Original Article

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Evaluation of the Efficacy and Safety of Chimeric Antigen Receptor-Modified T (CAR-T) Cell Therapy in Leukemia: A Five-Year Updated Systematic Review and Meta-analysis

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Abstract:

Objective: Despite the progress made by conventional treatments in reducing mortality rates, the significant number of relapsed or refractory patients necessitates the exploration of novel therapies. Recent studies on chimeric antigen receptor T-cell therapy (CAR-T) cells have shown promising outcomes for individuals battling blood cancers. However, the outcomes are still inconsistent due to the structural complexity of CAR-T cells and the rapid development of more advanced versions. This study evaluated the efficacy and safety of various CAR-T cells in Leukemia patients.

Material and Methods: The preferred reporting items for systematic reviews and meta-analysis 2020 protocol was used for the literature search and systematic review. Studies reporting CAR-T cell therapy's efficacy and safety in Leukemia patients were included. Statistical analyses were performed using R statistical software v.3.3. P-values≤0.05 were considered statistically significant.

Results: Eighteen single-arm clinical trials were included based on the inclusion criteria. Most of the studies involved patients with acute Lymphoblastic Leukemia. CAR-T cell therapy in Leukemia achieved a 79% (95% confidence interval [CI] [69%-87%], I^2=74%) complete response, 79% (95% CI [59%-91%], I^2=87%) cytokine release syndrome event, 18% (95% CI [9%-33%], I^2=72%) immune effector cell-associated neurotoxicity syndrome event rate, 69% (95% CI [47%-85%], I^2=82%) minimal residual disease-negative, and a 9% (95% CI [8%-13%], I^2=37%) mortality rate.

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Conclusion: CAR-T therapy has demonstrated efficient responses in Leukemia patients, reinforcing the positive outcomes observed with favorable toxicities. Further data regarding the durability of CAR-T cell therapy are essential for strengthening our understanding of CAR-T cell efficacy and safety in Leukemia patients.

Keywords: CAR-T cell therapy, efficacy, Leukemia, safety

Introduction

Acute Leukemia is an aggressive type of Leukemia with a high rate of proliferation accompanied by symptoms such as fatigue, bruising, and frequent infections. In contrast, chronic Leukemia tends to develop slowly and is not accompanied by significant clinical symptoms[1]. In 2023, a total of 59,610 cases of Leukemia were diagnosed, accounting for 3% of all cancer cases. The high morbidity is also in line with the relative survival rate of patients at 66.7%, and thus in line with the high mortality rate of 23,710 cases. This number represents 3.9% of all deaths due to cancer[2].

Given the high morbidity and mortality of Leukemia, management efforts are crucial to increasing the patient survival rate. Conventional management performed on Leukemia patients includes radiation, hematopoietic stem cell transplantation, chemotherapy, or supportive therapy[3]. In Leukemia therapy, high-dose chemotherapy can more effectively kill cancer cells. This systemic management can cause long-term use resistance on regular targets and is only used for early-stage treatment. Not only that, but resistance to Leukemia therapy also poses a severe threat in the form of resistant cells with a long-term early renewal capacity that can also drive clonal growth, namely Leukemia stem cells: these cells are considered triggers for Leukemia recurrence, especially the AML type.

Recently, chimeric antigen receptor-modified T cell (CAR-T) cells have been used for the curative immunotherapeutic targeting of CD19 and GD2 for cancer management. CAR is an immune receptor made in the laboratory by modifying lymphocytes to target and eliminate cells that express specific antigens on their surface. In contrast, T cells are genetically engineered to express a particular CAR. CARs expand the potential application of adaptive cell therapy with genetically modified cells that overcome more cancers, such as CAR-T cells targeting GD2 antigens capable of mediating a moderate clinical impact if applied to patients with neuroblastoma disease. Then, the most efficient use of CAR is in targeting the CD19 molecule because it is expressed in almost all B cell lymphomas and normal B cells. Therefore, an immunological approach such as CAR-T therapy was developed[4].

However, previous studies on the effectiveness and safety of CAR-T cells are still inconsistent. This is supported by studies related to resistance to CAR-T cell-based therapy, which shows that remission will be short-lived in some patients due to the persistence of deteriorating CAR-T cells or cancer cell resistance as a result of antigen modulation. Not only that, challenges in CAR-T cell therapy also include making specific therapies for patients. Several factors, such as loss of target antigen, tumor resistance, immunosuppression, tumor bulk, therapy toxicity, patient biology, and variability of CAR-T cells, can also influence the emergence of these challenges. This study aimed to evaluate the efficacy and safety of various CAR-T cells in patients with Leukemia.

Material and Methods

This systematic review and meta-analysis are presented in compliance with the preferred reporting items for systematic reviews and meta-analyses 2020 guidelines[5]. This study has been registered in PROSPERO (ID CRD42024573724).

Identification of relevant literature

A systematic literature search was conducted on databases such as PubMed, ScienceDirect, and Cochrane in order to identify the relevant topics, starting from the 15th of October 2023, through to the 19th of January 2024. Our primary focus was on clinical trials pertinent to our meta-analyses. Boolean operators were used by keywords such as ("CAR-T") AND ("Leukemia") AND ("*Efficacy*" OR "*Safety*"). All authors took part in the screening process, followed by an independent and individual assessment of each study based on the predetermined eligibility criteria. The final list of included trials was agreed upon through discussions between all the authors. A complete agreement was required before inclusion. Disagreement amongst reviewers was resolved through consensus.

Eligibility criteria

Observational clinical trials that evaluated the outcomes of Leukemia patients. The study population was children and adult ALL patients, with most of the patients categorized as relapsed or refractory ALL within a timeframe of the included study between 2018 and 2023. Patients who were treated with CD19 and bispecific CD19/CD22 CAR-T cells accompanied by evaluation of complete reports on complete responses (CR), minimal residual disease (MRD), adverse events such as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), and mortality were included. Exclusion criteria in this study were studies with antigen recognition domains other than CD19 and/or bispecific CD119/CD22 and Leukemia types other than ALL.

Data extraction

The following data were gathered for every study included within this meta-analysis: publication characteristics (article authors, year of publication), study design (trial design, clinical setting, recruitment period, follow-up duration), population characteristics (age, gender, and other baseline data), intervention (CAR-T cell used, lymphodepletion therapy used), and outcome data (summary information about treatment effects, i.e., clinical response and adverse effects).

Assessment of risk of bias

The cochrane risk of bias 2 tool (ROB 2)[6] was used to assess methodological and reporting biases within the included studies. This tool is attributed to 5 domains for evaluating bias within clinical trial studies. These include the randomization process, deviations from the intended intervention, missing outcomes/missing data, measurements of the outcome, and selective reporting of results. Based on the biases in each domain, 2 independent reviewers judge the overall risk of bias in order to receive either low bias, some concerns, or high bias.

Outcome of interest

Microsoft Excel was used to compile the data extracted from the studies. The primary outcomes were the number of CR alongside mortality and the number of adverse events CRS and ICANS. Conversely, our secondary data were set only to the MRD.

Statistical analysis

All the studies on this topic do not have controls as a comparator; thus, our primary and secondary outcomes are presented in proportions or percentages. Dichotomous outcomes (proportions) were assessed using the Mantel–Haenszel method applying fixed/random-effects models based on the heterogeneity in order to generate a percentage with 95% confidence interval (CI).

The following formula was used for calculating all the study parameters analyzed within this meta-analysis:

$$Proportion = \frac{Event}{Total\ Sample\ size}$$

Heterogeneity was assessed using the I^2; a heterogeneity below 50% represents low heterogeneity, and a heterogeneity of 50% or more represents high heterogeneity. Fixed effects were used for low heterogeneity, while high heterogeneity used the random effects. Further analyses using funnel plots and Peters tests were conducted in order to detect any small study biases between the included studies because some studies had very small sample sizes. All statistical analysis were performed using the R statistical software v.3.3 and R studio version 2023.03.0-daily+82.pro2.

Results

Literature search

We found 6,049 studies using a keyword-only systematic search of the literature. All authors then removed duplicates from the search results and carefully reviewed the titles and abstracts to ensure they were relevant. Eighteen studies fulfilled the eligibility criteria. After conducting individual and independent assessments of the entire texts of the remaining publications based on the predefined inclusion and exclusion criteria, 6 research studies were deemed insufficiently data-driven. These meta-analyses comprise 18 (2018–2023) papers that met the qualifying criteria. Figure 1 is a flowchart that summarizes the search and screening methodology.

