T cells through the co-stimulatory effects of CD28, albeit no objective therapeutic response was observed in patients with DLBCL.[18] More importantly, these patients were administered CAR-T cell therapy for at least 6 weeks after the last chemotherapy and were not administered any pre-treatment conditioning chemotherapy.

The first significant efficacy of anti-CD19-CD28 CAR T cells against DLBCL was observed following Cy/Flu chemotherapy pre-treatment.[22] Among the 11 recruited patients with refractory aggressive B-cell lymphoma, five achieved complete remission and three showed partial remission. The duration of the response of responsive patients ranged from 38 to 56 months.[22] Additionally, a clinical trial in which CD19 CAR-T cells with a 4-1BB co-stimulatory domain was administered with a 1:1 CD4+/CD8+ ratio to 32 adults with relapsed and/or refractory B cell NHL following Cy-based lymphodepletion chemotherapy with or without Flu yielded a total objective RR and CRR of 63% and 33%, respectively.[24]

Although numerous studies have confirmed the marked efficacy of CAR-T cells in B-cell lymphoma, severe toxicity remains the biggest obstacle when incorporating this technology. Common adverse events include CRS (the most frequent), neurotoxicity, off-target effects, and TLS. CRS is derived from the formation of cytokine storms that can lead to tissue damage, with grade 3/4 CRS especially requiring immediate intervention.[34] In the trial of the CAR-T cell plus co-stimulatory domain therapy,[24] 12.5% of patients developed severe CRS and were admitted to the intensive care unit for immunosuppressant treatment. In another trial,[27]13.6% of patients required anti-hypertensive drugs and 9% needed mechanical ventilation to assist with breathing. Neurotoxicity represents a series of reversible nervous system syndromes of unknown pathogenesis. Particularly, grade 3/4 neurotoxicity may be life-threatening. Suggested etiologies include a lack of conditioning chemotherapy pre-treatment[35] or interleukin (IL)-6 concentration.[36]

