

Efficiency and safety of autologous chimeric antigen receptor T-cells therapy used for patients with lymphoma

A systematic review and meta-analysis

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Abstract

Background: Chimeric antigen receptor (CAR) T-cell therapy has produced promising response rates in patients with B cell malignancies. However, previous meta-analyses have demonstrated that CAR T-cell efficacy is unsatisfactory in patients with lymphoma unlike in patient with other hematological malignancies, but these studies included insufficient numbers of studies and patients with lymphoma. Furthermore, clinicians are interested in the effects of infusion dose, CAR structure, interleukin-2 (IL-2), and conditioning therapy regimen.

Methods: All clinical trials administering autologous CAR T-cell therapy in lymphoma patients were searched in medical databases. A traditional meta-analysis was performed to assess the safety and efficacy of CAR T-cells in lymphoma treatment. Subgroup analysis was performed to determine the relationships between potential factors and efficacy. The best overall response rate (ORR), 6 month ORR (6m ORR), and severe cytokine release syndrome (sCRS) rate were calculated by Stata 14.0.

Results: A total of 411 patients across all the studies were included. Our analysis showed a best ORR of 0.71, a 6m ORR of 0.63, and an overall CRS (grade ≥ 3) rate of 0.18. The subgroup analysis showed that increased response rates and reduced CRS (grade ≥ 3) rates were associated with a low dose of CAR T-cells. No IL-2 administration and the use of a fludarabine-containing lymphodepletion regimen led to improved efficacy, while anti-CD19 CAR T cells led to a more successful outcome than anti-CD20 CAR T cells. In addition, 2nd- and 3rd-generation CAR T cells exhibited increased effectiveness in clinical studies, and no significant effect diversity was found between the 2nd- and 3rd-generation CAR T cells. sCRS was associated with a high dose of infused CAR T cells when IL-2 and fludarabine were excluded from the positive factors for sCRS.

Conclusion: CAR T cells are promising in the treatment of relapsed or refractory lymphoma. Doses lower than $10^8/m^2$, no IL-2 administration, fludarabine administration, and anti-CD19 CAR T cells were related to improved efficacy and safety.

Abbreviations: 95% CI = 95% confidence interval, CAR = chimeric antigen receptor, CR = complete response, IL-2 = interleukin-2, NHL = non-Hodgkin lymphoma, OR = overall response, ORR = overall response rate, sCRS = severe cytokine release syndrome, PR = partial response, scFv = single-chain variable fragment, SD = stable disease.

Keywords: CAR T cells, lymphoma, meta-analysis

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Data availability: All included studies' data are available from medical databases.

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1. Introduction

First, patients diagnosed with B-cell lymphoma accept first-line anthracycline-based chemotherapy regimens, especially rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP), incorporating rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.^[1,2] After receiving first-line therapy, numerous patients face the dilemma of relapsed or refractory lymphoma. Then, they are given salvage chemotherapy followed by autologous hematopoietic stem cell transplantation. However, Telio et al illustrated poor outcomes for salvage chemotherapy plus autologous hematopoietic stem cell transplantation in primary refractory diffuse large B-cell lymphoma,^[3] with a 23% to 29% response rate and a median progression-free survival time of only 3 months. Compared with conventional carcinoma-targeted treatment regimens such as chemotherapy, radiotherapy, and immunotherapy, chimeric antigen receptor (CAR) T cell therapy shows encouraging efficacy in relapsed and refractory B-cell malignancies, such as acute lymphocytic leukemia, chronic lymphocytic leukemia, and non-Hodgkin lymphoma (NHL).^[4-6] CAR T-cells are T cells that have been

genetically modified to express CARs, especially CARs against CD19 or CD20 (B-cell-specific tumor-associated antigens), that produce CAR T-cell activation, proliferation, cytokine production, and tumor cell killing.^[7,8] CAR T-cells have evolved via the addition of costimulatory domains.^[9] In addition, CAR T cells targeting CD30,^[10] κ light chains,^[11] and HER2^[12] have been manufactured for the treatment of Hodgkin lymphoma, B cell malignancies, and breast cancer, respectively.

Since the first clinical trial conducted by Till et al in 2008,^[13] dozens of clinical trials investigating CAR T-cell therapy have been conducted, with most occurring in America or China.^[14–16] Most of these trials have shown a sustained response in patients suffering from relapsed or refractory B-cell malignancies. However, the results of previous meta-analyses could be unsubstantiated, that is, they could be restricted by high costs and the inclusion of early phase 1 or 2 studies, as a majority of the early studies had a small sample size, recruiting fewer than 20 patients. Riaz et al included studies using anti-CD19 and anti-CD20 CAR-modified T cells for all B-cell malignancies and discussed different efficiencies among subtypes of B-cell malignancies.^[17] Zhang et al only included anti-CD19 CAR T cells for all B cell malignancies and found that no interleukin-2 (IL-2) administration and lymphodepletion could improve response rates.^[20] With only 178 patients evaluated, Zhou et al included no multicenter trials and discussed all B cell malignancies, making their result weak.^[19] Of note, all these previous meta-analyses demonstrated heterogeneity higher than 70% and failed to provide a plausible explanation. Here, we mainly ascribe their substantial heterogeneity to the diversity of B-cell malignancy subtypes and the discrepancies in T-cell derivation. For instance, allogeneic or autologous CAR T-cells should be taken into consideration.