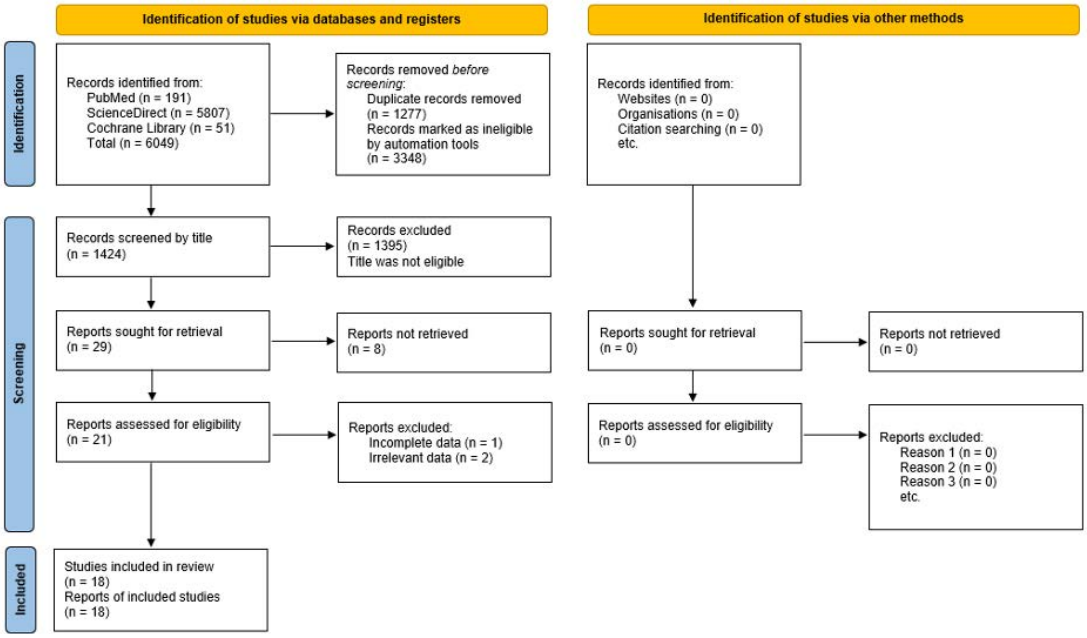

Figure 1 PRISMA flowchart[5]

Study characteristics

Characteristics of the studies included within this meta-analysis are listed in Table 1. There were 18 single-arm clinical trial studies included in this meta-analysis. Across these single-arm clinical trials, 694 patients were involved in this meta-analysis. Out of the 18 studies included, there seem to be only 4 studies conducted outside of China; 2 were conducted in America, while the rest were conducted in Israel and Spain. The age of the samples in the studies differs quite significantly, with the median age as low as 6 years and the highest median age as high as 51 years. The studies prominently used all patients as their samples, including B-cell and T-cell ALL. It should be noted that most of the studies involved patients who suffered from relapse or were refractory to conventional treatment. The vectors used in making CAR-T cells are also documented; the studies included seem to prefer lentivirus in producing CAR-T cells, with only 2 studies using retroviruses. Among all CAR-T cells used, CAR-T cells targeting CD19 are the most common. Other moieties that the CAR-T targets include cells targeting CD19 and CD22 as bispecific CAR-T cells. Additionally, most studies used 4-1 BB (CD137) as their costimulatory domain. Most of the studies we included did not mention the generation of CAR-T cells in use; however, through the conventional grouping of CAR-T cell generations, most fit into the second generation of CAR-T cells based on using only one costimulatory domain. Fludarabine and cyclophosphamide were used primarily for lymphodepletion therapy before CAR-T cell administration. However, other medications such as busulfan, clofarabine, methotrexate, cytarabine, vincristine, epirubicin, dexamethasone, and doxorubicin were also administered in some studies.

Risk of bias assessment results

All 18 studies were assessed using the cochrane ROB 2 tool. There were concerns about the overall risk of bias in only 4 studies, mostly due to the randomization process and selection of reported results. Otherwise, there was no significant or high risk of bias in any of the domains. However, the domain of the randomization process had some concerns caused by the high number of studies (16 studies) either not informing the randomization process or stating that the clinical trial was not randomized. A traffic light plot (Supplementary material) and an overall risk of bias throughout all the studies (Supplementary Figure 1) have been plotted in order to further visualize each study's risk of bias.

Outcome results

Complete response

Based on our proportional meta-analysis results, CAR-T cell therapy has shown a favorable clinical response in Leukemia patients, with 79% (95% CI [69%-87%], I^2=74%) of all the involved patients experiencing a CR (Figure 2A). Sub-group analysis based on the antigen recognition domain on all the parameters was completed; the evaluated recognition domain consisted of CD19 and a combination of CD19 and CD22 (bispecific CD19/CD22). We ran into similar problems with our previous sub-analysis; some subgroups only consisted of 1 study, making it ineligible. Here, we found that patients receiving CD19 CAR-T cells achieved a complete response of 79% (95% CI [68%-87%], I^2=76%), while other recognition domains, bispecific CD19/CD22, showed a similar CR of about 79% (95% CI [69%-87%], I^2=74%) compared to CD19. Other than the CR of patients receiving CD19 CAR-T cells, the

CR of bispecific CD19/CD22 CAR-T cells was not eligible due to each only consisting of one study (Figure 5A). Sub-analysis for all the previous parameters was also performed by Leukemia type. Based on the results of our sub-analysis on each Leukemia type of each Leukemic patients in each study, which consisted of B-cell ALL or unspecified ALL, we found that there was no significant difference in the pooled effect of incidence of CR between B-cell ALL or unspecified ALL patients treated with CAR-T cell therapy (80%, 95% CI [66%-89%], I^2=78% vs 81%, 95% CI [69%-89%], I^2=0%, respectively), with only a 1% difference leading to better outcomes in unspecified ALL patients (Figure 6A). Despite the better outcomes, this difference might be caused by including unspecified ALL patients in each study, which might have skewed the conclusion.

Minimal residual disease

Minimal residual disease negative (MRD-negative) refers to the absence of detectable cancer cells following treatment, typically determined through susceptibility tests. Since there is a high number of studies that include Leukemic patients who have relapsed, MRD was also assessed in order to determine if patients receiving this therapy were prone to relapse or not. Our analysis of MRD showed 69% of the total sample had no residual Leukemic cells in their bodies after treatment, which occurred in 69% (95% CI [47%-85%], I^2=82%) of all patients (Figure 2B), indicating the same probability of recurrence and non-recurrence.

In the sub-analysis by antigen recognition domain, the incidence of MRD was slightly better when using the CD19/CD22 antigen compared with CD19, showing a difference of 2% (67%, 95% CI [44%-84%], I^2=84% vs 69%, 95% CI [47%-85%], I^2=82%). In the sub-analysis based on type of ALL (Figure 5B), the incidence of MRD was slightly better in patients with B-cell ALL compared to unspecified ALL, showing a difference of 4% (73%, 95% CI [47%-89%], I^2=85% vs 69%, 95% CI [47%-85%], I^2=82%) (Figure 6B).

Cytokine release syndrome

However, despite its favourable rate of CR, patients who received CAR-T cell therapy also suffered from adverse events, which occurred in the majority of patients. Among these adverse events, CRS had the highest rate of occurrence, with around 79% (95% CI [59%-91%], I^2=87%) of all Leukemic patients suffering from this side effect (Figure 2C). Further sub-analysis of CRS based on its grade, which was categorized as lower (Grade 0-2) and higher (Grade 3-4) grade CRS, has revealed that lower grade CRS affected around 0.58 (95% CI [0.36; 0.77], I^2=91%) of all patients (Figure 3A), while higher grade CRS only affected 0.19 (95% CI [0.12; 0.29], I^2=73%) (Figure 4A). Even though CRS had a high occurrence in patients who received CAR-T cell therapy, most patients suffered only from lower grade CRS, comprised of only grade 0 up to grade 2 CRS. The event of CRS appeared to be less frequent in patients receiving CD19 CAR-T (78%, 95% CI [52%-92%], I^2=89%) compared to those receiving bispecific CD19/CD22 CAR-T cells (83%, 95% CI [65%-93%], I^2=0% (Figure 5C). The incidence of CRS appeared to be less frequent in patients with B-cell ALL (79% 95% CI [59%-90%], I^2=85%) compared to patients with unspecified ALL (81%, 95% CI [7%-100%], I^2=94%) (Figure 6C).

