Current clinical outcome of CD19/CD22 dual-targeting CAR T-cell therapy in refractory or relapsed B-cell acute lymphoblastic leukemia

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Abstract. To enhance clinical outcomes for hematologic malignancies, dual-targeting CAR T-cell therapies were devised. This study seeks to generalize the safety and efficacy of CD19/CD22 dual-targeting CAR T-cell therapy for the treatment of refractory or relapsed B-cell acute lymphoblastic leukemia in published clinical studies to cast lights on current advances and future challenges of this therapeutic. Manual searches were performed on PubMed and Science Direct to identify relevant articles for inclusion in the report. Forest plots were utilized to display data from individual studies as well as pooled estimates derived based on random effect model for complete remission rate, relapse rate, overall survival rate, and incidences of adverse events, and all data were presented along with their respective 95% confidence intervals. As per result, the pooled estimates for complete remission rate (CR), CR with negative minimal residual disease, relapse rate, overall survival rate (OS), incidences of cytokine release syndrome (CRS), severe CRS, neurotoxicity (NT) and severe NT are as follows: 89% (95% CI: 83.4%, 93.1%), 86.5% (95% CI: 83.4%, 93.1%), 43.3% (95% CI: 26.4%, 60.3%), at most 64.1% (95% CI: 50.7%, 75.3%) at 1 year, 80.4% (95% CI: 66.4%, 91.1%), 10.7% (95% CI: 0%, 30.3%), 9% (95% CI: 2.9%, 15%), 6.5% (95% CI: 0%, 25.5%), suggesting that CD19/CD22 dual-targeting CAR T-cell therapy has a great potential for treating patients with refractory or relapsed B-cell acute lymphoblastic leukemia.

Keywords: CD19/CD22, dual-targeting CAR T-cell therapy, refractory or relapsed B-cell acute lymphoblastic leukemia.

1. Introduction

Immunotherapy, especially the advent of CAR T-cell therapy, has truly revolutionized the field of cancer therapy and represents a significant advancement in the fight against this devastating disease. Combining antibody fragments with T-cell receptors led to its initial elucidation in 1987 by Yoshihisa Kuwana and colleagues in Japan. In 1989, two Israeli researchers, Gideon Gross and Zelig Eshhar, independently illustrated the concept of CAR-T [1]. Since then, CAR T-cell therapy has advanced consistently over the course of the last few decades, whose mechanism involves genetically redirecting and reprogramming T lymphocytes to express chimeric antigen receptors (CARs). These synthetic receptors incorporate the effector functions of T lymphocytes with the specificity of antibodies, which enables them to identify and eliminate cancerous cells expressing predefined surface antigens in a non-major histocompatibility complex dependent manner [2].

Over time, CAR T-cell therapy has shown exceptional clinical outcomes in patients with refractory or relapsed (R/R) hematologic malignancies, such as B-cell acute lymphoblastic leukemia (B-ALL), who have exhausted standard treatment options of chemotherapy and hematopoietic stem cell transplantation [3]. In this case, both CD19 and CD22 are promising targets for R/R B-ALL by CAR T-cell therapy given their expression solely on B-lineage cells and absence on most normal tissues [4]. Despite the outstanding response/remission rate of 70% - 90% achieved by these single-targeted CAR T cells, patients have not been able to remain in remission [5]. Relapses can occur in more than 50% of the responders within 1 year of treatment [6]. Typically, antigen escape, resulting in down-regulation or loss of CD19 and CD22 expression on leukemic stem cells, is the predominant factor that contributes to such disease progression and relapse [7].

Therefore, to improve the efficacy of CAR T-cell therapy and to prolong relapse-free survival, researchers proceeded to take a step further with the previous treatments and constructed CD19/CD22 dual-targeting CAR T-cell therapies. It was hypothesized that such dual-targeting strategies could be a viable countermeasure to antigen escape due to the low probability for one leukemic stem cell to down-regulate CD19 and CD22 at the same time [7]. While there also exists variability in the level of antigenic expression among patients, which may change over time in response to various target-specific treatments, a dual-targeting strategy may be an overall more effective therapy [8].

After preclinical studies confirming the viability of the treatment, in the past few years, multiple clinical trial studies on CD19/CD22 dual-targeting CAR T-cell therapies have been conducted to access their overall safety and efficacy in treating R/R B-ALL. This study aims to summarize this published information for potential comparison and generalization such that the current advances and future challenges of this therapy may be better understood.

1.1. Background

As previously mentioned, CAR T-cell therapy has demonstrated exceptional outcome in the treatment of certain blood cancers. However, its common association with adverse events such as cytokine release syndrome (CRS) and neurotoxicity (NT)/immune effector cell-associated neurotoxicity syndrome (ICANS) has precluded its widespread application [4]. Consequently, the safety profile has always been a primary concern when accessing the viability of any subtypes of CAR T-cell therapy; this will be addressed in subsequent analysis for CD19/CD22 dual-targeting CAR T-cell therapies.

There are typically five strategies employed to perform a dual-targeting CAR T-cell therapy: 1. Cocktail/Sequential infusion of two types of single-targeted CAR T cell (Si-CAR T-cell), 2. Bicistronicity CAR-T cell, 3. Co-transduction of Si-CAR T-cell and bicistronicity CAR T-cell, 4. Bivalent tandem CAR T-cell, 5. Bivalent loop CAR T-cell, as displayed in Figure 1 using CD19/CD22 dual-targeting CAR T-cell construct as an example [5]. T-cell products in strategy 2, 4, and 5 are also referred to as bispecific CAR T-cells (Bi-CAR T-cell) given their ability to target two different antigens simultaneously. Different arrangements for the light and heavy chain (VL & VH) of the two-antigen binding single-chain variable fragment (scFv) define two distinct types of bivalent CAR: bivalent tandem and bivalent loop, as demonstrated for strategies 4 and 5 [6].

The cocktail/sequential infusion approach is usually associated with high expenditure due to the manufacturing of two Si-CAR T-cell products and a higher quantity requirement of engineered cells [4]. Besides, an uneven expansion can occur between the two CAR T-cell population, which is a risk factor for incomplete elimination of cancer cells and disease progression. The same downsides also apply to strategy 3. However, these strategies that involves Si-CAR T-cell products may benefit from the available CAR constructs in the FDA approved products, whose functionality has been optimized. Strategies employing Bi-CAR T-cells, on the other hand, are less expensive and produce more homogenous cell products [6].