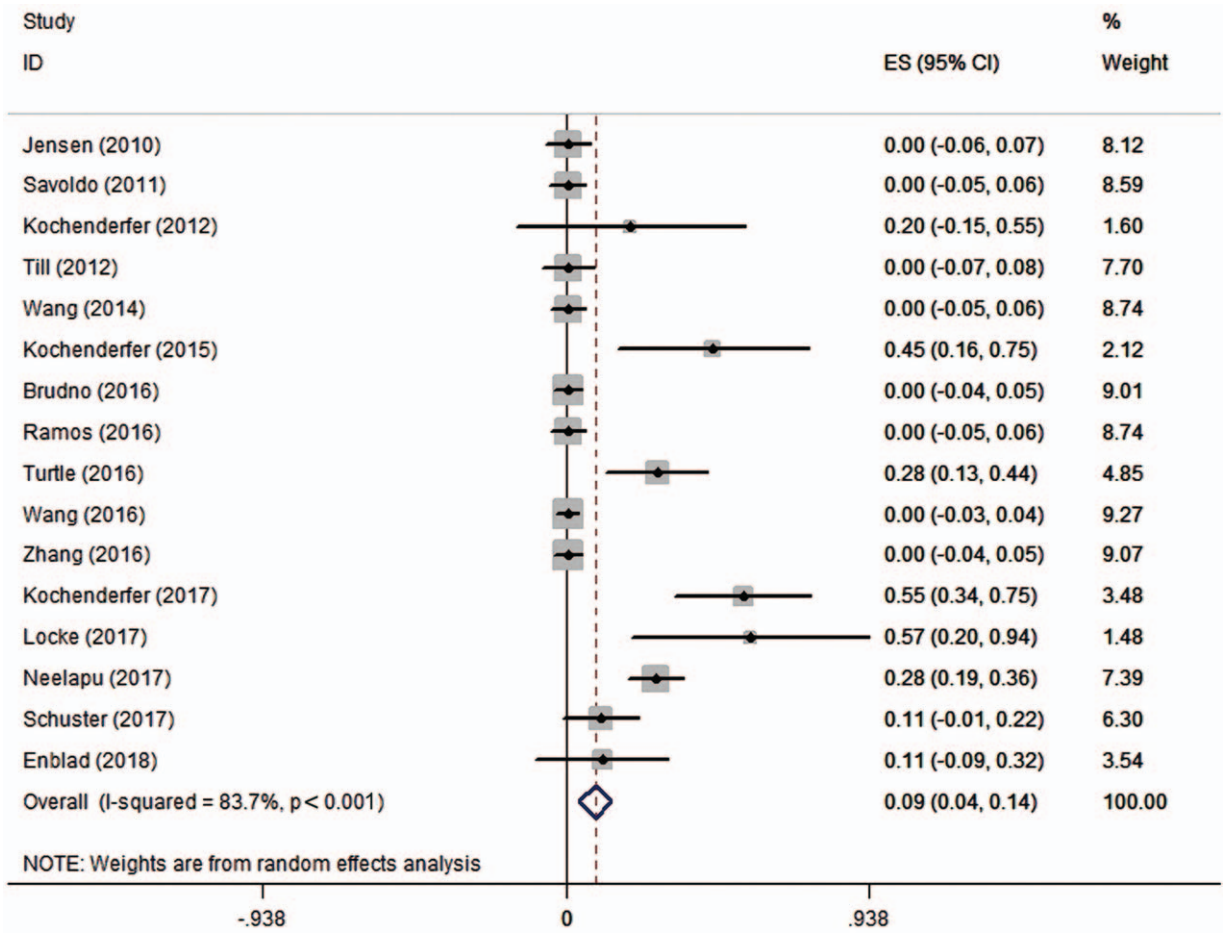

Figure 5: Forest plot of the total incidence rate of neurological toxicity (random effect model). The analysis showed that the total incidence rate of neurological toxicity 9% (95% CI: 0.04–0.14). CI: Confidence interval; Effect size (ES): Neurological toxicity incidence rate.

Most studies of CAR-T cell therapy of B-cell lymphoma indicated that many CAR-T treatment-related toxicities could be eliminated within two weeks of CAR-T cell infusion. Turtle *et al*[24] reported that the anti-IL-6 receptor antibody tocilizumab could successfully reduce CRS toxicity in patients with B-cell lymphoma; this agent has since become the first-line drug for CRS treatment. Steroid hormones are considered to be the alternative first-line drug for neurotoxicity therapy as they can penetrate the blood-brain barrier.[37]

Our meta-analysis showed that the overall RR and CRR of CAR-T cell therapy for B-cell lymphoma was 63% and 39%, respectively, confirming its good efficacy. According to the results of sub-group analysis, the following CAR-T cell therapy conditions yielded higher efficacy: ≥65 years old, DLBCL pathological type, non-CD19 target antigen, 4-1BB with or without CD28 as the co-stimulatory molecule (s), and Cy/Flu pre-treatment regime. The incidence rates of grade 3/4 CRS and grade 3/4 neurotoxicity were 21% and 9%, respectively during CAR-T cell treatment for B-cell lymphoma. Although the incidence of toxicity was elevated, few fatal adverse events occurred during the trial and no impact on patient quality of life was observed following active and timely treatment, demonstrating a fair degree of safety of CAR-T cell immunotherapy.

Based on the results of the Chi-square test (Q statistic) and I^2 statistic, the random model was selected to pool the effect size. At the same time, the sub-group analysis results did not reduce the heterogeneity, which may be attributed to the differences in the sample size of each study and individual heterogeneity. The sample size of each study was significantly different. There were eight studies with sample sizes of less than 10,[11,13,18-21,28,31] whereas one study involved 101 patients.[29] Additionally, CAR-T cell infusion was administered at different doses, ranging from 2.0×10^5cells/kg[24] to 3×10^7cells/kg.[19] This indicates that the source of article heterogeneity may be individual heterogeneity.

Compared with other meta-analyses regarding CAR-T treatment,[16,17] the study offers several advantages. First, we included more studies, and this should have improved outcome reliability. Second, we performed sub-group analyses on age, pathological type, target antigen, co-stimulatory molecule, and conditioning regimen, Hence the analysis is more comprehensive and the results are more convincing. Third, we included only patients with B cell lymphoma among B-cell hematologic malignancies, rendering the study more specifically targeted. Furthermore, we have not only analyzed anti-CD19 CAR-T cells, but also anti-CD20 CAR-T cells.

Table 2: Summary of the meta-analysis.