Immune effector cell-associated neurotoxicity syndrome

Another concerning adverse effect of CAR-T cell therapy was the development of ICANS; it was revealed that 18% (95% CI [9%-33%], I^2=72%) of all patients developed ICANS during the therapy, which occurred at a significantly lower rate than CRS events (Figure 2D). In contrast to CRS, ICANS had less frequent events in patients

receiving bispecific CD19/CD22 CAR-T cells compared to CD19 alone, which differed by 13% (7%, 95% CI [1%-37%], I^2=0% vs 20%, 95% CI [10%-37%], I^2=78%) (Figure 5D). The incidence of ICANS was also less common in patients with unspecified ALL compared to B-cell ALL, which differed only by 2% (16%, 95% CI [1%-88%], I^2=91% vs 18%, 95% CI [11%-29%], I^2=45%) (Figure 6D).

Mortality

Due to the concerning rate of adverse events, mortality rates were also assessed to consider the possibility of lethal effects from CAR-T cell therapy. Here, we found that the overall mortality rates were meager despite the concerning rates of adverse events, affecting only 9% (95% CI [8%-13%], I^2=37%) of patients who received CAR-T cell treatment (Figure 2E). All parameters show a high heterogeneity (>50%) between the study results, except for the overall mortality rates that show a low heterogeneity (<50%). Based on the sub-group analysis, mortality rates of CD19 were found to be lower than bispecific CD19/CD22 CAR-T cells by a margin of 6% (6%, 95% CI [2%-14%], I^2=56% vs 12%, 95% CI [6%-22%], I^2=0%) (Figure 6E). All subgroup analyses showed very high heterogeneity with some exceptions. Mortality rates were also lower in patients with unspecified ALL, with a difference of 9% (4%, 95% CI [2%-8%], I^2=19% vs 13%, 95% CI [8%-21%], I^2=0%) (Figure 6E).

Study bias evaluation

The evaluation of small studies' biases using funnel plots and the Peters test was also conducted in light of some studies employing a concerningly low sample size. Surprisingly, Peters test of all the parameters, which included CR (p-value=0.3949), MRD (p-value=0.4094), CRS (p-value=0.0622), ICANS (p-value=0.1933), and mortality rates (p-value=0.4030), have p-values above 0.05 that show insignificant value for funnel plot asymmetry, indicating the absence of any study bias in all the parameters of our meta-analysis. Funnel plots of each parameter are represented in Figure 7. Note that the final sub-group analysis pooled effects may not have reached the same value as the original pooled effect value; this is due to the fact that some of the studies included in the original pooled effect were not included in the subsequent sub-analysis due to undisclosed data that were essential to the grouping of said studies into the sub-analysis.

Table 1 Characteristics of senior recreational golfers (n=100)

Author (Year)	Country	Sample size	Age	Leukemia subtype	Duration Follow Up (month)	Vector	Ag Recognition Moieties	Costimulatory Domain	Lymphodepleting Therapy
Jacoby (2018)[10]	China	21	11[a]	ALL*	20	Gamma Retrovirus	CD19	CD28	Fludarabine+ cyclophosphamide
Ma (2019)[24]	China	13	6[a]	B-Cell ALL*	18	Lentivirus	CD19	4-1BB	Fludarabine+ cyclophosphamide
Zhang (2019)[25]	China	52	6[a]	B-Cell ALL*	15	Lentivirus	CD19/CD22	4-1BB	busulfan/fludarabine
Jiang (2019)[26]	China	60	18-30[a]	B-Cell ALL*	34	Lentivirus	CD19	4-1BB	Fludarabine+ cyclophosphamide
Noelle (2019)[27]	America	49	34[a]	B-Cell ALL	24	Lentivirus	CD19	4-1BB	cyclophosphamide, fludarabine, clofarabine, methotrexate, cytarabine, vincristine, and doxorubicin.
Itzhaki (2020)[28]	Israel	38	17±14[b]	ALL	38	Retrovirus	CD19	CD28	Fludarabine+ cyclophosphamide
Ortiz (2020)[16]	Spain	38	24.5[a]	ALL*	12	Lentivirus	CD19	4-1BB	Fludarabine+ cyclophosphamide
Zhao (2020)[13]	China	122	26[a]	ALL*	53	Lentivirus	CD19	4-1BB	Fludarabine+ cyclophosphamide
Wang (2020)[14]	China	23	42[a]	B-Cell ALL*	12	Lentivirus	CD19	4-1BB	Fludarabine

Table 1 (countinued)

Author (Year)	Country	Sample size	Age	Leukemia subtype	Duration Follow Up (month)	Vector	Ag Recognition Moieties	Costimulatory Domain	Lymphodepleting Therapy
Heng (2020)[7]	China	10	16[a]	ALL*	18	Lentivirus	CD19	4-1BB	Fludarabine+ cyclophosphamide OR cyclophosphamide +vincristine+ Epirubicin+ dexamethasone
Dai (2020)[15]	China	6	28[a]	ALL*	11	Lentivirus	CD19/CD22	4-1BB	Fludarabine+ cyclophosphamide
Hu (2021)[12]	China	6	49[a]	B-Cell ALL*	8	Lentivirus	CD19/CD22	4-1BB	Fludarabine+ cyclophosphamide
Lu (2021)[21]	China	47	29[a]	B-Cell ALL	31	Lentivirus	CD19	CD28	Fludarabine+ cyclophosphamide
Roddie (2021)[22]	America	25	41.5[a]	ALL*	3	Lentivirus	CD19	4-1BB	Fludarabine+ cyclophosphamide
Yang (2022)[8]	China	25	20[a]	B-Cell ALL	23	Lentivirus	CD19	CD28	Fludarabine+ cyclophosphamide
Li (2022)[18]	China	78	31[a]	B-Cell ALL*	39	Lentivirus	CD19	CD28	Fludarabine+ cyclophosphamide
Gong (2022)[11]	China	61	32[a]	B-Cell ALL*	43	Lentivirus	CD19	4-1BB	Fludarabine+ cyclophosphamide
Niu (2023)[29]	China	19	51[a]	B-Cell ALL	16	Lentivirus	CD19/CD22	-	N/A

[a]Median Age, [b]Mean Age *R/R=relapsed/refractory, CRS=cytokine release syndrome, ICANS=immune effector cell-associated neurotoxicity syndrome, MRS=minimal residual disease, Ag=antigen