Zhang et al^[20] and Irbaz et al^[17] demonstrated that the overall response rates (ORRs) for lymphoma were only 0.36 and 0.53, and these studies were unconvincing because the included trials were insufficient and the analyses lacked multicenter and large-sample studies. Therefore, a more detailed and comprehensive systematic review focusing on lymphoma is necessary, especially since updated clinical studies have been reported. The factors potentially affecting efficacy are complex and were summarized by Brudno et al^[16]; these factors include but are not limited to long-term persistence, CAR design (costimulatory domain, hinge, and trans-membrane portions), conditioning chemotherapy regimen choice, immunological rejection, infusion dosage, loss of target, multitarget strategy, T-cell subpopulations, etc. In our study, overall efficacy and safety in lymphoma were calculated, and factors affecting response rates and severe cytokine release syndrome (sCRS) rates were discussed.

2. Materials and methods

2.1. Eligibility criteria and search strategy

Two authors independently searched relevant literature published between January 1, 2002 and November 1, 2018, among the following medical databases: Medline, Embase, Cochrane Central Register of Controlled Trials, and ClinicalTrials.gov. Clinical studies recruiting patients with NHL, performing autologous CAR T-cell therapy, and reporting outcomes and safety-related events were included preliminarily. Then, articles without intact outcomes, unreported or ongoing trials, studies using allogeneic CAR T-cells, and articles not in English were abandoned.

2.2. Data extraction

Literature reports with a title and an abstract meeting our eligibility criteria were identified by 2 authors independently. Then, full texts were carefully identified and reevaluated. We extracted related information in the following fields: author/year, CAR design and generation, conditioning or lymphodepleting chemotherapy, IL-2 administration, total infused CAR T-cell number, response rates, and relevant adverse events.

2.3. Outcome measures

Among the outcomes of patients undergoing CAR T-cell therapy, which included complete response (CR), partial response (PR), stable disease, and progressive disease, an effective overall response (OR) was considered to be a CR or PR outcome. We assessed both the best ORR and 6 months ORR (6m ORR) as evaluation indicators. Unevaluable outcomes were considered progressive disease. Adverse events were defined as grade 3–4 CRS and grade 3–4 neurotoxicity, because grade 1–2 adverse events were common immediately after infusion and were not life-threatening, so there was no worth in analyzing them. Studies reporting adverse events without a clear grading system were recorded as “not reported.” In studies using the Common Terminology Criteria for Adverse Events version 3.0/4.0, we checked all reported adverse events one by one in accordance with Lee et al’s criteria.^[21] B-cell aplasia analysis was not included in our work due to mere reports.

2.4. Statistical analysis

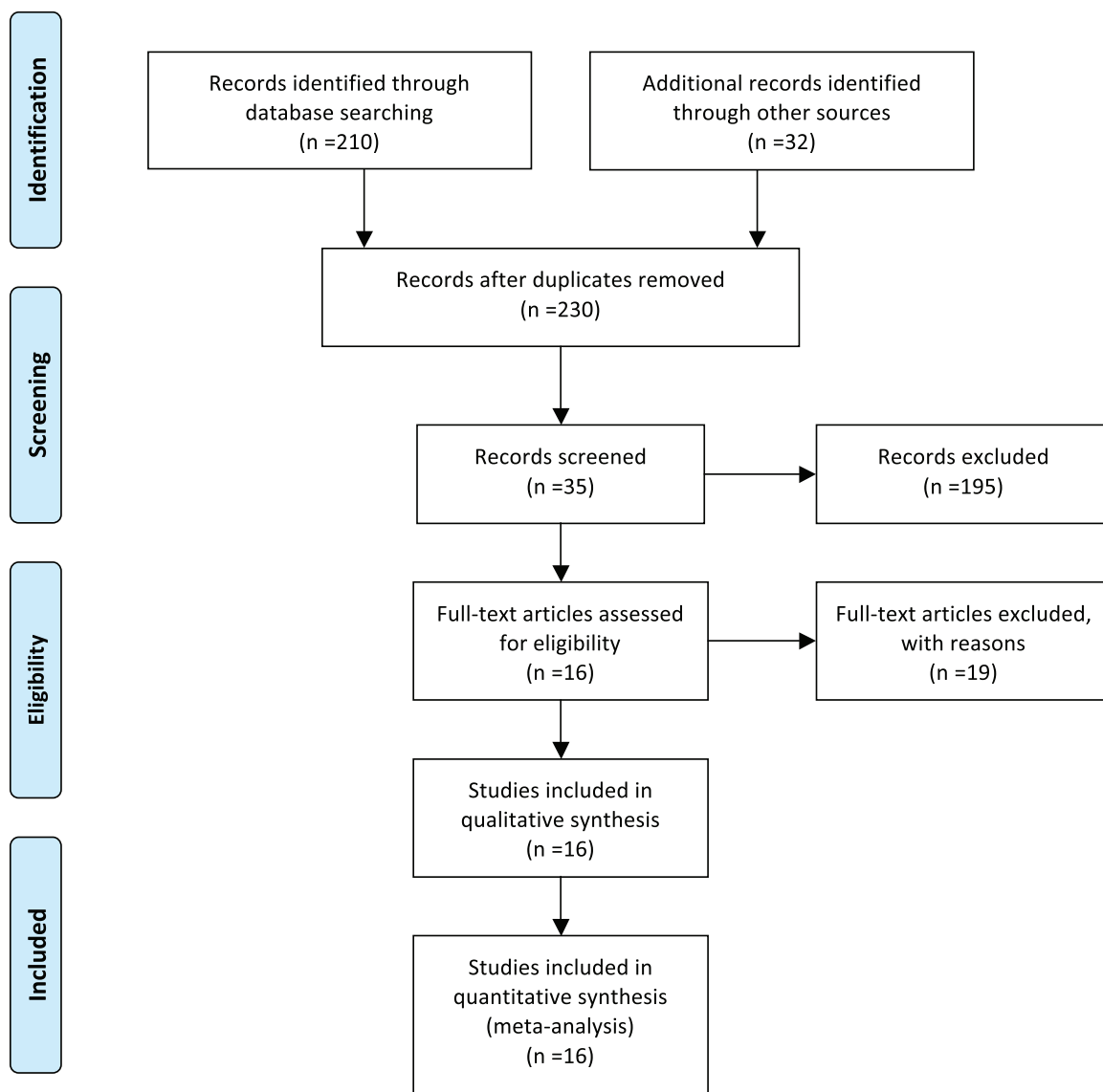
A meta-analysis was implemented with Stata 14.0. We used I^2 to assess heterogeneity among studies. OR and 95% confidence intervals (95% CIs) were calculated to evaluate the efficacy of CAR T-cell therapy in lymphoma using both a fixed-effects model and a random-effects model. From our perspective, I^2 values $\leq 25\%$, between 25% and 50%, and $\geq 50\%$ were equal to low, moderate, and high heterogeneity, respectively. The fixed-effects model was used to calculate heterogeneity among groups.