Parameters	I^2 value (%)	*P* value (*Q test*)	*P* value (Begg)	*P* value (Egger)
Overall RR	97.3	<0.001	0.118	0.352
Overall CRR	92.8	<0.001	0.934	0.005
Age-RR	97.2	<0.001	0.138	0.170
Young	98.2	<0.001	0.032	0.727
Senior				
Age-CRR	84.9	<0.001	0.199	<0.001
Young	98.2	<0.001	0.345	0.762
Senior				
Pathological type-RR	96.7	<0.001	0.153	0.615
DLBCL	97.8	<0.001	0.488	0.923
N-DLBCL				
Pathological type-CRR	95.4	<0.001	0.582	0.727
DLBCL	98.3	<0.001	0.124	0.568
N-DLBCL				
Target antigen-RR	97.6	<0.001	0.583	0.193
CD19	68.9	0.012	0.027	0.012
N-CD19				
Target antigen-CRR	94.6	<0.001	0.428	0.010
CD19	92.3	<0.001	1.000	0.109
N-CD19				
Co-stimulatory molecule-RR	98.8	<0.001	0.474	0.936
CD28	84.0	<0.001	0.452	0.031
N-CD28				
Co-stimulatory molecule-CRR	99.0	<0.001	0.858	0.835
CD28	30.7	0.205	0.707	0.352
N-CD28				
Pretreatment scheme-RR	19.3	0.271	0.917	0.023
Cy/Flu	98.2	<0.001	0.283	0.482
N-Cy/Flu				
Pretreatment scheme-CRR	93.8	<0.001	0.076	0.062
Cy/Flu	89.0	<0.001	0.088	0.037
N-Cy/Flu				
CRS incidence rate	99.1	<0.001	<0.001	0.902
Neurotoxicity incidence rate	83.7	<0.001	<0.001	0.001

RR: Response rate; CRR: Complete remission rate; DLBCL: Diffuse large B-cell lymphoma; N-DLBCL: Non-diffuse large B-cell lymphoma; Flu: Fludarabine; Cy: Cyclophosphamide; CR: Complete remission; CRS: Cytokine release syndrome.