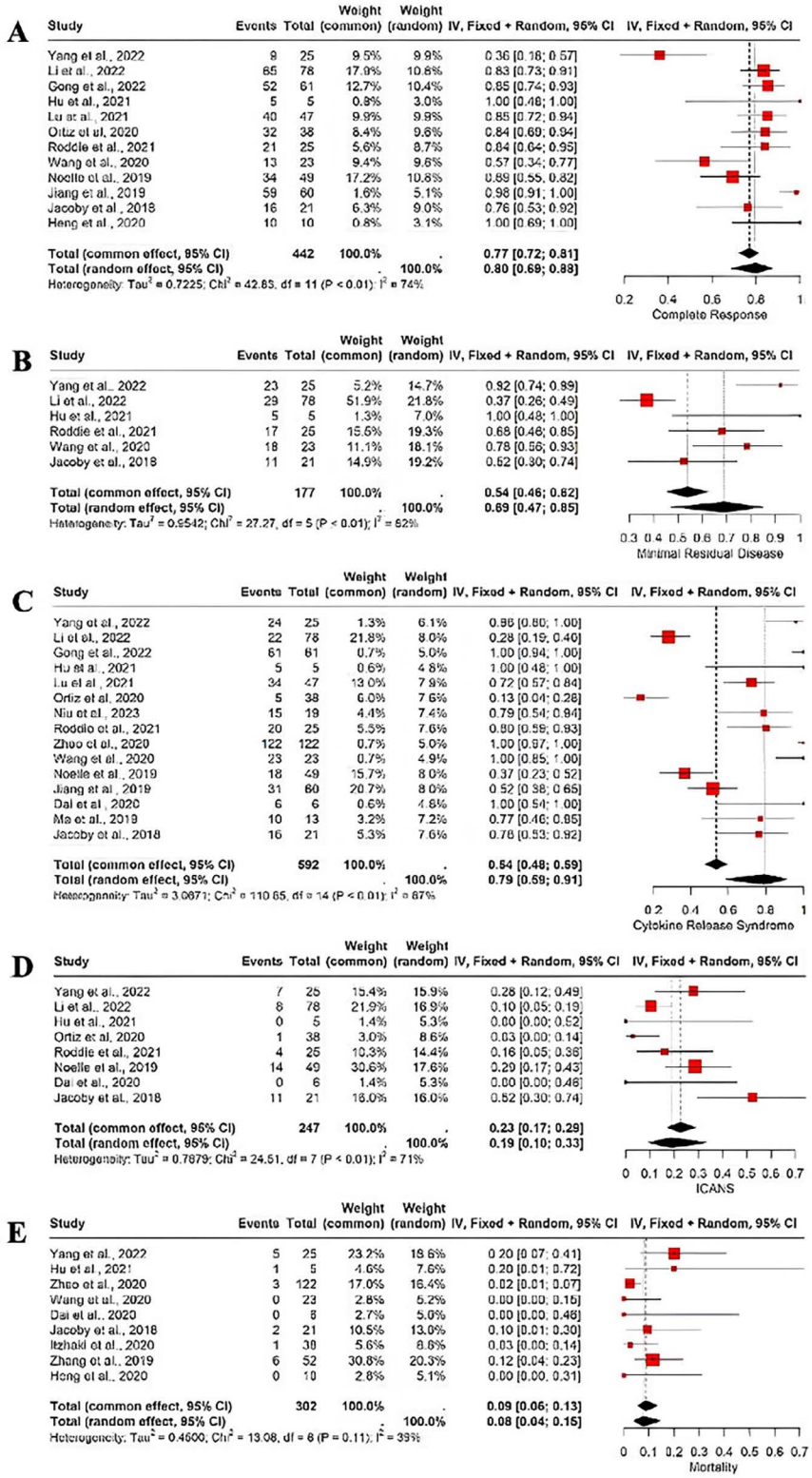

Figure 2 Forest plot of (A) CAR-T cell therapy complete response, (B) Minimal residual disease event, (C) Cytokine Release Syndrome, (D) Immune cell-mediated associated neurotoxicity syndrome event, (E) Mortality.

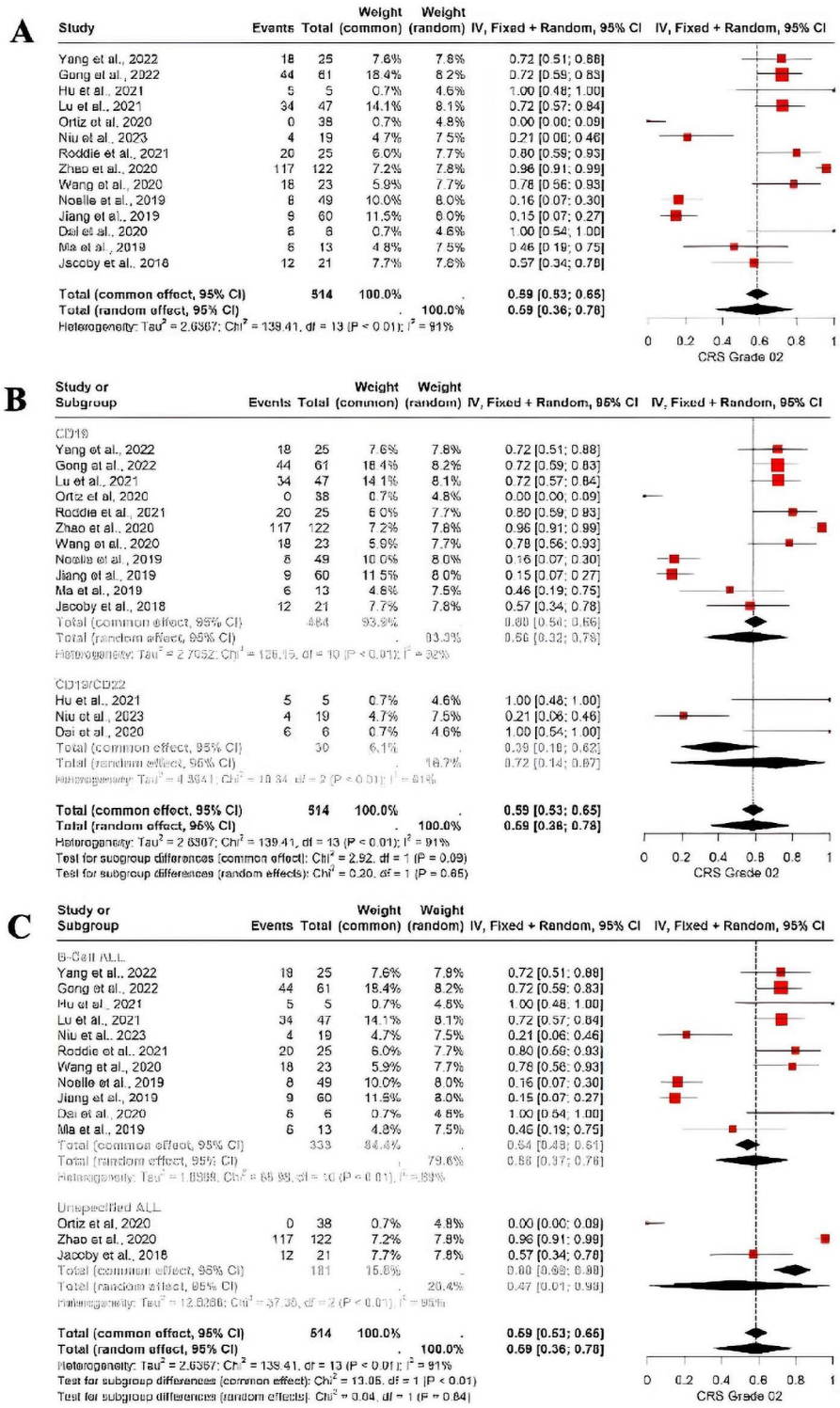

Figure 3 Forest plot of Cytokine Release Syndrome grade 0–2. (A) Overall analysis, (B) Sub–analysis based on antigen recognition, (C), Sub–analysis based on Leukemia type.

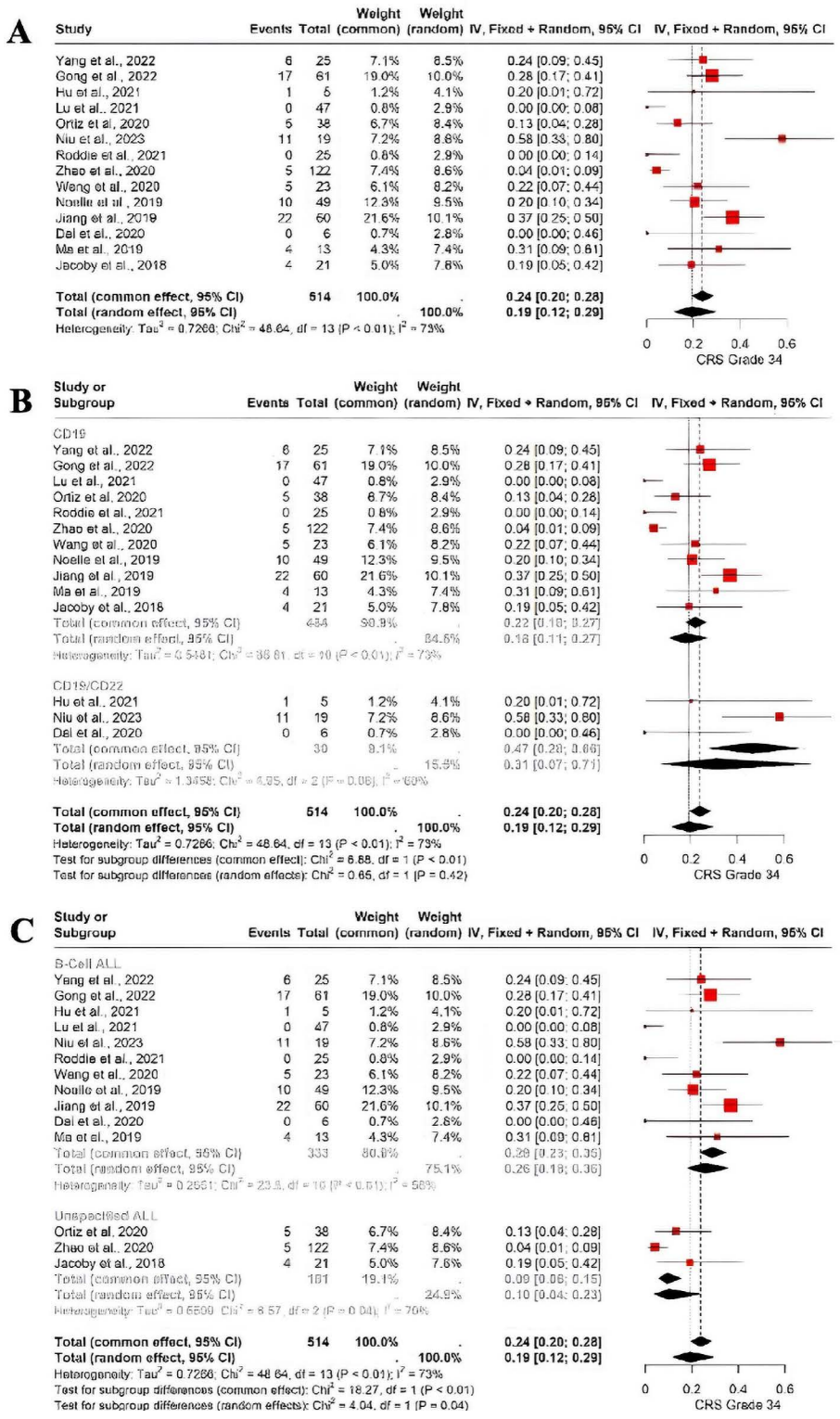

Figure 4 Forest plot of Cytokine Release Syndrome grade 3–4. (A) Overall analysis, (B) Sub–analysis based on antigen recognition, (C), Sub–analysis based on Leukemia type.