2.5. Ethical approval

This systematic review is based on published studies; therefore, ethical approval is not required. We have received an exemption from Institutional Review Board of Shanxi Medical University. And this article does not contain any studies with human participants or animals performed by any of the authors.

3. Results

A total of 230 articles that potentially met our criteria were identified after a comprehensive manual search of Medline, Embase, Cochrane CENTRAL, ClinicalTrials.gov, and other sources. Then, 195 studies were removed by screening titles and abstracts. In total, 183 were not clinical studies, and 12 did not address lymphoma. After full-text evaluations of the remaining 35 articles, 16 were included in the systematic review and meta-analysis. Two were removed because they used allogeneic CAR T-cells. Five were not full-text articles or had incomplete data. Three used repeated data from one study. One study not only evaluated a too-short period of treatment but also lacked details needed to make the treatment strategy clear. One study used a CAR specific for the κ light chain, which may cause heterogeneity. Five were review articles. Additionally, 2 studies published as meeting



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Figure 1. Flow chart of the literature search.

abstracts were included in our studies (Fig. 1, flow chart). Two studies found in a review were also incorporated. The basic information and characteristics of the included 16 studies published between 2008 and 2017 and conducted in several countries are summarized in Table 1 and are presented in the same order in the bibliography.^[6,13,22–35] In total, 241 patients with lymphoma who received standard CAR T-cell infusion were included in our study. The dose of infused CAR T-cells ranged from $0.66 \times 10^6/\text{kg}$ to $3.3 \times 10^9/\text{m}^2$. For subsequent subgroup analysis by dose, data in cells/kg were multiplied by 60 kg and divided by 1.73 m^2 to convert the data into cells/ m^2 .

3.1. Response rate

Among the included clinical trials, the best response rates of CAR T-cell therapy for lymphoma ranged from 0.31 to 0.82. The best

ORR was 0.71 (95% CI: 0.67–0.76), demonstrating a higher efficacy than that reported in a previous meta-analysis. No substantial heterogeneity was observed ($I^2=45.8\%$, $P=.031$) (Fig. 2). Furthermore, the 6m ORR was relatively stable and reached 0.54 (95% CI: 0.41–0.66) (Fig. 2). According to the subgroup analysis protocol designed at the beginning of the study, we compared both the best ORRs and 6m ORRs among subgroups divided by target, CAR generation, infused cell dose, IL-2 regimen, or lymphodepletion regimen. A large efficacy difference appeared in 6m ORR between the anti-CD19 (0.66, 95% CI: 0.61–0.71) and anti-CD20 groups (0.34, 0.19–0.50) (see Fig. S1A, Supplemental Content, which illustrates subgroup analysis of best ORR by target, <http://links.lww.com/MD/D287>) but disappeared in the best ORR (see Fig. S1B, Supplemental Content, which illustrates subgroup analysis of 6m ORR by target, <http://links.lww.com/MD/D287>), indicating that anti-

Table 1
Basic studies characters.

Study [reference]	Num	Cell dose	CAR	Best ORR	6m ORR	CRS ≥3	CRS criteria	IL-2	Chemotherapy
Till 2008 ^[13]	7	1.00E+08–3.30E+09/m ²	CD20 (1st)	3	1	NP	CTCAE3.0	Injection	CVP,FND, 131 I-tositumomab
Zhang 2016 ^[22]	11	0.41E+07–1.46E+07/kg	CD20 (2nd)	9	5	1	CTCAE3.0	Expansion	Cy
Jensen 2010 ^[23]	4	1.00E+8–2.00E+9/m ²	CD19/20 (1st)	2	2	3	CTCAE3.0	Injection	Flu
Till 2012 ^[24]	3	1.00E+8–3.30E+9/m ²	CD20 (3rd)	3	3	1	CTCAE3.0	Injection	Cy
Wang 2014 ^[25]	7	2.00E+7–9.00E+7/kg	CD20 (2nd)	5	2	3	CTCAE3.0	No	COD,COED, CHODE,ESHAP
Neelapu 2017 ^[26]	101	2.00E+6/kg	CD19 (2nd)	83	83	13	Lee	No	Cy, Flu
Turtle 2016 ^[27]	32	2.00E+5–2.00E+7/kg	CD19 (3rd)	19	19	4	CTCAE4.03	Injection	(Cy, Flu)/Cy
Wang 2015 ^[28]	16	8.41E+7 (total)	CD19 (2nd)	10	10	NP	NP	Rituximab	Rituximab
Kochenderfer 2017 ^[29]	22	1.00E+6–6.00E+6/kg	CD19 (2nd)	16	14	5	NP	Expansion	Cy, Flu
Kochenderfer 2015 ^[30]	11	1.00E+6–5.00E+6/kg	CD19 (2nd)	8	7	3	NP	Expansion	Cy, Flu
Kochenderfer 2012 ^[6]	4	0.50E+7–4.00E+7/m ²	CD19 (2nd)	3	3	2	CTCAE3.0	Injection	Cy, Flu
Brudno 2016 ^[31]	9	0.66E+6–2.00E+6/kg	CD19 (2nd)	6	NP	3	Lee	No	Cy, Flu
Enblad 2015 ^[32]	7	1.00E+8–2.00E+8/m ²	CD19 (3rd)	2	NP	NP	NP	Expansion	Cy, Flu
Schuster 2017 ^[33]	28	3.08E+6–8.87E+6/kg	CD19 (2nd)	18	16	5	Penn	Expansion	Cy mainly
Juliet trial ^[34]	81	1.00E+7–6.00E+7 (total)	CD19 (2nd)	43	17 (of 46)	47	Penn	NP	Cy/Flu or bendamustine
Juno transfer ^[35]	68	Not available	CD19 (2nd)	51	14 (of 35)	1	NP	NP	Cy, Flu