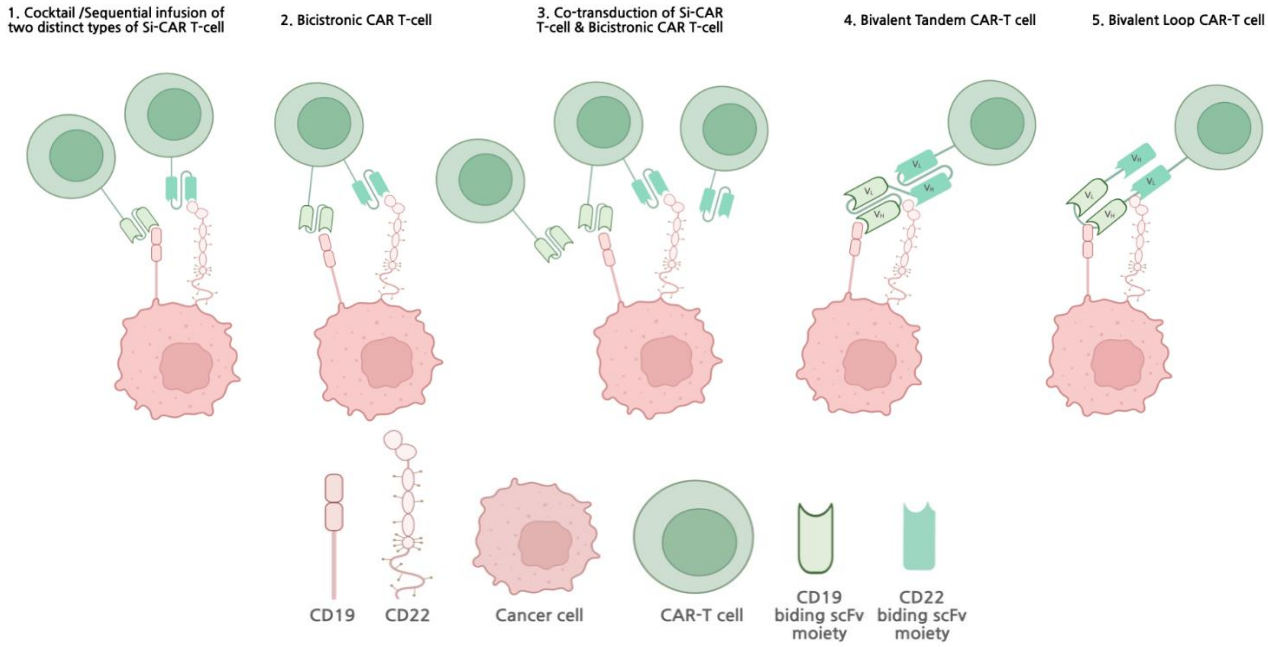

Figure 1. Schematic diagram of the five strategies for dual-targeting CAR T-cell therapy

2. Method

2.1. Eligibility & Exclusion Criteria

Clinical trial studies evaluating efficacy and safety of CD19/CD22 dual-targeting CAR T-cell therapies for the treatment of relapsed/refractory Acute Lymphoblastic Leukemia;

Research published between 2018 and 2023;

At least one of the following measures of success is included in the published work: Complete remission rate (CR), time till relapse/relapse rate, overall survival rate (OS);

Adverse event incidence data for CRS and ICANS/ NT must be included in published results;

Clinical trials that are still in progress are excluded;

In cases when one study builds upon the findings of another by the same author, the more recent study is utilized.

2.2. Literature search

Two online databases, PubMed and Science Direct, were searched manually for eligible publications using keywords: Clinical trial, phase 1 study, CD19/CD22, bispecific/sequential/ cocktail/tandem, dual-targeting CAR T-cell therapy, relapsed/refractory acute lymphoblastic leukemia, hematological malignancies.

2.3. Data extraction

Basic information and characteristics of the enrolled studies, including the first author, year of publication, strategy for dual-targeting CAR T-cell therapy, trial size, age of enrolled patients and dose levels are organized in table for reference. Eligible studies are mined for post-treatment clinical response/outcome data, and adverse event statistics. Data is also presented in tables.

2.4. Statistical Analysis

The adverse event incidence statistics are presented as stacked column graphs to provide a clear visual depiction of data for observational analysis. Forest plots are used to present overviews for all five data groups (CR rate, time till relapse/relapse rate, OS rate, incidence for CRS and neurotoxicity) concluded from clinical studies in form of effect sizes (incidences) and their respective 95%

confidence intervals (CI). Meanwhile, as the majority of the clinical studies collected were non-randomized single-arm cohort studies, a random effects model is utilized, along with the Der Simonian-Laird method for assigning weights. It involves weighting each study based on its sample size and the estimated between-study variance, and is used to generalize the pooled estimates with 95% confidence intervals for the five data groups. The pooled estimates are also displayed on the same forest plots. The process also includes heterogeneity assessment that uses I^2 to quantify the percentage of the total variation across studies attributable to heterogeneity as opposed to chance. Python (version 3.11.2) is utilized to conduct the above statistical analysis and construct forest plots.

3. Results

3.1. Studies enrolled

An overview of the basic information of the 14 relevant published clinical studies enrolled in this study is provided in Table 1. Despite all five strategies of dual-targeting CAR T-cell therapies being included, the number of trials available for each of the strategies are not sufficient for inter-comparison.