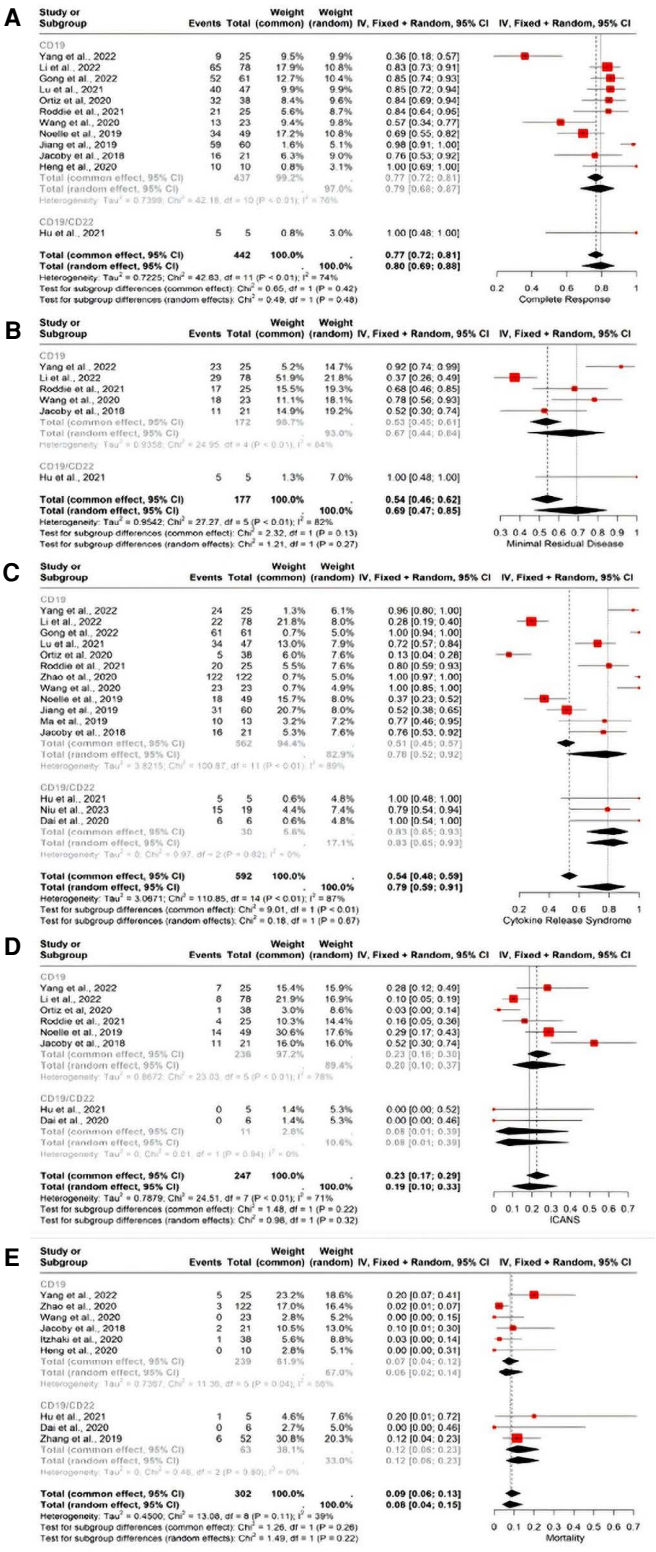

Figure 5 Forest plot based on antigen recognition sub-analysis. (A) CAR-T cell therapy complete response, (B) Minimal residual disease event, (C) Cytokine Release Syndrome, (D) Immune cell-mediated associated neurotoxicity syndrome event, (E) Mortality.

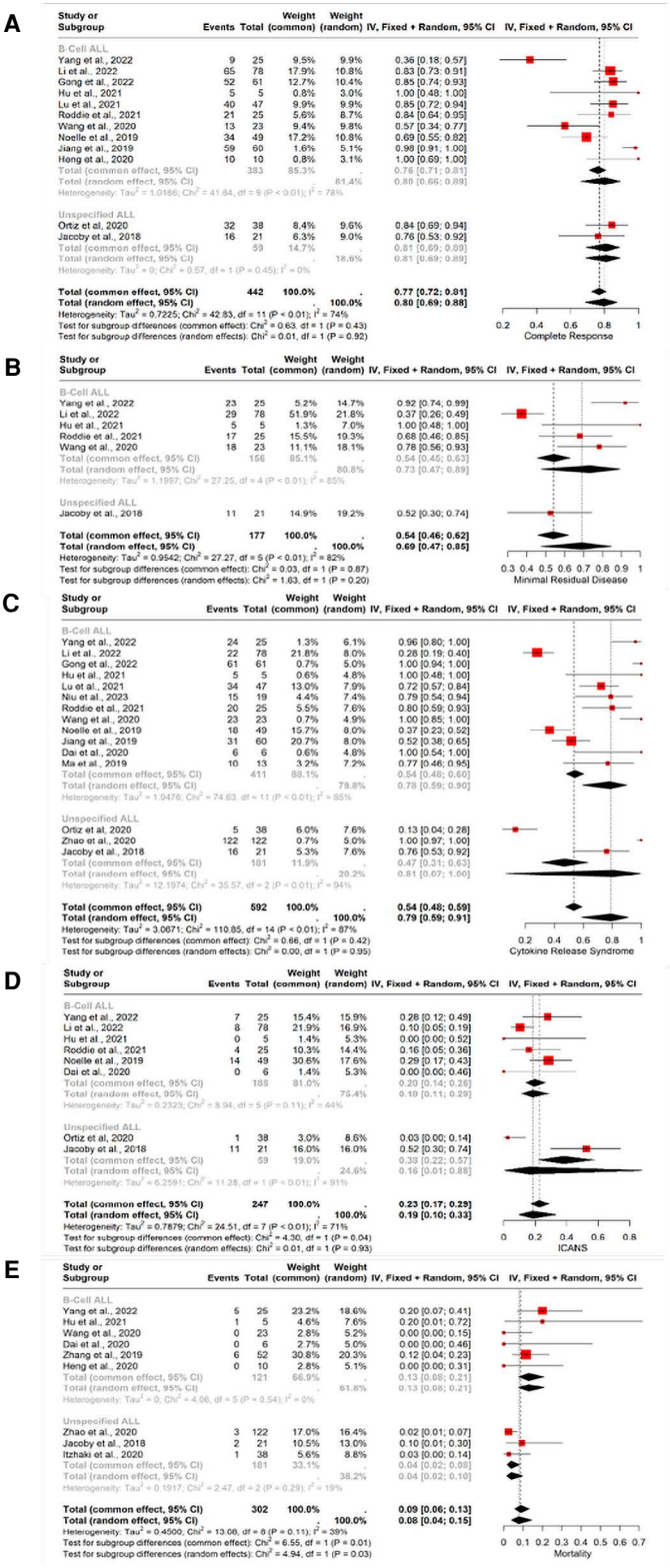

Figure 6 Forest plot based on Leukemia type sub-analysis. (A) CAR-T cell therapy complete response, (B) Minimal residual disease event, (C) Cytokine Release Syndrome, (D) Immune cell-mediated associated neurotoxicity syndrome event, (E) Mortality.