CAR = chimeric antigen receptor, CHODE = cyclophosphamide, doxorubicin, vincristine, dexamethasone, and etoposide, COD = cyclophosphamide, vincristine, and dexamethasone, COED = cyclophosphamide, vincristine, etoposide and dexamethasone, CRS = severe cytokine release syndrome, CVP = cyclophosphamide, vincristine and prednisone, ESHAP = methylprednisolone, etoposide, carboplatin, and high-dose cytosine arabinoside, FND = fludarabine, mitoxantrone, and dexamethasone, Num = patients number, CTCAE = Common Terminology Criteria for Adverse Events, Cy = cyclophosphamide, Flu = fludarabine, IL-2 = interleukin-2, Lee = Lee et al's CRS grading system, NP = not report, ORR = overall response rate, 6m ORR = 6 months overall response rate, Penn = University of Pennsylvania's CRS grading system.

CD19 CAR T cells produced more durable remission than anti-CD20 cells. Between groups of 1st-, 2nd-, and 3rd-generation cells, the best ORR of the 2nd-generation cells (0.73, 95% CI: 0.68–0.77) was the highest, followed by that of the 3rd-generation cells (0.65, 95% CI: 0.46–0.77) and then the 1st-generation cells (0.47, 95% CI: 0.25–0.70) (see Fig. S2A, Supplemental Content, which illustrates subgroup analysis of best ORR by CAR generation, <http://links.lww.com/MD/D287>). Of note, the 2nd- and 3rd-generation CAR T cells showed familiar outcomes of 0.65 in 6m ORRs, while the 1st-generation CAR T cells achieved a 50% lower response rate (0.31, 95% CI: 0.11–0.50) (see Fig. S2B, Supplemental Content, which illustrates subgroup analysis of 6m ORR by CAR generation, <http://links.lww.com/MD/D287>).

When considering the effect of a high dose versus a low dose, similar efficacy was observed for the best ORR (low-dose group: 0.72, 95% CI: 0.66–0.77; high-dose group: 0.67, 95% CI: 0.56–0.77) (see Fig. S3A, Supplemental Content, which illustrates subgroup analysis of best ORR by dose, <http://links.lww.com/MD/D287>). However, the 6m ORR of the high-dose group (0.45, 95% CI: 0.34–0.57) was dramatically lower than that of the low-dose group (0.70, 95% CI: 0.64–0.76) (see Fig. S3B, Supplemental Content, which illustrates subgroup analysis of 6m ORR by dose, <http://links.lww.com/MD/D287>).

IL-2 was administered to support CAR T cell expansion in vitro or injected in vivo. In our analysis, the no IL-2 group had a better best ORR and more durable 6m ORR than the IL-2