Table 1. Basic information of enrolled studies

Author	Year of publication	Taget (Therapy name)	Strategy	Disease	Trial size n	Age	Dose (Cells per kg body weight)	References
Amrolia et al.	2019	CD19/CD22 AUTO3	Bicistronic	R/R B-ALL	10	Median 8.5, Range: 5-16	3 × 106 (n=5) 5 × 106 (n=5)	[9]
Cordoba et al.	2021	CD19/CD22 AUTO3	Bicistronic	R/R B-ALL	15	Median 8, Range: 4-16	5 × 106	[7]
Cui et al.	2022	CD19/CD22	Bivalent Tandem	R/R B-ALL	47	Median 28, Range 6-56	Median: 1 (0.5 ~ 2.5) × 107	[10]
Dai et al.	2020	CD19/CD22	Bivalent Tandem	R/R B-ALL	6	Median 23.5, Range: 17-44	1.7 × 106 to 3 × 106	[11]
Gardner et al.	2018	CD19/CD22	Co-transduction	R/R B-ALL	7	Range: 1-26	1 × 106 (n=4) 3 × 106 (n=3)	[12]
Hu et al.	2021	CD19/CD22 CTA101	Bivalent Tandem	R/R B-ALL	6	Median 49, Range: 26-56	1 × 106 (n=3) 3 × 106 (n=3)	[13]
Pan et al.	2020	CD19/CD22	Sequential	R/R B-ALL	20	Median 6,	CD19 median:	[14]

						Range: 1-16	10 (3.3 ~ 42.8) × 105 CD22 median: 10 (0.25 ~ 47.4) × 105	
Spiegel et al.	2021	CD19/CD22	Bivalent Loop	R/R B-ALL	17	Median 47, Range: 26–68	1 × 106 (n=2) 3 × 106 (n=15)	[15]
Schultz et al.	2019	CD19/CD22	Bivalent (Tandem/Loop not specified)	R/R B-ALL	14	Median 23, Range: 2–68	1 × 106 (n=7) 3 × 106 (n=7)	[16]
Wang (Na) et al.	2020	CD19/CD22	Cocktail	R/R B-ALL	51	Median 27, Range 9-62	CD19: 2.6 ± 1.5 ×106 CD22: 2.7 ± 1.2 ×106	[17]
Wang (Yiyun) et al.	2020	CD19/CD22	Bivalent Tandem	R/R B-ALL	15	Median 27, Range 16-65	Median: 4.85 (1.04 ~ 7.02) × 106	[18]
Liu (Sining) et al.	2021	CD19/CD22	Bivalent Tandem	R/R B-ALL	49	Not reported	Not reported	[19]
			Sequential		13	Not reported	Not reported	
Liu (Shuangyou) et al.	2021	CD19/CD22	Sequential	R/R B-ALL	27	Median 21, Range, 1.6-55	CD19 Median: 1 (0.486 ~ 5) × 105 CD22 median: 2 (0.32 ~ 5) × 105	[20]
Yang et al.	2018	CD19/CD22	Cocktail	R/R B-ALL	15	Median 19, Range 4-45	CD19 median: 2 (0.9 ~ 5) × 105 CD22 median: 0.5 (0.4 ~ 12) × 105	[21]

3.2. Response and Outcome

Table 2 summarizes the clinical response and outcome statistics (CR, Relapse rate, OS, Event free survival/Progression-free survival) of R/R B-ALL patients treated by CD19/CD22 dual-targeting CAR T-cell therapies. On the last row, arithmetic means are deduced for each category to provide a rough overview of the pooled data.

Table 2. Clinical response and outcome statistics of CD19/CD22 dual-targeting CAR T-cell therapies

First author (Last name)	n*	CR/CRi	Relapse	OS	EFS/PFS
Amrolia	7	7/10 evaluated: 100% (7/7) MRD- CR	3/7 at 1 year CD19-/CD22dim in 1	N/A	N/A
Cordoba	15	At 1 months: 86.67% (13/15) MRD- CR	8/15 at 1 year 9/15 at 18 months CD19-/CD22dim in 2 CD19- in 1	At 1 year: 60% (9/15)	EFS At 1 year: 33% (5/15)
Cui	47	At 1 months 85.11% (40/47) MRD- CR	6/47 at 6 months 12/47 at 1 year CD19- in 2	At 6 months: 93.65% (44/47) At 1 year: 80.85% (38/47)	N/A
Dai	6	At 1 months: 100% (6/6) MRD- CR	6/6 at 1 year CD19- & CD22dim in 1	N/A	N/A
Gardner	7	By Day 21: 71.43% (5/7) CR 57.14% (4/7) MRD- CR	N/A	N/A	N/A
Hu	6	At 1 months: 83.33% (5/6) MRD- CR	1/6 at half year CD19+/CD22dim in 1	N/A	N/A
Pan	20	At 1 months: 100% (20/20) MRD- CR	3/20 at 1 year CD22dim in 1 CD19- in 2	At 1 year: 90% (18/20)	N/A
Spiegel	17	At 1 months: 82.35% (14/17) MRD- CR	10/17 at half year CD19-/dim in 5	At 1 year: ~20% (3/17) Median: 11.8 months	PFS At 1 year: 0 Median: 5.8 months
Schultz	12	12/14 evaluated At 1 months: 83.33% (10/12) Eventually: 91.67% (11/12)	3/12 All CD19+	At 9.5 months: 91.67% (11/12)	N/A
Wang (Na)	51	At 1 months: 94.12% (48/51) MRD- CR Eventually:	14/51 at 1 year 24/51 at 18 months CD19+/CD22+ in 23 CD19-/CD22dim in 1	At 1 year: 62.75% (32/51) Median: 31.0 months	PFS At 1 year: 52.94% (27/51)

		96.08% (49/51) MRD- CR			Median: 13.6 months
Wang (Yiyun)	15	86.67% (13/15) MRD- CR	11/15 at 1 year	Median: 21.7 months Range: 13.3-30.2 months	N/A
Liu (Sining)	49	95.92% (47/49) CR 81.63% (40/49) MRD- CR	12/49 at half year	At half year: 89.8% (44/49)	N/A
	13	69.23% (9/13) CR 61.54% (8/13) MRD- CR	1/13 at half year	At half year: 92.31% (12/13)	N/A
Liu (Shuangyou)	21	At 1 months: 95.24% (20/21) MRD- CR	7/21 at 2 year CD19- in 2	At 1 year: 85.71% (18/21)	PFS At 1 year: 61.90% (13/21)
Yang	15	By 20-30 days: 100% (15/15) CR 93.33% (14/15) MRD- CR	2/15 at 1 year Both CD19+	At 1 year: 6.67% (1/15)	N/A
Summary	301	90.37% (272/301) CR Studies reported MRD assessment: 88.46% (253/286) MRD- CR	Studies that followed up at 1 year: 59/176 CD19-/dim/CD22lo/dim occurred in 19 out of 80 reported relapse	OS rate of at most 71.54% (186/260) by 1 year	N/A

n*: Number of patients being checked for the following categories; CR: Complete remission; CRi: Complete remission with incomplete count recovery; OS: Overall survival rate; EFS: Event free survival; PFS: Progression-free survival; Superscript (+): Normal antigenic expression; Superscript (-): No antigenic expression; Superscript (dim): Diminished antigenic expression below the threshold needed for inducing anti-tumor functions

3.2.1. Complete remission

Comparable to single-targeted CAR T-cell therapies, among the 301 R/R B-ALL patients who received CD19/CD22 dual-targeting CAR T-cell therapies, 272 of them have reached complete remission by the conclusion of the studies, for a CR rate of 90.37%. Here, minimal residual disease (MRD) is reported as an indicator of treatment efficacy and recurrence risk, given that patients with an MRD-negative CR usually have an improved prognosis and a greater chance of remaining disease-free over time. In studies that reported MRD assessment alongside CR, 253 out of 286 were able to attain MRD- CR, suggesting a likely positive outcome for these patients.