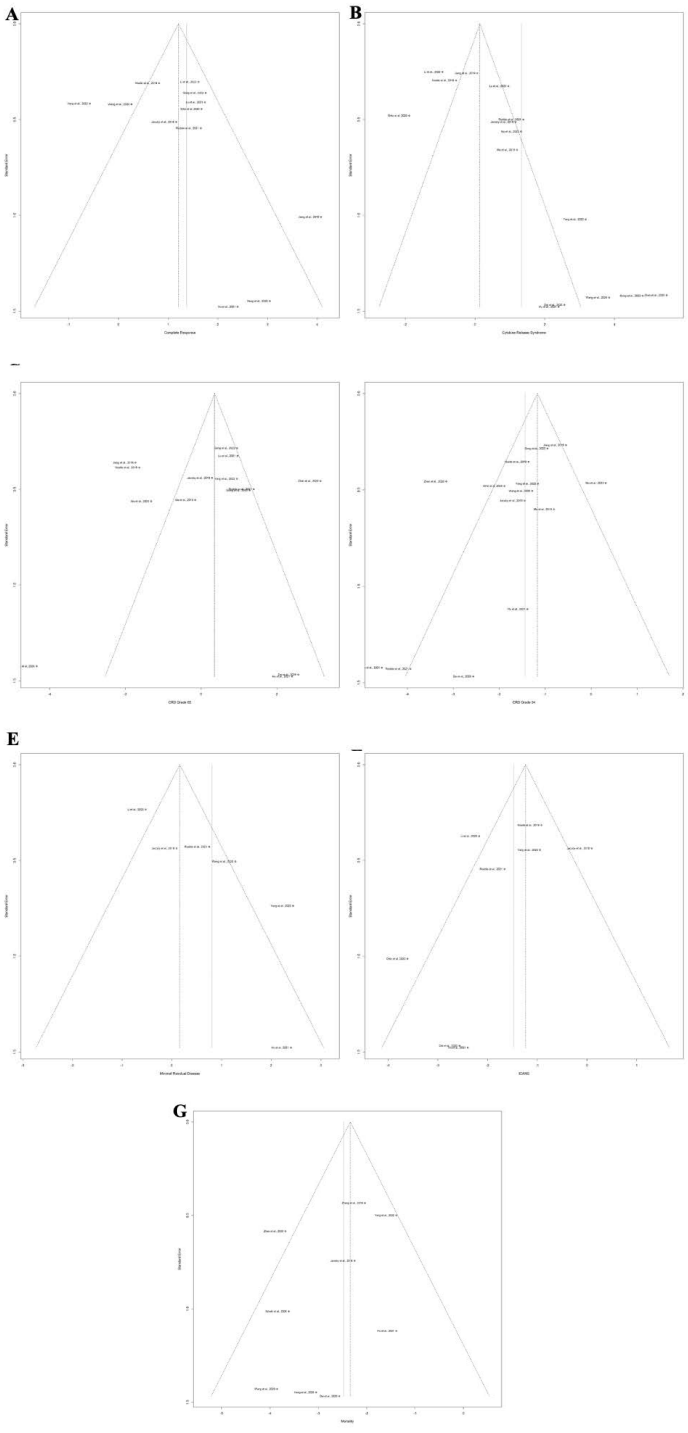

Figure 7 Funnel plots of (A) CAR-T cell therapy complete response, (B) Minimal residual disease event, (C) Cytokine Release Syndrome, (D) Immune cell-mediated associated neurotoxicity syndrome event, (E) Mortality.

Discussion

CAR-T cell therapy has shown significant efficacy alongside low mortality, even though the adverse events are still widespread; this therapy shows excellent potential as an alternative treatment for Leukemia. CAR-T cell therapy shows relatively high efficacy in treating Leukemia. In the analysis with CR parameters, the percentage of patients achieving CR even reached 79%. This finding aligns with Heng et al. with 100% of patients achieving CR[7]. In contrast, Yang et al. (2022) reported a significantly lower CR rate of 36%[8]. The administration of a higher CAR-T dose in Heng et al. (2020) showed a median effective dose of 2.47×10^6 cells/kg (2.3×10^5 – 4.17×10^7)[7].Meanwhile, the lower CAR-T doses in Yang et al. (2022) were associated with a lower clinical response, with a dose range of 1×10^4–1×10^5 cells/kg[8]. Result variability may also be due to the differences in patient characteristics, including age, type of Leukemia, and the differences in lymphodepletion therapy undergone. The younger patients in Heng et al. (mean age 16 years) exhibited a better response to CAR-T therapy compared to the older patients in Yang et al. (mean age 20 years), though the difference was not substantial[7,8]. Differences in the type of Leukemia in the sample may introduce bias, as the B-cell ALL population in Yang et al. showed a lower efficacy compared to those with unspecified ALL[8]. Furthermore, the structural differences in CAR-T cells could explain the observed discrepancies. The study demonstrated that the 4-1BB costimulatory domain used in Heng et at. had a better efficacy than CD28[7]. This is in accordance with research that has shown CAR-T cells with the 4-1BB costimulatory domain are more persistent than those with CD28. This is due to the more continuous CAR signaling that leads to increased exhaustion, while the 4-1BB domain is able to mitigate this exhaustion[9]. Sub-analysis based on antigen type was also carried out, CAR-T with dual CD19/CD22 antigen target exhibited lower efficacy (CR=83%) than CAR-T with CD19 antigen alone (CR=79%). It should be noted that the analysis based on bispecific CD19/CD22 antigens only involved one study, and thus, the results may not be reliable as a reference for comparison. Conversely, the analysis based on Leukemia type showed consistent efficacy against unspecified ALL (CR=81%) and B-cell ALL patients (CR=80%). In the analysis with unspecified ALL, higher results were reported by Ortiz et al. with a percentage of patients achieving CR of 84%. A lower proportion was reported by Jacoby et al. with 76%[10]. Variations in patient demographics may introduce minor biases, influencing these efficacy outcomes. Additionally, the use of gamma retroviral vectors with CD28 costimulatory domains showed lower clinical responses compared to the use of lentivirus vectors combined with 4-1BB costimulatory domains. Yet, considering the lack of studies in the analysis focusing on unspecified ALL types, it may not adequately represent the group in the real world.

CAR-T cell therapy, despite its favorable efficacy, is accompanied by a significant incidence of adverse effects, particularly CRS, which occurs in approximately 79% of patients. This aligns with Gong et al., Hu et al., Zhao et al., Wang et al., and Dai et al., which reported 100% of patients experiencing CRS[11–15]. In contrast, Ortiz et al. documented a significantly lower incidence of CRS at 13%[16]. Such discrepancies may be influenced by demographic variations, including ethnicity and age. For instance, Ortiz et al. indicated that younger Spanish patients (median age 24.5 years) exhibited a more favorable clinical response[16]. Conversely, a study focusing on an older Chinese population reported a higher incidence of CRS[11,12,14]. Interestingly, even in younger patients with a mean age of 28 years, Dai et al. reported a high CRS occurrence, potentially impacted by their small sample size (n=6)[15]. Zhao et al. also found elevated CRS rates in a young patient group (mean age 26 years), likely due to the administration of higher

CAR-T doses (3×10^6 and 5×10^6 cells) and the inclusion of patients with relapsed/refractory ALL, who may have experienced exacerbated side effects[13]. Sub-analysis based on target antigens was performed; the results revealed that CAR-T therapy with CD19 antigen had a lower incidence of CRS (78%) than CD19/CD22 (83%). This may demonstrate that the more CAR-T targets the more likely it is to have side effects. The results of the CD19/CD22 analysis align with Hu et al., and Dai et al.[12,15], while a lower percentage of CRS events were reported by Niu et al., with 79% of patients experiencing CRS[17]. Yet, sample size differences may introduce survivorship bias, particularly in studies with limited populations (n=6). Sub-analysis of Leukemia type was also performed, demonstrating more consistent results among the 2 groups. At least 79% of patients in the B-cell ALL population experienced CRS, in line with Gong et al., Hu et al., Wang et al., and Dai et al.[11,12,14,15]. However, Li et al. reported a significantly lower incidence of CRS at 28%, which may be attributed to the use of the CD28 costimulatory domain, in contrast to the 4-1BB domain used in the other studies[18]. In vitro studies have shown higher cytokine release in CAR-T with 4-1BB compared to CD28. In addition to showing stronger activation, this may also support the higher incidence of CRS[19]. In the group of patients with unspecified ALL, the incidence of CRS reached 81%, in line with Zhao et al. (2020), but significantly lower results were reported by Ortiz et al. (2020)[13,16].