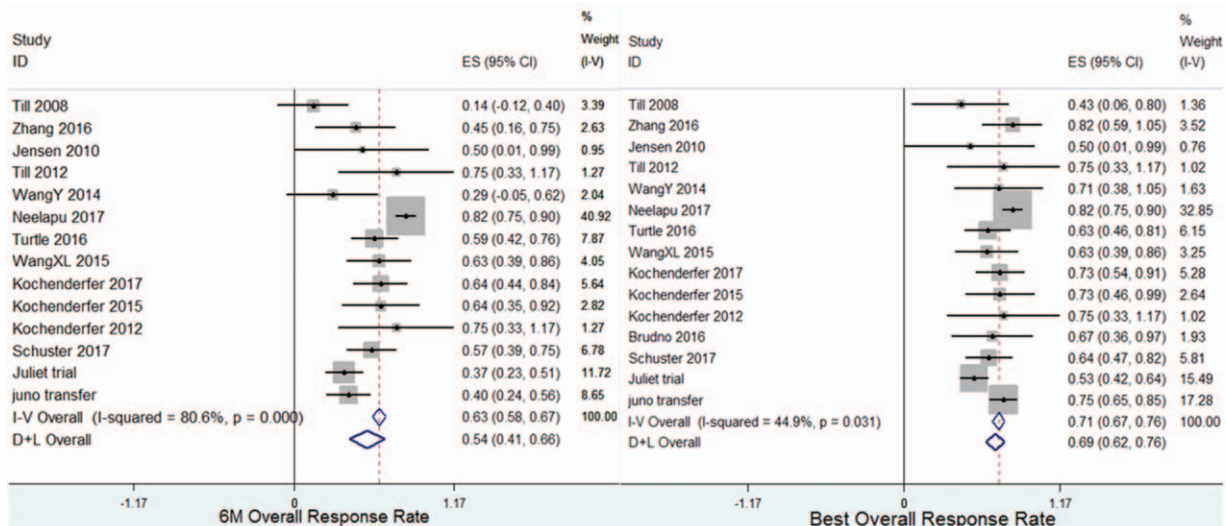


Figure 2. Forest plot of the best ORR and 6m ORR. Best ORR=0.71 (95% CI: 0.67–0.76). Heterogeneity: $I^2=41.3\%$, $P=.043$. 6m ORR=0.63 (95% CI: 0.58–0.67). Heterogeneity: $I^2=80.6\%$, $P=.000$. CI=confidence interval, ORR=overall response rate, 6m ORR=6 months overall response rate.

expansion group and the IL-2 injection group, which had the worst outcomes, although the efficacy gap was small (see Fig. S4A, B, Supplemental Content, which illustrate subgroup analysis of best ORR and 6m ORR by IL-2 administration, <http://links.lww.com/MD/D287>). Furthermore, all trials that performed lymphodepletion before CAR T cell infusion and groups using fludarabine during lymphodepletion achieved a better effect than those that used other regimens. Taking both the best ORR and 6m ORR into consideration, especially the 6m ORR, the no flu group achieved a disappointing response rate (0.48, 95% CI: 0.38–0.58), while the flu group outcomes were promising (0.73, 95% CI: 0.67–0.79) (see Fig. S5A, B, Supplemental Content, which illustrate the subgroup analysis of best ORR and 6m ORR by lymphodepletion, <http://links.lww.com/MD/D287>).

3.2. Cytokine release syndrome (grade ≥ 3)

The rates of sCRS ranged widely, spanning from 0.01 to 0.75. The overall rate was 0.26 (95% CI: 0.14–0.38) (see Fig. S6, Supplemental Content, which illustrates the overall sCRS rate, <http://links.lww.com/MD/D287>). Enormous heterogeneity was observed ($I^2=90.9\%$, $P=.000$). In the following subgroup analyses, we found that studies that used a high dose of CAR T-cells had a higher rate of sCRS (0.27, 95% CI: 0.12–0.43) than studies that used a low dose of CAR T-cells (0.16, 95% CI: 0.07–0.25) (see Fig. S7, Supplemental Content, which illustrate subgroup analysis of sCRS rate by dose, <http://links.lww.com/MD/D287>). In addition, our results illustrated that IL-2 administration and lymphodepletion were not observed to have any impact on the sCRS rate (see Fig. S8, 9, Supplemental Content, which illustrate subgroup analysis of sCRS rate by IL-2 administration and lymphodepletion, <http://links.lww.com/MD/D287>).

4. Discussion

Although previous systematic reviews have demonstrated that the efficacy of CAR T-cell therapy for solid tumors is greatly inferior to the efficacy of CAR T-cell therapy for hematologic malignancies,^[17,20] our systematic review analyzed more comprehensive and updated literature and showed a promising outcome: the best ORR of CAR T-cell therapy for lymphoma was as high as 0.71, and the stable 6m ORR was 0.54. However, there is still a substantial margin for improvement because some studies have shown a tremendous CR rate of more than 90% in acute lymphocytic leukemia. Over the last several years, clinicians have been trying to optimize CAR T-cell therapy to improve clinical efficacy and reduce the incidence of adverse events. Factors and variables that may influence efficacy or safety include but are not limited to CAR structure design, methods to introduce CARs into T cells, original T cell sources (autologous or allogeneic), T-cell culture conditions, lymphodepletion regimens, cytokine support for T cell infusion, CAR T cell dosages, subpopulations of cells used to generate CAR T cells, and the biology and severity of the targeted malignancy.

The dosage of infused CAR T-cells that can generate the best curative effect remains unknown. Some investigators believe that infusing more CAR T-cells will lead to a higher response rate, but whether this approach could lead to an increased incidence of adverse events is still unknown.^[9,24] In Ramos et al' study, sCRS was observed with the highest level of anti-CD30 CAR T-cells.^[11]

Turtle et al considered a CAR T-cell dose of $2 \times 10^7/\text{kg}$ (high dose) to be excessively toxic in NHL patients, but a lower dose was well tolerated.^[27] In Park et al's review, a high disease burden was correlated with sCRS and associated with increased levels of CAR T-cells, but this study was unable to infer a relationship between T-cells and the severity of CRS on account of the small number of patients treated with the high dose of T-cells.^[14] In addition, Neelapu et al reported a large number of enrolled patients and concluded that efficacy is correlated with CAR T cell numbers in patients. Notably, they adopted a therapeutic regimen with a low infusion dose of $2 \times 10^6/\text{kg}$, which meant that the number of alive CAR⁺ T cells in their patients was even lower; consequently, their conclusion is only appropriate in their specific low-dose range. Apparently, the infused CAR T cell doses in most studies are much higher than those reported by Neelapu et al (Table 1). Turtle et al injected 3 doses ($2 \times 10^5/\text{kg}$, $2 \times 10^6/\text{kg}$, and $2 \times 10^7/\text{kg}$) of CAR T cells, and the low-dose group ($2 \times 10^5/\text{kg}$ and $2 \times 10^6/\text{kg}$) showed a 6m ORR of 15/23, while the $2 \times 10^7/\text{kg}$ group reported a lower response rate of 4/7. Hence, we decided to explore the virtual correlation and separate all studies reporting their infused dose by the bisection method. Thus, our results indicate that a lower dose of CAR T-cell infusion could effectively improve the response rate and avert the increasing incidence of sCRS (grade ≥ 3). Anti-CAR immune responses may be a negative factor. Single-chain variable fragment (scFv) regions and junctions between different CAR domains may be immunogenic, which means that as the CAR⁺ T cell number in the body increases, the anti-CAR immune response becomes stronger. Of note, this conclusion could be uncertain because among 7 high-dose studies, 4 administered anti-CD20 CAR T cells, so the influence could derive from the use of different targets. Thus, we suggest setting a range from $2 \times 10^6/\text{kg}$ to $5 \times 10^6/\text{kg}$ as the recommended infusion dose.