Below, the forest plot illustrates the variation in effect estimates across studies for the CR rate post dual CAR T treatment, which is eventually accounted for by a random effects model in the analysis. As shown in Figure 2, the overall CR is estimated to be 89%, with a 95% confidence interval of (83.4%, 93.1%). The diamond at the foot of the graph depicts the pooled estimate with a 95% confidence interval. It gives a weighted, more conservative, and representative interpretation of the

treatment effect across different studies, comparing to any particular study or arithmetic mean calculation.

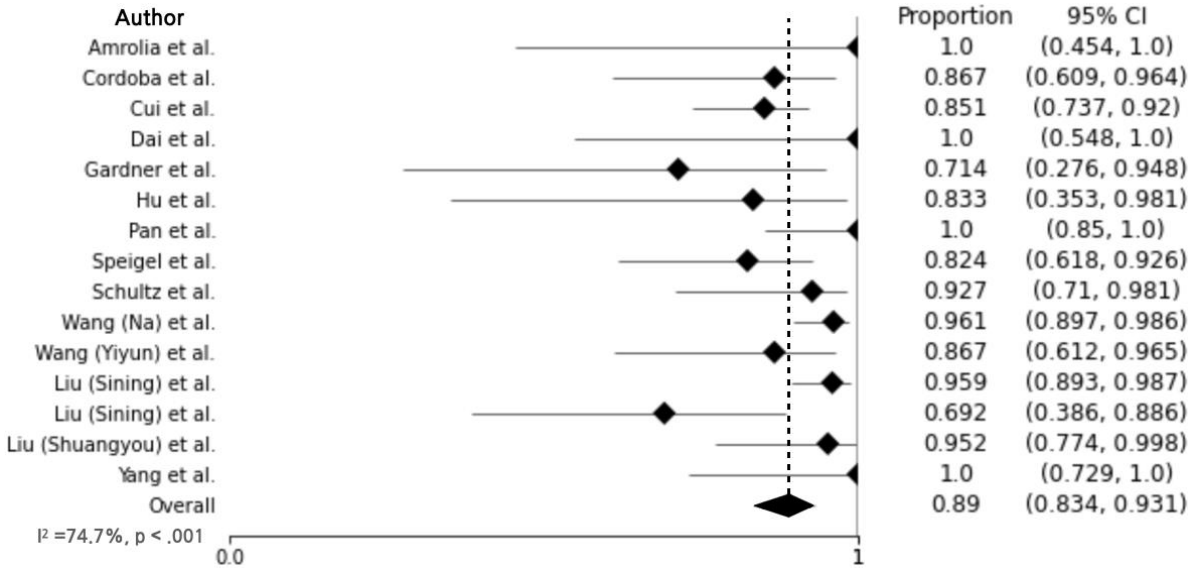

Figure 2. Complete remission rate and its pooled estimate

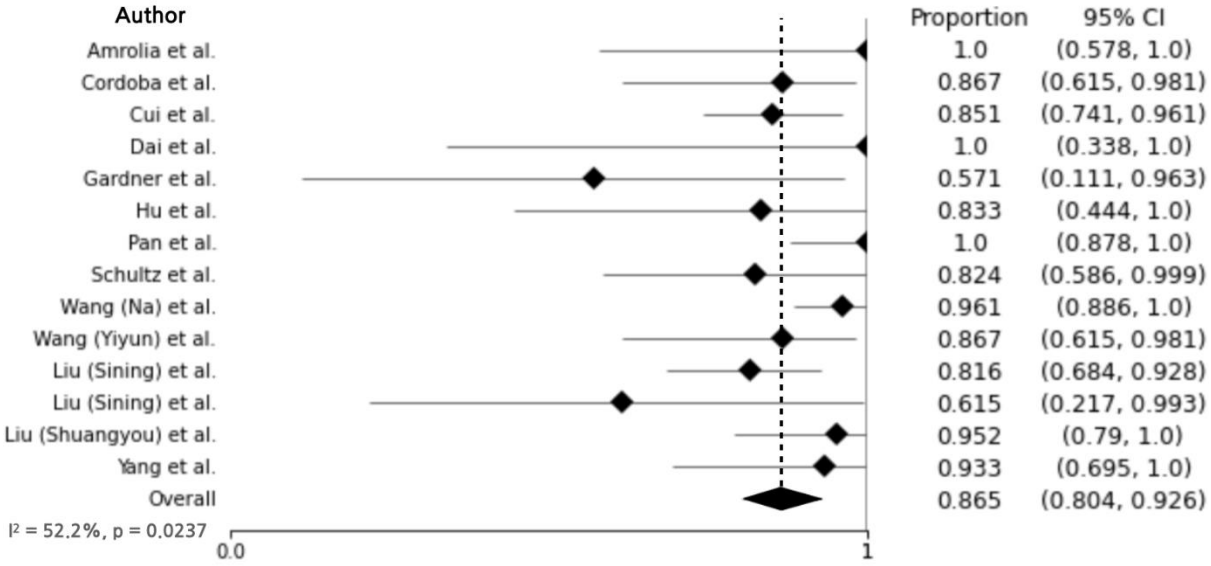

Figure 3. Rate of complete remission with negative minimal residual disease and its pooled estimate

Similarly, in Figure 3, a pooled estimate has shown that 86.5% of patients reached MRD- CR, with a 95% confidence interval of (83.4%, 93.1%).

3.2.2. Relapse rate

The length of time that patients were followed up with after receiving the treatment varied greatly throughout the 14 enrolled clinical studies. Hence, only clinical results that included a return check at 1 year are used for the assessment of relapse rate; among them, 59 of 176 (33.52%) patients have experienced a relapse by then. Based on the meta-analysis using a random effects model, the estimated proportion of patients experiencing relapse within one year is 43.3% (95% CI: 26.4%, 60.3%), as shown in Figure 4.