Specifically, the incidence of grade 2 CRS among patients was reported to be 58%. This finding aligns with Dai et al. with 100% of 6 patients experiencing grade 2 CRS[15]. In contrast, Ortiz et al. reported no cases among the 38 patients involved[16]. Sub-analyses based on target antigens consistently indicated a lower incidence of grade 2 CRS in patients treated with CD19-targeted CAR-T therapy (56%). This is in line with Zhao et al. who reported that 96% of patients achieved grade 2 CRS[13]. The incidence was reported to be higher in the CAR-T with CD19/CD22 antigens group (67%), corroborating the findings of Dai et al., which noted 100% incidence among 6 patients[15]. Conversely, Niu et al. reported a lower incidence of 21% among 19 patients[17]. Differences in side effect responses were also evident in the sub-analyses, based on Leukemia type. The B-cell ALL patient group exhibited a higher tendency to experience grade 2 CRS (57% of cases), which aligns with Dai et al.[15]. However, significantly lower results were reported by Jiang et al., with 15% of cases out of 60 patients[20]. This discrepancy may be due to the difference in recognition moieties, as the use of CD19 alone in Jiang et al. resulted in fewer side effects compared to the dual-targeting approach with CD19/CD22. Meanwhile, the unspecified ALL patient group had a lower incidence percentage (47% of cases), with Zhao et al. reporting the highest number of cases, while Ortiz et al. reported no cases[13,16].

Furthermore, the percentage of more severe CRS, grade 3 or 4, was reported at 19%. This is in line with Niu et al. with 58% of patients reaching grade 3/4 CRS[17]. While Dai et al. and Lu et al. reported no cases[15,21]. The older patient population (median age 51 years) in Niu et al. may correlate with the increased incidence of serious adverse events, despite the overall CRS percentage being lower than in other studies. The administration of higher CAR-T doses, reaching up to 5×10^6 cells/kg (range: $1-5\times10^6$ cells/kg), may also contribute to variations in clinical responses[17]. In contrast, the lower CAR-T doses used in Lu et al. (1×10^6 cells/kg) and Dai et al. ($1.7-3\times10^6$ cells/kg) were associated with improved safety profiles, though this could also have been influenced by the limited number of patients in Dai et al.[15,21]. In the sub-analysis based on target antigen, CAR-T with CD19 showed lower cases of grade 3/4 CRS (18%) compared to CD19/CD22

(29%), which is consistent with previous analysis. Results for the CD19-targeted group aligned with Jiang et al., who reported that 37% of patients experienced grade 3/4 CRS, while Lu et al. and Roddie et al. reported no cases[20–22]. Variability in patient demographics and CAR-T constructs may have introduced bias, affecting these outcomes. In a sub-analysis based on Leukemia type, the B-cell ALL group had a percentage of the grade 3/4 CRS incidence, whereas the unspecified ALL group reported 10%. Niu et al. supported the findings in the B-cell ALL group, while Lu et al., Roddie et al., and Dai et al. observed more favorable responses[15,17,21,22]. In the unspecified ALL group, the result aligns with Jacoby et al., who reported 19% of 21 patients experienced grade 3/4 CRS with a lower incidence of 4%, as reported by Zhao et al.[10,13].

In addition to CRS, ICANS has also been reported as a side effect. Overall analyses indicate a relatively high incidence of ICANS events, reaching 18%. This aligns with Jacoby et al., who reported an ICANS incidence of 52%[10]. Conversely, Hu et al. and Dai et al. reported no cases of ICANS[12,15]. Despite the comparability of the doses used, this variability may be attributed to the differences in CAR-T cell structure. The poor outcome in Jacoby et al. may be related to the use of the CD19 recognition domain rather than dual CD19/CD22 targets and the CD28 costimulatory domain instead of 4-1BB[10]. Surprisingly, this contradicts the incidence of CRS, which shows dual antigen targets or 4-1BB domain use tends to have worse outcomes. This was supported by a sub-analysis based on target antigens, which revealed that the incidence of ICANS was 20% in the CD19 group, aligning with the findings of Jacoby et al.[10]. In contrast, Ortiz et al. reported a lower incidence of 3%[16]. Differences in patient ethnicity, vectors, and costimulatory domains may have caused this difference. Meanwhile, in the CD19/CD22 group, which was based on only 2 studies (Hu et al. and Dai et al.) although showing a favorable outcome, the analysis is likely to show survivorship bias due to the lack of samples in each study and the lack of comparative analyses[12,15].

This study assessed the MRD negative in order to determine whether patients receiving this therapy are prone to relapse or not. Our analysis of MRD showed a relatively high incidence, 69% (95% CI [47%-85%], I^2=82%) of all patients. This result is supported by the incidence of negative MRD in the studies of Hu et al. (5 patients) by 100%, Yang et al. (23 patients) by 92%, Wang et al. (18 patients) by 78%, Roddie et al., (17 patients) by 68%, and Jacoby et al., (11 patients) by 52%[8,10,14,22]. These results show that half of the sample population did not have residual Leukemia cells after treatment. Linear with this, the possibility of relapse will be smaller after CAR-T cell therapy. However, a study conducted by Jacoby et al. found that 1 out of 11 patients who were MRD-negative experienced relapse after 21 months. This occurred because the patient experienced extramedullary (EM) relapse in the CNS and bone marrow[10].

In the sub-analysis by antigen recognition domain, the incidence of MRD was slightly better when using the CD19/CD22 antigen compared with CD19, showing a difference of 2% (67%, 95% CI [44%-84%], I^2=84% vs 69%, 95% CI [47%-85%], I^2=82%). CD19 is a suitable target antigen for CAR-T cell therapy in ALL because it is widely expressed on the surface of ALL cells. CAR T cells targeted with CD19 induced complete remission of the disease in up to 90% of patients with relapsed or refractory B-cell ALL[23]. Thus, a negative MDR outcome in patients after CD19 CAR-T cell therapy was favorable. In contrast, the results of CD19/CD22 CAR-T cell MRD cannot be said to be reliable due to the lack of comparative studies. In addition, it should be noted that studies with a small sample size allow for interpretation bias that shows incompatible results. In the sub-analysis based on type of ALL, the incidence of MRD was slightly better in patients

with B-cell ALL compared to unspecified ALL, showing a difference of 4% (73%, 95% CI [47%-89%], I^2=85% vs 69%, 95% CI [47%-85%], I^2=82%). This reflects that patients with ALL, including unspecified ALL, have a small risk of recurrence. It should be noted that the lack of studies in unspecified ALL means the results of the analysis cannot be used as a reference, as there is no comparator to validate them.

The common effect model shows a proportion of events at 0.0872 with a 95% CI ranging from 0.0563 to 0.1327. The random effects model indicates a proportion of events at 0.0767 with a 95% CI ranging from 0.0396 to 0.1434. Heterogeneity is quantified with a tau^2 of 0.4368 and a tau of 0.6609, with an I^2 of 37.5%, indicating moderate heterogeneity. The H value of 1.26 suggests slight variation between studies. The heterogeneity test yields a Q value of 12.79 with 8 degrees of freedom and a p-value of 0.1192, indicating that the heterogeneity is not statistically significant. Given these results, the lack of significant funnel plot asymmetry suggests that the meta-analysis results are not likely to be heavily influenced by any publication bias. The moderate heterogeneity (τ^2=2.3288) indicates that while there is some variability in the effect sizes, it is not extreme. For mortality outcomes, this means that the pooled estimate of the effect on mortality is likely to be reliable and not significantly skewed by unpublished studies or small-study effects. However, the moderate heterogeneity suggests that the impact of the interventions on mortality may vary somewhat between different studies, possibly due to the differences in study populations, interventions, or other factors.

Conclusion

It can be concluded that CAR-T therapy is associated with efficient responses and tolerable side effects in Leukemia patients. However, due to limited data and some data source limitations, additional studies on the efficacy of CAR-T cell therapy and further randomized controlled clinical trials are needed. Further analysis focusing on the role of co-stimulatory domains is essential to enhance CAR-T cell therapy's efficacy and safety profile. Conducting long-term follow-up studies is crucial in order to assess the durability of responses and identify late-onset toxicities. Additional randomized controlled trials are necessary to validate the early-phase findings and establish robust evidence of CAR-T cell therapy's comparative efficacy and safety versus standard treatments. By addressing these recommendations, future research can build on the promising results of CAR-T cell therapy, ultimately improving outcomes for Leukemia patients and expanding the therapeutic potential of this innovative treatment.

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Conflict of interest

The authors declare no conflicts of interest.