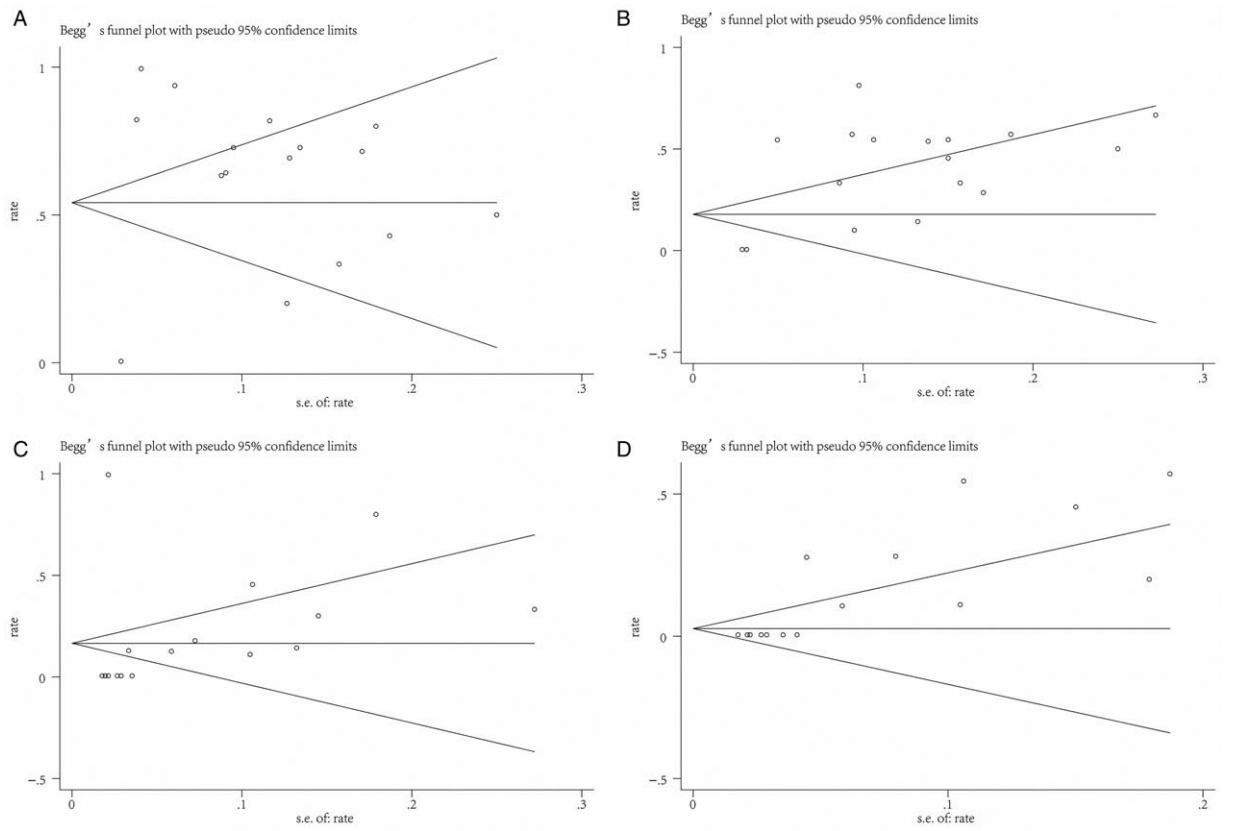

Figure 6: (A) The funnel plot of the total overall response rate. (B) The funnel plot of the total complete remission rate. (C) The funnel plot of cytokine release syndrome incidence rate. (D) The funnel plot of neurological toxicity incidence rate.

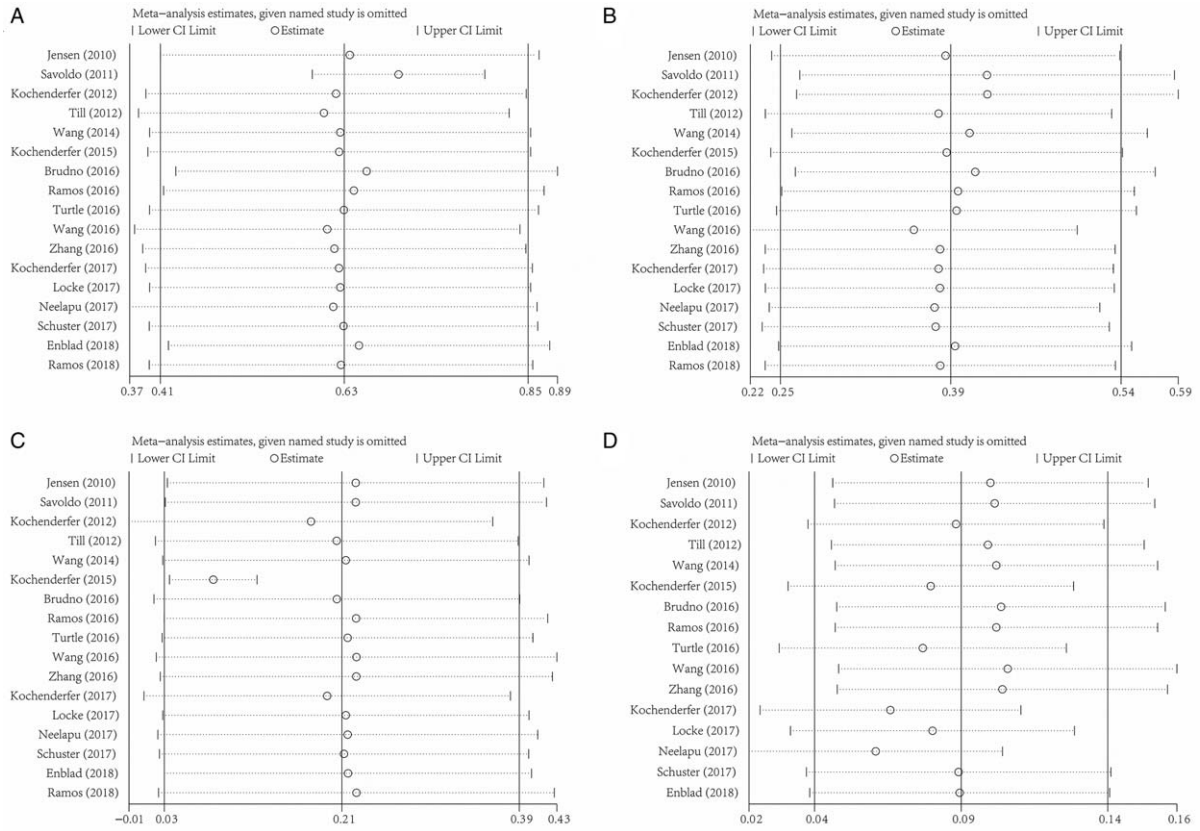

Figure 7: The graph of sensitivity analysis (the vertical line on the left indicated the total lower CI, the vertical line in the middle indicated the total pooled effect size, and the vertical line on the right indicated the total higher CI. The circle indicated the pooled effect size after deleting the study.) (A) The sensitivity analysis of the overall response rate. (B) The sensitivity analysis of an overall complete remission rate. (C) The sensitivity analysis of cytokine release syndrome incidence rate. (D) The sensitivity analysis of neurological toxicity incidence rate. CI: Confidence interval.