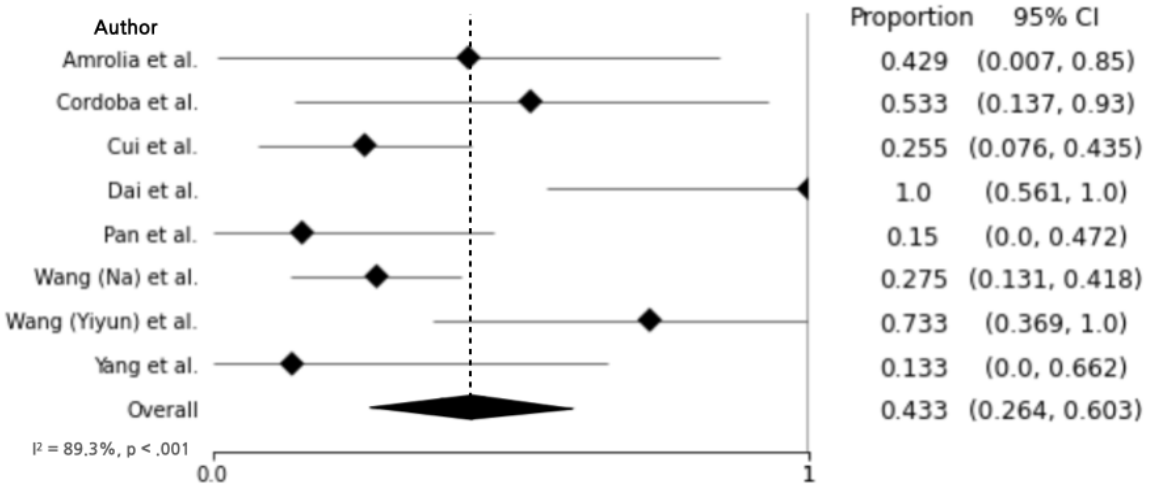

Figure 4. Relapse rate at 1 year and its pooled estimate

Furthermore, antigen expression on leukemic cell of relapsed patients is critical for evaluating the efficiency of CD19/CD22 dual-targeting CAR T-cell treatments, which were conceived as a preventative strategy for disease progression due to antigen escape. Table 3 displays a compilation of clinical data on abnormal antigenic expression seen in relapsed patients as reported by 11 of the 14 studies. Diminished or absent expression of either CD19 or CD22 is thought to be a failure of these novel therapies in combatting the immune escape mechanism. According to the count, a total of 19 in 80 relapsed patients exhibited some form of lowered CD19/CD22 expression on B-ALL cells post treatment.

Table 3. Incidences of relapse with CD19-/dim/CD22-/dim

First author (Last name)	Total count for relapsed patients	$CD19^{-/dim}/CD22^{-/dim}$ occurrence
Amrolia	3	1
Cordoba	9	3
Cui	12	2
Dai	6	1
Hu	1	1
Pan	3	3
Speigel	10	5
Schultz	3	0
Wang (Na)	24	1
Liu (Shuangyou)	7	2
Yang	2	0
Summary	80	19

3.2.3. Overall survival

For the most accurate description of survival rates, a one-year time stamp is used: all survival data collected within this period following treatment is incorporated. To account for the incidences of deaths occurring after clinical follow-up but within the 1-year time frame, the phrase "at most" is employed. As concluded in the summary row of Table 2, the 260 patients being followed up within time had a 1-year survival rate of at most 71.54%. According to the summary estimate presented, R/R B-ALL patients had an OS of at most 64.1% (95% CI: 50.7%, 75.3%) within 1 year following CD19/CD22 dual-targeting CAR T-cell therapies, as shown in Figure 5.

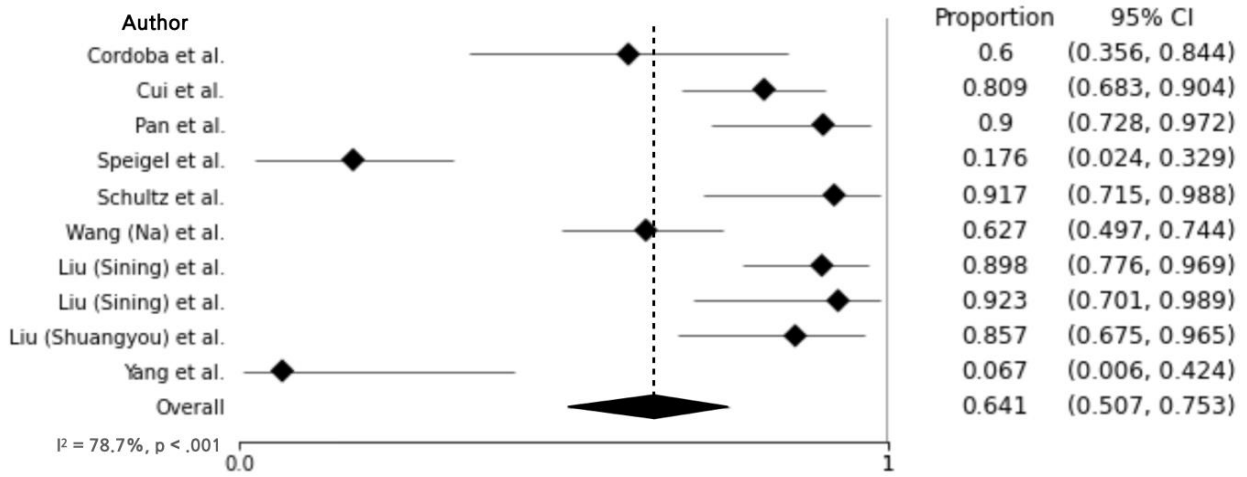

Figure 5. Overall survival rate at 1 year and its pooled estimate

3.3. Safety profile

Presented in Table 4 is a summary of the safety statistics, the occurrence of cytokine release syndrome and neurotoxicity related to CD19/CD22 dual-targeting CAR T-cell therapies. The Lee criteria and the National Cancer Institute Common Terminology Criteria for Adverse Events are used to quantify the severity of CRS and NT, respectively [22]. In general, any incidence of CRS or NT equal or exceeding Grade 3 is deemed a severe adverse event.