During the expansion of CAR T-cells, cytokines are usually administered to improve the expansion of the cells. As a kind of cytokine, IL-2 is usually used to stimulate the proliferation of CAR T-cells. It was believed that a higher response rate would be generated through this process. However, in Zhang et al's systematic review, IL-2 was associated with a decreased response rate in anti-CD19 CAR T-cell-treated patients in all B cell malignancies.^[20] Till et al concluded that IL-2 contributed to the increased incidence of partial adverse events.^[24] In our analysis, 4/12 trials did not use IL-2 for CAR T-cell expansion, and 4/12 used IL-2 for in vitro expansion only, while 4/12 used IL-2 for expansion in vivo. Our subgroup analysis indicated that IL-2 administration was not related to sCRS but did attenuate both initial and durable efficacy, especially in trials where IL-2 was injected directly into patients.

Compared with 1st-generation CARs that contain only a CD3 ζ intracellular signaling domain plus an scFv,^[13] 2nd-generation CARs that have an added costimulatory domain, such as CD28 or 4-1BB, show a stronger absolute effect depending on the results.^[26] Third-generation CARs incorporate 2 costimulatory domains derived from different costimulatory proteins, such as CD28 and 4-1BB.^[24,27] However, it is controversial whether 3rd-generation CAR T cells are more effective than 2nd-generation CAR T cells. Although theoretically 2 costimulatory domains would lead to more-intensified specific recognition, our subgroup analysis demonstrated that 3rd-generation CAR T cells produced a lower best ORR than 2nd-generation CAR T cells and the same 6m ORR as that produced by 2nd-generation CAR T cells.

Another function of 2nd-generation CARs is the induction of IL-2 secretion. IL-2 might promote activation-induced cell death to restrict the expansion and accumulation of CAR T-cells, while CD3/CD28 has a positive effect. One of our limitations is that IL-2 use for expansion may be unreported, making it difficult to distinguish the IL-2 expansion group from the no IL-2 group. Consequently, our conclusion is reliable only for the IL-2 infusion group. Although the reason why IL-2 could be barrier to treatment efficacy is still unknown, our advice for clinical work is to not use direct IL-2 administration for cell expansion.

Each patient in our studies, before the infusion of CAR T cells, received a conditioning regimen for several days to deplete lymphocyte numbers and increase serum cytokine levels. We observed that almost all of the trial patients received cyclophosphamide, but only half of the studies utilized cyclophosphamide plus fludarabine as a lymphodepletion regimen (Table 1). However, flu-containing conditioning chemotherapy seems to be relatively successful.^[26,27,29,31] A lymphodepletion regimen containing fludarabine is supposed to prevent immunological rejection of CAR T cells by recipient anti-CAR immune responses, especially when a high dose of T cells is infused. Turtle et al noted that flu increased CD4⁺ and CD8⁺ T cell numbers.^[27] As our forest plots illustrated, fludarabine-containing conditioning chemotherapy significantly optimized the effectiveness of CAR T-cell therapy compared with regimens lacking fludarabine. Additionally, in our sCRS analysis, no evidence was found to support the idea that the addition of fludarabine could lead to an increased incidence of sCRS. Hence, cyclophosphamide plus fludarabine should be considered a safe and promising standard lymphodepletion regimen.

Cytokine release syndrome (CRS), neurotoxicity, and B cell aplasia are major adverse events in CAR T-cell therapy. The most common and serious adverse event, CRS, refers to a constellation of symptoms resulting from significant production of inflammatory cytokines (especially IFN γ and TNF) as a result of a high level of T cell proliferation.^[6] Common symptoms include fever, myalgia, nausea, and vomiting. Severe symptoms that threaten patients' lives include but are not limited to hypoxia, hypotension, severe fever, and organ toxicities. Infrequent symptoms, such as dermatological rash and disseminated intravascular coagulation, were also reported by Wang et al^[25] and Kochenderfer et al.^[6] Among the trials included in our systematic review, studies that did not report CRS had no clear classification. Usually, transient grade 1 or grade 2 CRS is observed in almost all patients several days after infusing CAR T-cells without causing serious damage to the patients,^[26] so our review considered only life-threatening grade 3 and grade 4 CRS.

The overall rate of grade 3 and grade 4 CRS was 0.26. In Turtle et al's study, the low-dose group's sCRS rate was 1/23, while the high-dose group's incidence was 3/9, indicating that a higher cell dose may increase the frequency of sCRS.^[27] Kochenderfer et al showed that grade 3 or 4 toxicities are associated with peak CAR⁺ peripheral blood mononuclear cell numbers and peak IL-10 and IL-15 levels.^[29] We concluded that a high dose of infused CAR T-cells has a positive correlation with a high incidence of sCRS, which supports many clinical investigators' hypotheses. Furthermore, our results negated the hypothesis that IL-2 is implicated as the cause of sCRS, but we still assume that IL-2 is related to low-grade CRS. Clinically, to control CRS, effective methods include reducing the dose level, administering the anti-IL-6 neutralizing antibody tocilizumab and corticosteroids, and using anti-CD19 CAR T cells instead of anti-CD20 CAR T-cells

for lymphoma. Neurotoxicity, including confusion, delirium, aphasia, seizure, and especially elevated intracranial pressure, is a tricky problem and might be associated with CAR T-cells in the CSF.^[29] Only 5 included studies clearly reported grade 3 or grade 4 neurotoxicity, but the overall incidence rate was as high as 1/3, prompting investigators to need to reduce this risk.