As a co-stimulatory molecule, CD28 significantly improves the efficacy of CAR-T cell immunotherapy. However, our meta-analysis shows that CAR-T cell immunotherapy was more effective in non-CD28 group patients. Several factors may have contributed to this discrepancy. First, the number of patients in the CD28 and non-CD28 groups varied greatly (almost 3:1). This large difference may lead to some deviation in the final results. On the other hand, there was no significant difference in the RR and CRR between the two groups, and small deviations may lead to opposite end results. Secondly, CAR-T cell immunotherapy has not been evaluated using randomized controlled trials (RCTs); thus, numerous other confounding factors may exist and affect the final outcome, which may also lead to contradictory findings. Thirdly, the non-CD28 group comprised two sub-groups: 4-1BB with or without CD28. It remains unknown whether 4-1BB alone functions better than with CD28 as a co-stimulatory molecule or the combination of CD28 and 4-1BB is required. Additional larger studies are required to address these issues.

This meta-analysis has several limitations. First, only one large multi-center research sample was included[29] as CAR-T cell immunotherapy is not yet widely clinically utilized. To some extent, the limited number of studies included may affect the strength of our study. Second, the conclusions of the meta-analysis are generally consistent with most of the current clinical studies; nevertheless, some results were slightly biased. For example, better efficacy was observed in elderly patients, which is obviously inconsistent with clinical practice results. Discrepancies in the results may be related to the different age boundaries. We chose the age of 65 years as the age boundary because this constituted the basis of the multi-center study included.[29] An older age-boundary (eg, 70 years) may result in more effective outcomes for younger patients. The better efficacy in the non-CD19 target antigen group is contrary to the accepted conclusion that the use of anti-CD19-CAR-T cells yields better B-cell lymphoma treatment efficacy. This may result from sample size differences, with the great majority incorporating the CD19 target antigen. Furthermore, as noted above, the lack of RCTs for CAR-T cell efficacy dose not guaranteed controlled variables, impacting the accuracy of the final results. The limited sample size likely contributes to all these issues. Third, studies with positive results have a greater chance of being published, which may cause an exaggeration of the clinical value of CAR-T cells in patients with B-cell lymphoma.

Overall, this meta-analysis verified the good efficacy of CAR-T cell treatment for B-cell lymphoma. Although the incidence rate of toxicity was elevated, few fatal adverse events occurred in the trials, confirming the excellent safety of CAR-T cell immunotherapy. Clinicians should pay more attention to the occurrence of toxicity and provide

timely prevention and intervention. Large multi-center studies and RCT should be conducted to verify our results and confirm the effect of CAR-T cell treatment of B-cell lymphoma. Further studies are also required to determine the optimal schedule of CAR-T cell treatment for B-cell lymphoma.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 81470139), and the Beijing Municipal Science & Technology Commission (No. Z181100001718127).