Table 4. Occurrence of cytokine release syndrome and neurotoxicity

First author (Last name)	n	CRS NOB	CRS Grade 1	CRS Grade 2	CRS Grade ≥ 3	NT NOB	NT Grade 1	NT Grade 2	NT Grade ≥ 3
Amrolia	10	1	8	1	0	9	1	0	0
Cordoba	15	3	11	1	0	11	4	0	0
Cui	47	6	15	18	8	46	0	1	0
Dai	6	0	4	2	0	6	0		
Gardner	7	2	5	0	0	5	2	0	0
Hu	6	0	3	2	1	6	0		
Pan	20	5	15		1	17	3	0	0
Spiegel	17	4	5	7	1	12	1	1	3
Schultz	12	3	8		1	10	1		1
Wang (Na)	50	0	40		10	44	6		0
Wang (Yiyun)	15	0	13		2	15	0		
Liu (Sining)	49	Not reported			9	Not reported			
	13	Not reported			3	Not reported			
Liu	21	10	8	3	0	21	0		
Yang	15	2	12	0	1	14	0		1
Sum	303	36	181		37	270	19		5

NOB: Not observed

The two studies by Liu (Sining) et al. are occasionally excluded in the following analysis since the number of patients experiencing CRS Grades 1-2 and NT are not reported.

3.3.1. Cytokine release syndrome

A stack column chart (Figure 6) facilitates the clear comparison of subgroups; CRS was observed in a high proportion of R/R B-ALL patients treated with anti-CD19/CD20 CAR T-cell therapies in all clinical trials enrolled, while only a smaller percentage reached a level of severe adverse event.

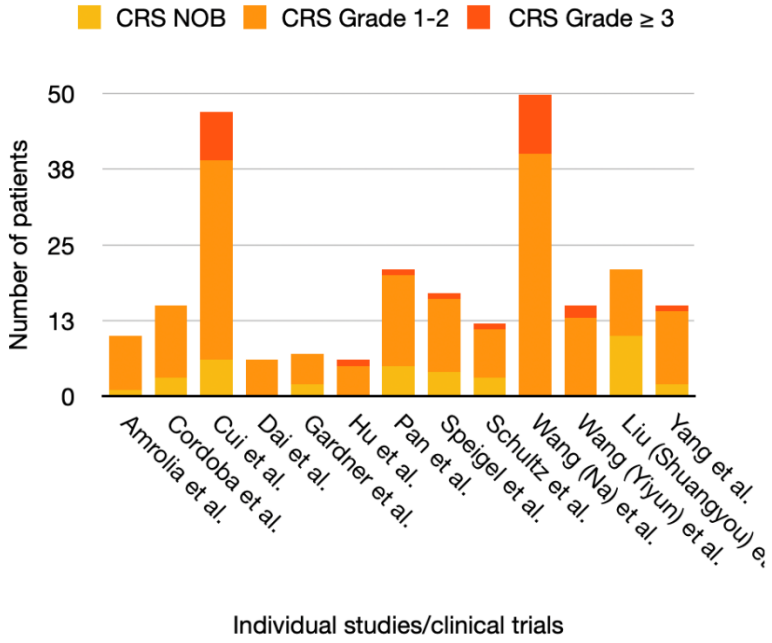

Figure 6. CRS cases divided by grades

The previous observation is confirmed by the forest plots as well as the pooled estimates. It is shown that CRS occurs at a rate of 80.4% (95% CI: 66.4%, 91.1%), whereas adverse CRS takes place at a substantially reduced rate of 10.7% (95% CI: 0%, 30.3%), as shown in Figure 7 and Figure 8. Even with cases of severe adverse reaction, the condition is often reversible with treatment of Tocilizumab and Corticosteroids, indicating an overall acceptable safety profile for this treatment approach [7,12, 15,17].