Although our study focused on autologous CAR T-cells, allogeneic CAR T-cells were administered in 2 excluded studies.^[36,37] One achieved 2 PRs in 10 patients with lymphoma, and 1 achieved 1 partial remission in 6 patients. The total response rate was lower than 0.2, which was far inferior to that of autologous CAR T-cells. Efficacy was further evaluated in a systematic review of donor-origin CAR T-cells by Anwer et al, who concluded that there is a very low risk of graft-versus-host disease flare with the use of donor-derived CAR T cells.^[18] Based on data from the excluded literature, we are not convinced of the efficacy and safety of allogeneic CAR T-cells.

To the best of our knowledge, this is the first meta-analysis of CAR T cells focusing on lymphoma. We respectfully disagree with mixing leukemia with lymphoma to evaluate efficacy. That is, while treating lymphoma, CAR T cells are confronted with more challenges, such as a physical barrier and an immunosuppressive tumor microenvironment. Based on sufficient samples, we powerfully indicated the advantage of fludarabine, while other systematic reviews have only concentrated on lymphodepletion. In addition, the effects of dosage and IL-2 administration were systematically discussed in our analysis. In contrast to conventional knowledge, high-dose infused CAR T cells attenuated rather than strengthened the ORR. Notably, we carefully divided IL-2 administration into an in vivo injection group and an in vitro expansion group.

Finally, some expectations have been brought forward. The long-term persistence of CAR T cells at the tumor site is crucial for patient progression-free survival. A preclinical trial indicated that the inclusion of both 4-1BB and CD28 costimulatory domains improves the long-term survival of T cells.^[38] Immunological rejection is another limitation that is a barrier to the therapeutic effectiveness of CAR T cells and can be resolved by improving CAR design. Replacing scFv-containing regions from murine antibodies with humanized scFv regions may attenuate the degree of immunogenicity. Inefficient recruitment by immature tumor blood vessels is a critical determinant of promising outcomes.^[39] Manipulating homing addressins on T-cells and vascular addressins on tumor blood vessels is a potential approach to intensify the recruitment of CAR T-cells. Long et al found that myeloid-derived suppressor cells can inhibit the response of CAR T-cells and that the application of ATRA reduces the quantity of myeloid-derived suppressor cells to enhance the efficacy of CAR T-cells against sarcoma.^[40] Therefore, a new regimen that integrates ATRA may strengthen the outcomes of CAR T-cells in solid tumors. Furthermore, bispecific CARs that simultaneously incorporate 2 kinds of receptors (such as an anti-CD19 receptor plus an anti-CD20 receptor) are being investigated and may eliminate disease progression caused by "antigen escape." Additionally, optimizing the CAR T cell subpopulation achieves improved outcomes. The ratio of CD4⁺:CD8⁺ CAR T cells and the use of T memory stem cells are under investigation because of the superior proliferative capacity and antitumor efficacy of these cell types.^[27,41]

In conclusion, our systematic review and meta-analysis showed a promising response rate for CAR T-cells in lymphoma.

Through subgroup analysis, we found 3 new results that are useful for future clinical decision-making: a CAR T-cell dose lower than $10^8/m^2$ is associated with increased response rates and reduced incidence rates of CRS (grade ≥ 3); no IL-2 administration is recommended because IL-2 has been proven to decrease the response rate; and anti-CD19 CAR T-cell therapy is recommended for lymphoma rather than anti-CD20 CAR T-cell therapy because anti-CD19 CAR T-cells produce fewer CRS events. Last but not least, we firmly believe that new immunotherapy methods will be ameliorated in many ways and applied in more kinds of malignancies.

4.1. Weaknesses

Our meta-analysis included 2 nonfull-text studies that only provided basic information and outcomes. This inclusion might produce inexactness. Wang et al's study enrolled 16 patients meeting the enrollment criteria; however, 13 of them were at a CR or PR status before CAR T cell therapy, resulting in deviations in the best ORR. Therefore, we adapted a 6m ORR in the best ORR analysis. The Juliet trial reported lymphodepleting chemotherapy with Cy/flu or bendamustine. However, we did not obtain the full data to divide the patients into 2 subgroups when flu was administered, so we did not add this trial to the subgroup analysis of the lymphodepletion regimen. Heterogeneity exists in CRS assessment criteria because 2 studies conducted at the University of Pennsylvania adopted their own standards. Between these 2 studies, the Juliet trial is considered the main source of heterogeneity, and its sCRS incidence rate was beyond 0.5 for unknown reasons.

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References

- [1] Vose JM, Zhang MJ, Rowlings PA, et al. Autologous transplantation for diffuse aggressive non-Hodgkin's lymphoma in patients never achieving remission: a report from the autologous blood and marrow transplant registry. *J Clin Oncol* 2001;19:406–13.
- [2] Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med* 1995;333:1540–5.
- [3] Telio D, Fernandes K, Ma C, et al. Salvage chemotherapy and autologous stem cell transplant in primary refractory diffuse large B-cell lymphoma: outcomes and prognostic factors. *Leuk Lymphoma* 2012;53:836–41.