Conflicts of interest

None.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5–29. doi: 10.3322/caac.21254.
2. Al-Hamadani M, Habermann TM, Cerhan JR, Macon WR, Maurer MJ, Go RS. Non-Hodgkin lymphoma subtype distribution, geodemographic patterns, and survival in the US: a longitudinal analysis of the National Cancer Data Base from 1998 to 2011. Am J Hematol 2015;90:790–795. doi: 10.1002/ajh.24086.
3. Teras LR, DeSantis CE, Cerhan JR, Morton LM, Jemal A, Flowers CR. 2016 US lymphoid malignancy statistics by the World Health Organization subtypes. CA Cancer J Clin 2016;66:443–459. doi: 10.3322/caac.21357.
4. Coiffier B, Thieblemont C, Van Den Neste E, Lepeu G, Plantier I, Castaigne S, *et al*. Long-term outcome of patients in the LNH-98. 5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. Blood 2010;116:2040–2045. doi: 10.1182/blood-2010-03-276246.
5. Crump M. Management of relapsed diffuse large B-cell lymphoma. Hematol Oncol Clin North Am 2016;30:1195–1213. doi: 10.1016/j.hoc.2016.07.004.
6. Gisselbrecht C, Glass B, Mounier N, Singh Gill D, Linch DC, Trneny M, *et al*. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. J Clin Oncol 2010;28:4184–4190. doi: 10.1200/jco.2010.28.1618.
7. Van Den Neste E, Schmitz N, Mounier N, Gill D, Linch D, Trneny M, *et al*. Outcomes of diffuse large B-cell lymphoma patients relapsing after autologous stem cell transplantation: an analysis of patients included in the CORAL study. Bone Marrow Transplant 2017;52:216–221. doi: 10.1038/bmt.2016.213.
8. Brentjens RJ, Latouche JB, Santos E, Marti F, Gong MC, Lyddane C, *et al*. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med 2003;9:279–286. doi: 10.1038/nm827.
9. Scheuermann RH, Racila E. CD19 antigen in leukemia and lymphoma diagnosis and immunotherapy. Leuk Lymphoma 1995;18:385–397. doi: 10.3109/10428199509059636.
10. Dai H, Wang Y, Lu X, Han W. Chimeric antigen receptors modified T-cells for cancer therapy. J Natl Cancer Inst 2016;108:kjv439. doi: 10.1093/jnci/djv439.
11. Jensen MC, Popplewell L, Cooper LJ, DiGiusto D, Kalos M, Ostberg JR, *et al*. Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. Biol Blood Marrow Transplant 2010;16:1245–1256. doi: 10.1016/j.bbmt.2010.03.014.
12. Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH, *et al*. Anti-CD22-chimeric antigen receptors targeting B-cell precursor acute lymphoblastic leukaemia. Blood 2013;121:1165–1174. doi: 10.1182/blood-2012-06-438002.
13. Ramos CA, Savoldo B, Torrano V, Ballard B, Zhang H, Dakhova O, *et al*. Clinical responses with T lymphocytes targeting malignancy-associated kappa light chains. J Clin Invest 2016;126:2588–2596. doi: 10.1172/jci86000.
14. Berger C, Sommermeyer D, Hudecek M, Berger M, Balakrishnan A, Paszkiewicz PJ, *et al*. Safety of targeting ROR1 in primates with chimeric antigen receptor-modified T cells. Cancer Immunol Res 2015;3:206–216. doi: 10.1158/2326-6066.cir-14-0163.
15. Lee WL, Slutsky AS. Sepsis and endothelial permeability. N Engl J Med 2010;363:689–691. doi: 10.1056/NEJMcibr1007320.
16. Riaz IB, Zahid U, Kamal MU, Husnain M, McBride A, Hua A, *et al*. Anti-CD 19 and anti-CD 20 CAR-modified T cells for B-cell malignancies: a systematic review and meta-analysis. Immunotherapy 2017;9:979–993. doi: 10.2217/imt-2017-0062.
17. Cao JX, Gao WJ, You J, Wu LH, Liu JL, Wang ZX. The efficacy of anti-CD19 chimeric antigen receptor T cells for B-cell malignancies. Cytotherapy 2019;21:769–781. doi: 10.1016/j.jcyt.2019.04.005.
18. Savoldo B, Ramos CA, Liu E, Mims MP, Keating MJ, Carrum G, *et al*. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. J Clin Invest 2011;121:1822–1826. doi: 10.1172/jci46110.
19. Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Maric I, *et al*. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 2012;119:2709–2720. doi: 10.1182/blood-2011-10-384388.
20. Till BG, Jensen MC, Wang J, Qian X, Gopal AK, Maloney DG, *et al*. CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. Blood 2012;119:3940–3950. doi: 10.1182/blood-2011-10-387969.
21. Wang Y, Zhang WY, Han QW, Liu Y, Dai HR, Guo YL, *et al*. Effective response and delayed toxicities of refractory advanced diffuse large B-cell lymphoma treated by CD20-directed chimeric antigen receptor-modified T cells. Clin Immunol 2014;155:160–175. doi: 10.1016/j.clim.2014.10.002.
22. Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, *et al*. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol 2015;33:540–549. doi: 10.1200/JCO.2014.56.2025.
23. Brudno JN, Somerville RP, Shi V, Rose JJ, Halverson DC, Fowler DH, *et al*. Allogeneic T cells that express an anti-CD19 chimeric antigen receptor induce remissions of B-cell malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease. J Clin Oncol 2016;34:1112–1121. doi: 10.1200/JCO.2015.64.5929.
24. Turtle CJ, Hanafi LA, Berger C, Hudecek M, Pender B, Robinson E, *et al*. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells. Sci Transl Med 2016;8:355ra116. doi: 10.1126/scitranslmed.aaf8621.
25. Wang X, Popplewell LL, Wagner JR, Naranjo A, Blanchard MS, Mott MR, *et al*. Phase 1 studies of central memory-derived CD19 CAR T-cell therapy following autologous HSCT in patients with B-cell NHL. Blood 2016;127:2980–2990. doi: 10.1182/blood-2015-12-686725.
26. Zhang WY, Wang Y, Guo YL, Dai HR, Yang QM, Zhang YJ, *et al*. Treatment of CD20-directed chimeric antigen receptor-modified T cells in patients with relapsed or refractory B-cell non-Hodgkin lymphoma: an early phase IIa trial report. Signal Transduct Target Ther 2016;1:16002. doi: 10.1038/sigtrans.2016.2.
27. Kochenderfer JN, Somerville RPT, Lu T, Shi V, Bot A, Rossi J, *et al*. Lymphoma remissions caused by anti-CD19 chimeric antigen receptor T cells are associated with high serum interleukin-15 levels. J Clin Oncol 2017;35:1803–1813. doi: 10.1200/jco.2016.71.3024.
28. Locke FL, Neelapu SS, Bartlett NL, Siddiqi T, Chavez JC, Hosing CM, *et al*. Phase 1 results of ZUMA-1: a multicenter study of KTE-C19 anti-CD19 CAR T cell therapy in refractory aggressive lymphoma. Mol Ther 2017;25:285–295. doi: 10.1016/j.ymthe.2016.10.020.
29. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, *et al*. Axicabtagene Ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 2017;377:2531–2544. doi: 10.1056/NEJMoa1707447.
30. Schuster SJ, Svoboda J, Chong EA, Nasta SD, Mato AR, Anak Ö, *et al*. Chimeric antigen receptor T cells in refractory B-cell lymphomas. N Engl J Med 2017;377:2545–2554. doi: 10.1056/NEJMoa1708566.
31. Enblad G, Karlsson H, Gammelgard G, Wenthe J, Lovgren T, Amini RM, *et al*. A phase I/IIa trial using CD19-targeted third-generation