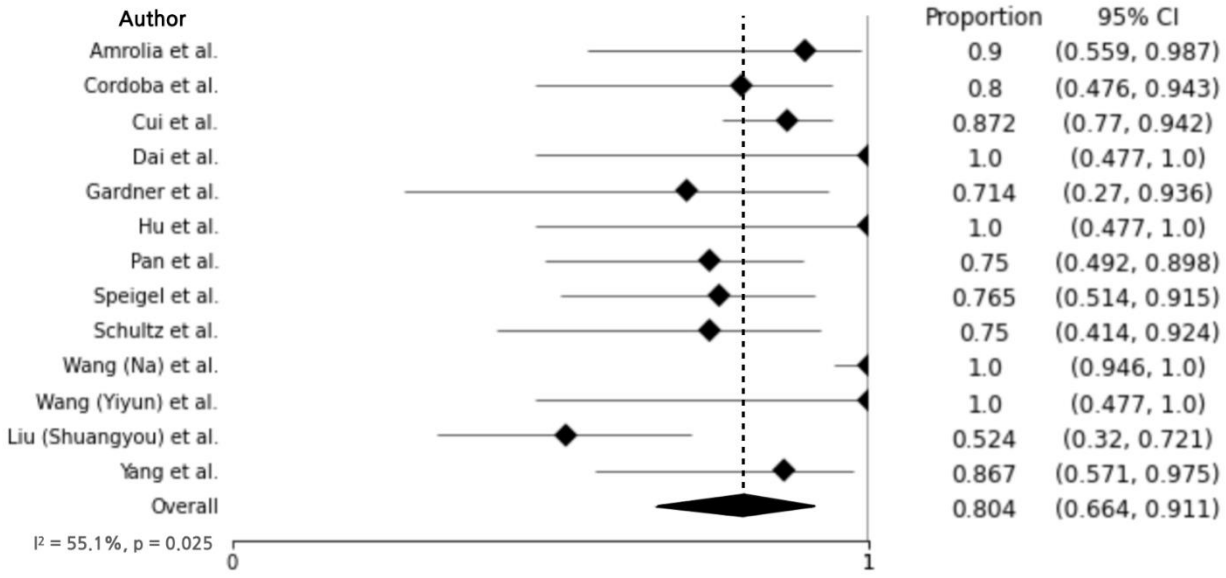

Figure 7. CRS occurrence rate and its pooled estimate

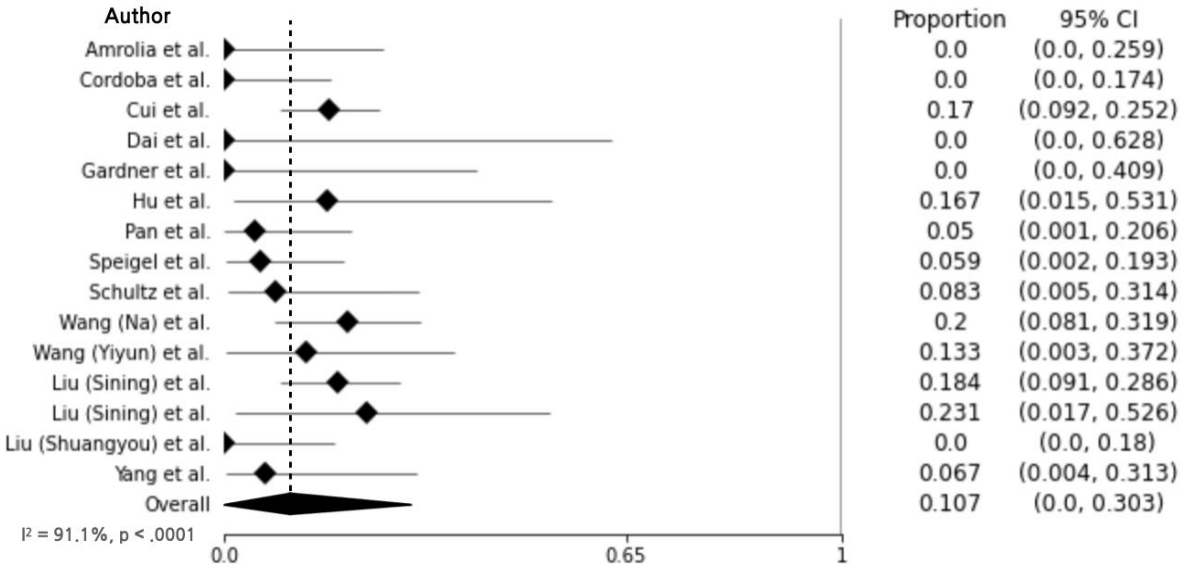

Figure 8. CRS (Grade ≥ 3) occurrence rate and its pooled estimate

3.3.2. Neurotoxicity

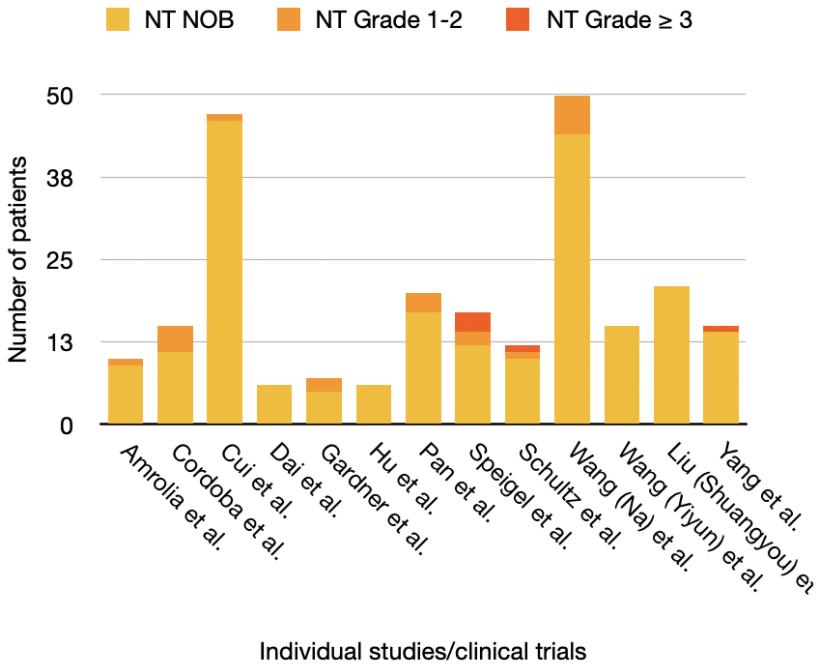

Figure 9. NT cases divided by grades

Clearly demonstrated in the stacked column graph (Figure 9), NT effects are generally uncommon among R/R B-ALL patients after exposure to CD19/CD22 dual-targeting CAR T-cell therapies, with both a low incidence of onset and progression. Those who have undergone neurotoxicity often have mild symptoms, early onset, and quick recovery. NT Grade ≥ 3 was only seen in 3 studies with a total of 5 cases and can be treated by corticosteroids, anakinra and anticonvulsants [15].

In accordance with previous observations, summary estimate by the random effect model in Figure 10 and Figure 11 indicates a 9% occurrence rate for neurotoxicity (95% CI: 2.9%, 15%) and a severe event occurrence at rate of 6.5% (95% CI: 0%, 25.5%), both of which supports the overall safety of the dual targeting therapy.