- [4] Dai H, Zhang W, Li X, et al. Tolerance and efficacy of autologous or donor-derived T cells expressing CD19 chimeric antigen receptors in adult B-ALL with extramedullary leukemia. *Oncoimmunology* 2015;4:e1027469.
- [5] Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med* 2014;6:224ra25.
- [6] Kochenderfer JN, Dudley ME, Feldman SA, 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;119:2709–20.
- [7] Eshhar Z, Waks T, Gross G, et al. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci U S A* 1993;90:720–4.
- [8] Michel S, Renier B, Isabelle R. The basic principles of chimeric antigen receptor design. *Cancer Discov* 2013;3:388–98.
- [9] Kochenderfer JN, Wilson WH, Janik JE, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood* 2010;116:4099–102.
- [10] Wang CM, Wu ZQ, Wang Y, et al. Autologous T cells expressing CD30 chimeric antigen receptors for relapsed or refractory Hodgkin's lymphoma: an open-label phase 1 trial. *Lancet* 2015;386:512–20.
- [11] Ramos CA, Savoldo B, Torrano V, et al. Clinical responses with T lymphocytes targeting malignancy-associated μ light chains. *J Clin Invest* 2016;126:2588–96.
- [12] Ahmed N, Brawley V, Hegde M, et al. HER2-specific chimeric antigen receptor-modified virus-specific T cells for progressive glioblastoma: a phase 1 dose-escalation trial. *JAMA Oncol* 2017;3:1094–101.
- [13] Till BG, Jensen MC, Wang J, et al. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. *Blood* 2008;112:2261.
- [14] Park JH, Geyer MB, Brentjens RJ. CD19-targeted CAR T-cell therapeutics for hematologic malignancies: interpreting clinical outcomes to date. *Blood* 2016;127:3312.
- [15] Singh N, Frey NV, Grupp SA, et al. CAR T cell therapy in acute lymphoblastic leukemia and potential for chronic lymphocytic leukemia. *Curr Treat Options Oncol* 2016;17:28.
- [16] Brudno JN, Kochenderfer JN. Chimeric antigen receptor T-cell therapies for lymphoma. *Nat Rev Clin Oncol* 2018;15:31–46.
- [17] Riaz IB, Zahid U, Kamal MU, 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.
- [18] Anwer F, Shaukat AA, Zahid U, et al. Donor origin CAR T cells: graft versus malignancy effect without GVHD, a systematic review. *Immunotherapy* 2017;9:123.
- [19] Zhou H, Luo Y, Zhu S, et al. The efficacy and safety of anti-CD19/CD20 chimeric antigen receptor- T cells immunotherapy in relapsed or refractory B-cell malignancies: a meta-analysis. *BMC Cancer* 2018;18:929.
- [20] Zhang T, Cao L, Xie J, et al. Efficacy of CD19 chimeric antigen receptor-modified T cells for treatment of B cell malignancies in phase I clinical trials: a meta-analysis. *Oncotarget* 2015;6:33961–71.
- [21] Lee DW, Rebecca G, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood* 2014;124:188–95.
- [22] Zhang WY, Wang Y, Guo YL, 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.
- [23] Jensen MC, Popplewell L, Cooper LJ, 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;16:1245–56.
- [24] Till BG, Jensen MC, Jinjuan W, 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;119:3940–50.
- [25] Wang Y, Zhang WY, Han QW, 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;155:160–75.

- [26] Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med* 2017;377:2531–44.
- [27] Turtle CJ, Hanafi LA, Berger C, 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.
- [28] Wang X, Popplewell LL, Wagner JR, 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.
- [29] Kochenderfer JN, Somerville RP, Lu T, 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;35:1803–13.
- [30] Kochenderfer JN, Dudley ME, Kassim SH, 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;33:540–9.
- [31] Brudno JN, Shi V, Stroncek D, et al. T cells expressing a novel fully-human anti-CD19 chimeric antigen receptor induce remissions of advanced lymphoma in a first-in-humans clinical trial. *Blood* 2016; 128:999.
- [32] Enblad G, Karlsson H, Gammelgard G, et al. A phase I/IIa trial using CD19-targeted third generation CAR T cells for lymphoma and leukemia. *Clin Cancer Res* 2018;24:6185–94.
- [33] Schuster SJ, Svoboda J, Chong EA, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N Engl J Med* 2017;377:2545.
- [34] Value in Using CAR T Cells for DLBCL. *Cancer Discovery*. 2017.
- [35] Matsuki E, Younes A. Checkpoint inhibitors and other immune therapies for Hodgkin and non-Hodgkin lymphoma. *Curr Treat Options Oncol* 2016;17:31.
- [36] Kochenderfer JN, Dudley ME, Carpenter RO, et al. Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation. *Blood* 2013;122:4129–39.
- [37] Brudno JN, Somerville RPT, Shi V, 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.
- [38] Zhao Z, Condomines M, Sj VDS, et al. Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. *Cancer Cell* 2015;28:415–28.
- [39] Ager A, Watson HA, Wehenkel SC, et al. Homing to solid cancers: a vascular checkpoint in adoptive cell therapy using CAR T-cells. *Biochem Soc Trans* 2016;44:377.
- [40] Long AH, Highfill SL, Cui Y, et al. Reduction of MDSCs with all-trans retinoic acid improves CAR therapy efficacy for sarcomas. *Cancer Immunol Res* 2016;4:869.
- [41] Sabatino M, Hu J, Sommariva M, et al. Generation of clinical-grade CD19-specific CAR-modified CD8+ memory stem cells for the treatment of human B-cell malignancies. *Blood* 2016;128:519–28.