References

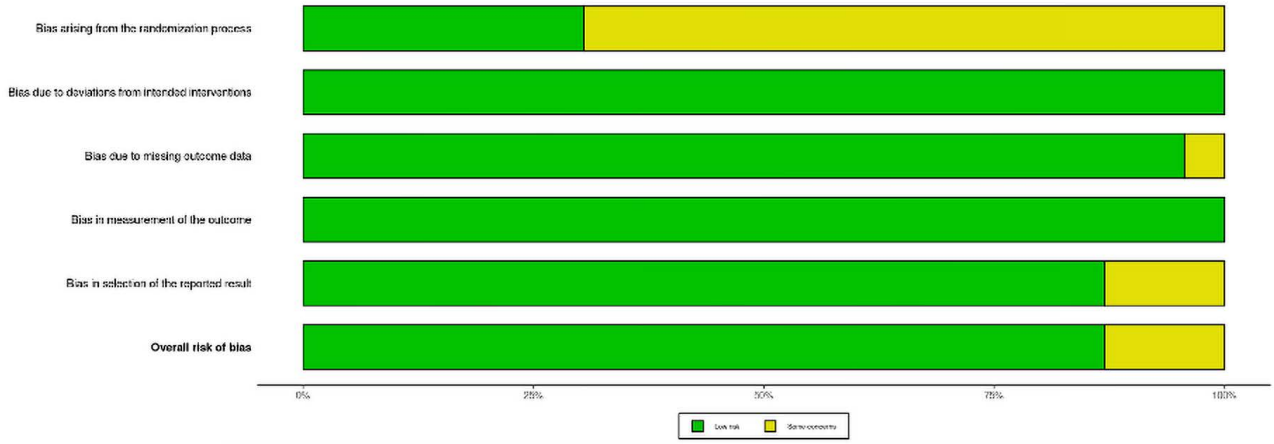
1. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood 2011;117:5019.
2. Leukemia — Cancer stat facts [homepage on the Internet]. Bethesda: National Institutes of Health; 2022 [cited 2023 Oct 30]. Avaliable from: https://seer.cancer.gov/statfacts/html/leuks.html.
3. Terwilliger T, Abdul-Hay M. Acute lymphoblastic leukemia: a comprehensive review and 2017 update. Blood Cancer J 2017;7:e577.
4. Sermer D, Brentjens R. CAR T-cell therapy: Full speed ahead. Hematol Oncol 2019;37:95–100.
5. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC,

Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ;2021:372. doi: 10.1136/BMJ.N71.

6. Sterne JAC, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. BMJ;366:l4898. doi: 10.1136/BMJ.L4898.
7. Heng G, Jia J, Li S, Zhang Y, Wang T, Liu F, et al. Sustained therapeutic efficacy of humanized anti-CD19 chimeric antigen receptor T cells in relapsed/refractory acute lymphoblastic leukemia. Clin Cancer Res 2020;26:1606-15.
8. Yang J, He J, Zhang X, Liu Y, Zhou S, Wang Z, et al. Next-day manufacture of a novel anti-CD19 CAR-T therapy for B-cell acute lymphoblastic leukemia: first-in-human clinical study. Blood Cancer J 2022;12:104.
9. Long AH, Haso WM, Shern JF, Lee W, Wu M, Wang X, et al. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. Nat Med 2015;21:581-90.
10. Jacoby E, Bielorai B, Avigdor A, Zuckerman T, Shouval R, Besser MJ, et al. Locally produced CD19 CAR T cells leading to clinical remissions in medullary and extramedullary relapsed acute lymphoblastic leukemia. Am J Hematol 2018;93:1485-92.
11. Gong WJ, Qiu Y, Li MH, Chen LY, Li YY, Yu JQ, et al. Investigation of the risk factors to predict cytokine release syndrome in relapsed or refractory B-cell acute lymphoblastic leukemia patients receiving IL-6 knocking down anti-CD19 chimeric antigen receptor T-cell therapy. Front Immunol 2022;13:922212. doi: 10.3389/fimmu.2022.922212.
12. Hu Y, Zhou Y, Zhang M, Wang Y, Chen X, Liu H, et al. CRISPR/Cas9-Engineered Universal CD19/CD22 dual-targeted CAR-T cell therapy for relapsed/refractory B-cell acute lymphoblastic leukemia. Clin Cancer Res 2021;27:2764-72.
13. Zhao H, Wei J, Wei G, Luo Y, Shi J, Cui Q, et al. Pre-transplant MRD negativity predicts favorable outcomes of CAR-T therapy followed by haploidentical HSCT for relapsed/refractory acute lymphoblastic leukemia: a multi-center retrospective study. J Hematol Oncol 2020;13:42.
14. Wang J, Mou N, Yang Z, Zhang L, Zhao X, Wei L, et al. Efficacy and safety of humanized anti-CD19 CAR T therapy following intensive lymphodepleting chemotherapy for refractory/relapsed B acute lymphoblastic leukaemia. Br J Haematol 2020;191:212-22.
15. Dai H, Wu Z, Jia H, Wang X, Wang Y, Zhang H, et al. Bispecific CAR-T cells targeting both CD19 and CD22 for therapy of adults with relapsed or refractory B cell acute lymphoblastic leukemia. J Hematol Oncol 2020;13:30.
16. Ortíz-Maldonado V, Rives S, Castellà M, Gutiérrez C, Salazar R, Granados I, et al. CART19-BE-01: a multicenter trial of ARI-0001 cell therapy in patients with CD19+ relapsed/refractory malignancies. Mol Ther 2021;29:636-44.
17. Niu J, Qiu H, Xiang F, Zhang Y, Liu Z, Wang X, et al. CD19/CD22 bispecific CAR-T cells for MRD-positive adult B cell acute lymphoblastic leukemia: a phase I clinical study. Blood Cancer J 2023;13:44.
18. Li M, Xue S-L, Tang X, Li Y, Wang Y, Xu Z, et al. The differential effects of tumor burdens on predicting the net benefits of ssCART-19 cell treatment on r/r B-ALL patients. Sci Rep 2022;12:378.
19. Zhao X, Yang J, Zhang X, Liu X, Sun Y, Zhang Y, et al. Efficacy and safety of CD28- or 4-1BB-Based CD19 CAR-T Cells in B Cell acute lymphoblastic leukemia. Mol Ther Oncolytics 2020;18:272–81.
20. Jiang H, Li C, Yin P, Wang H, Zhang Y, Liu Y, et al. Anti-CD19 chimeric antigen receptor-modified T-cell therapy bridging to allogeneic hematopoietic stem cell transplantation for relapsed/refractory B-cell acute lymphoblastic leukemia: an open-label pragmatic clinical trial. Am J Hematol 2019;94:1113–22.
21. Lu W, Wei Y, Cao Y, Zhang Y, Chen Y, Wang H, et al. CD19 CAR-T cell treatment conferred sustained remission in B-ALL patients with minimal residual disease. Cancer Immunol Immunother 2021;70:3501–11.
22. Roddie C, Dias J, O'Reilly MA, Reshmi S, Maloney D, Hosing C, et al. Durable responses and low toxicity after fast off-rate CD19 chimeric antigen receptor–T therapy in adults with relapsed or refractory B-cell acute lymphoblastic leukemia. J Clin Oncol 2021;39:3352–63.
23. Davila ML, Brentjens RJ. CD19-Targeted CAR T cells as novel cancer immunotherapy for relapsed or refractory B-cell acute lymphoblastic leukemia. Clin Adv Hematol Oncol 2016;14: 802–8.
24. Ma F, Ho JY, Du H, Li J, Zhang Y, Wang H, et al. Evidence of long-lasting anti-CD19 activity of engrafted CD19 chimeric antigen receptor-modified T cells in a phase I study targeting pediatrics with acute lymphoblastic leukemia. Hematol Oncol 2019;37:601–8.
25. Zhang Y, Chen H, Song Y, Liu W, Xu X, Li X, et al. Chimeric

antigen receptor T-cell therapy as a bridge to haematopoietic stem cell transplantation for refractory/relapsed B-cell acute lymphoblastic leukemia. Br J Haematol 2020;189:146–52.

26. Jiang H, Li C, Yin P, Zhang X, Liu Y, Wang J, et al. Anti-CD19 chimeric antigen receptor-modified T-cell therapy bridging to allogeneic hematopoietic stem cell transplantation for relapsed/refractory B-cell acute lymphoblastic leukemia: an open-label pragmatic clinical trial. Am J Hematol 2019;94:1113–22.
27. Frey NV, Shaw PA, Hexner EO, Lacey SF, Porter DL, Schuster SJ, et al. Optimizing chimeric antigen receptor T-Cell therapy for adults with acute lymphoblastic leukemia. J Clin Oncol 2020;38:415–22.
28. Itzhaki O, Jacoby E, Nissani A, Baruch R, Shapira-Frommer R, Schachter J, et al. Head-to-head comparison of in-house produced CD19 CAR-T cell in ALL and NHL patients. J Immunother Cancer 2020;8:e000148. doi: 10.1136/JITC-2019-000148.
29. Niu J, Qiu H, Xiang F, Zhang Z, Zhou H, Li Y, et al. CD19/CD22 bispecific CAR-T cells for MRD-positive adult B cell acute lymphoblastic leukemia: a phase I clinical study. Blood Cancer J 2023;13:1–4.



Supplementary Figure 1 The overall risk of bias plot