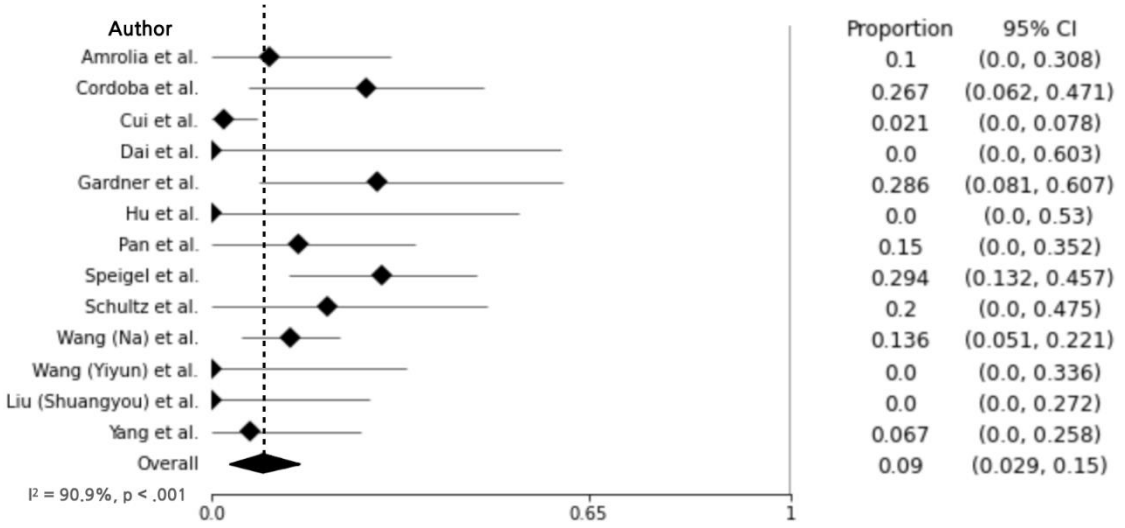

Figure 10. Forest plot for NT occurrence rate and its pooled estimate

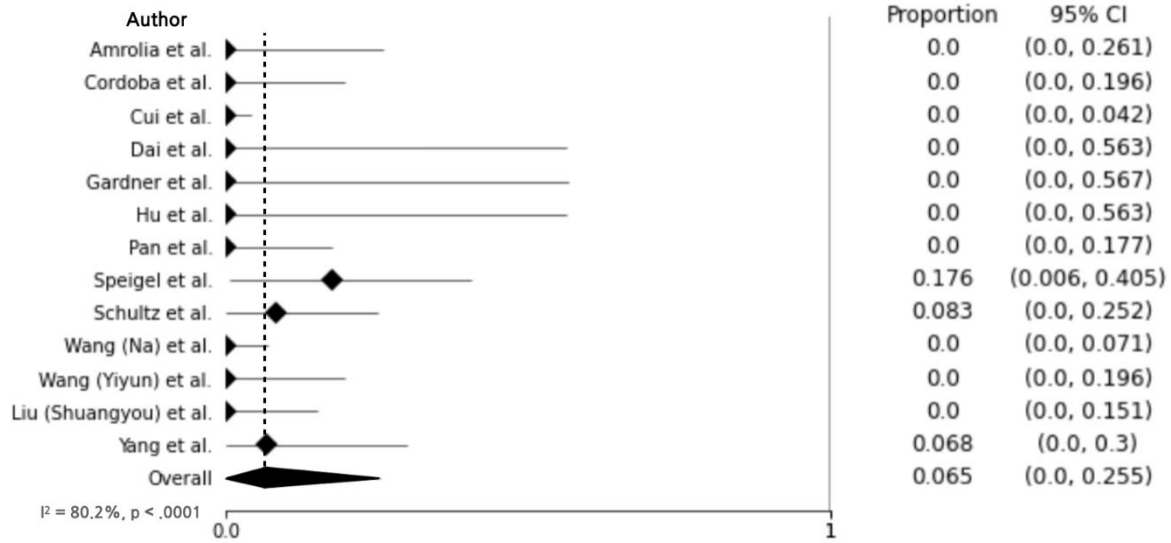

Figure 11. Forest plot for NT (Grade ≥ 3) occurrence rate and its pooled estimate

4. Discussion

CD19/CD22 dual-targeting CAR T-cell therapy, which is an adaptation of anti-CD19 CAR T-cell therapy that aims to safeguard patients from disease progression marked by negative expression of the targeted antigen, demonstrated impressive results on R/R B-ALL patients. Although the overall rate of relapse did not decrease significantly after one year, 19 of the 80 relapsed patients exhibited a reduction in CD19/CD22 expression on B-ALL cells after treatment, with only 5 cases relapsing with decreased or absent expression of both CD19 and CD22 antigens. In addition, CD19/CD22 dual-targeting CAR T-cell therapy achieved complete remission rate of 89% (95% CI: 83.4%, 93.1%) in R/R B-ALL patients and an MRD- CR of 86.5% (95% CI: 83.4%, 93.1%), which is comparable to, and potentially superior then the CR of 81% (95% CI: 69%, 90%) achieved by CD19 directed therapy [23]. Nonetheless, the pooled overall survival rate at one year follow-up of at most 64.1% (95% CI: 50.7%, 75.3%) does not show significant statistical difference with that of anti-CD19 therapy, 70% (95% CI: 67.7%, 72.8%) [24].

Following decrease in relapse rate with negative target antigen expressions, notable, however, is the prevalence of antigen-positive relapse and its common association with low CAR T-cell counts at disease progression. It underscores the urgent need of optimizing CAR construct and dual-targeting strategy to promote CAR T cell persistence and expansion in vivo and thus prevent subsequent

treatment failure, which is necessary for long-term disease management [7]. Besides, reducing the immunogenicity of CAR T-cells by employing humanized antibody fragments instead of murine-derived CARs may assist in the long-term persistence of these cells [11].

Meanwhile, aside from the immediate side effects, later/long-term toxicities following dual CAR T-cell therapy, including prolonged cytopenias, B cell aplasia, abnormal hemogram and late infections, are not reported by most studies due to short follow-up duration [3,25]. Further research is needed for assessment and management of these conditions, given these are also crucial factors that determines the safety profile of the treatment.

Lastly, it is important to discuss the limitation of this study. The pooled overall survival and relapse rate may not fully reflect the efficacy of the CD19/CD22 dual-targeting CAR T-cell therapy due to patients' differential baseline condition and whether they received additional treatments, such as allo-HSCT, following the clinical trials [11,13,17,18,19,21]. Furthermore, as the total number of enrolled studies is rather small, interpretation of the results should be proceeded with caution. Most pooled estimates using a random effect model display a moderate or high heterogeneity between different trials with an I^2 of 52% - 91%, indicating that the variation is likely due to differences in study design, patients enrolled, interventions, or other factors, rather than chance alone. For this instance, heterogeneity may be primarily attributable to the different dual-targeting strategies employed. However, despite inclusion of all five strategies in enrolled studies, the number of trials available for each strategy is insufficient to generate reliable subgroup estimates. Hence, more clinal trials are anticipated to improve pooled estimate generalizations of clinical data as well as enable statistics-based identification of a more effective and safe dual targeting strategy.

5. Conclusion

Both pooled estimates of clinical outcome data and safety profile statistics suggest a great potential of CD19/CD22 dual-targeting CAR T-cell therapy in treating refractory or relapsed B-cell acute lymphoblastic leukemia, while this therapeutic also has ample room for improvement in terms of potency, CAR T cell persistence and expansion in vivo, CAR construct, targeting strategy, manufacture expenditure etc. For more accurate and representative interpretations of the efficacy and safety of dual CAR-T cell therapy, future clinical studies with larger sample size, extended follow-up time, better assessment and management of adverse events are required.

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