CAR T cells for lymphoma and leukemia. Clin Cancer Res 2018;24:6185–6194. doi: 10.1158/1078-0432.ccr-18-0426.
32. Ramos CA, Rouce R, Robertson CS, Reyna A, Narala N, Vyas G, *et al*. In vivo fate and activity of second- versus third-generation CD19-specific CAR-T cells in B cell non-Hodgkin's lymphomas. Mol Ther 2018;26:2727–2737. doi: 10.1016/j.ymthe.2018.09.009.
33. Zhou Z, Sehn LH, Rademaker AW, Gordon LI, Lacasce AS, Crosby-Thompson A, *et al*. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. Blood 2014;123:837–842. doi: 10.1182/blood-2013-09-524108.
34. Neelapu SS. Managing the toxicities of CAR T-cell therapy. Hematol Oncol 2019;37 (Suppl 1):48–52. doi: 10.1002/hon.2595.
35. Kochenderfer JN, Dudley ME, Carpenter RO, Kassim SH, Rose JJ, Telford WG, *et al*. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. Blood 2013;122:4129–4139. doi: 10.1182/blood-2013-08-519413.
36. Turtle CJ, Hanafi LA, Berger C, Gooley TA, Cherian S, Hudecek M, *et al*. CD19 CAR-T cells of defined CD4+: CD8+ composition in adult B cell ALL patients. J Clin Invest 2016;126:2123–2138. doi: 10.1172/JCI85309.
37. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. Blood 2016;127:3321–3330. doi: 10.1182/blood-2016-04-703751.

How to cite this article: Zheng XH, Zhang XY, Dong QQ, Chen F, Yang SB, Li WB. Efficacy and safety of chimeric antigen receptor-T cells in the treatment of B cell lymphoma: a systematic review and meta-analysis. Chin Med J 2020;133:74–85. doi: 10.1097/CM9.0000000000000568