

## Emerging role of autologous CD19 CAR T-cell therapies in the second-line setting for large B-cell lymphoma: A game changer?

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# Emerging Role of Autologous CD19 CAR T-Cell Therapies in the Second-Line Setting for Large B-cell Lymphoma: A Game Changer?

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## Abstract

Chimeric antigen receptor T-cell (CAR T) therapy has been proven effective in the third-line (and beyond) setting in patients with large B-cell lymphoma (LBCL). Until recently, high-dose chemotherapy followed by autologous hematopoietic cell transplantation (auto-HCT) was considered the standard of care in the second-line setting in patients demonstrating an objective response before the procedure. The ZUMA-7 and TRANSFORM studies showed the benefit of axicabtagene ciloleucel and lisocabtagene maraleucel, respectively, in patients refractory to or relapsing within 12 months of first-line anthracycline-based chemoimmunotherapy. However, a third trial (BELINDA study) using tisa-genlecleucel failed to show a benefit in the same setting compared to standard salvage chemoimmunotherapy followed by auto-HCT. Several differences exist between these trials, including trial designs, patient population, crossover permissibility, bridging therapy, and end-point definitions. In this review, we summarize the current evidence for the treatment of patients with LBCL in the third line and beyond and standard treatment in the second line before CAR T therapy approval and interpret outcomes of the three trials examining the role of CAR T therapy in the second line and their impact in reshaping future practice.

**Keywords:** Chimeric antigen receptor T-cell therapy, Second line, Large B-Cell lymphoma

## 1. Introduction

High-dose therapy (HDT) followed by autologous hematopoietic cell transplantation (auto-HCT) represents the standard treatment option for patients with large B-cell lymphomas (LBCLs) that relapse after frontline anthracycline-based chemoimmunotherapy and demonstrate an objective response to platinum-based second-line therapy [1,2]. Approximately 50% of cases are treated with auto-HCT [1,2]. Unfortunately, for patients who receive auto-HCT in less than a partial response (PR), the efficacy of auto-HCT is limited, with an anticipated 3-year survival <20% [3]. Moreover, the SCHOLAR-1 collaborative study showed that prognosis of DLBCL is dismal in patients who failed two or more lines of therapy, with an anticipated

median overall survival (OS) of 6.3 months and 1-year OS rate of only 28% [4].

Chimeric antigen receptor T-cell (CAR T) therapy has revolutionized the treatment of relapsed or refractory (R/R) LBCL, and several products targeting CD-19 are already commercially available, namely, axicabtagene ciloleucel (axi-cel), tisa-genlecleucel, and lisocabtagene maraleucel (liso-cel) [5–7]. These products have demonstrated impressive complete remission rates (CRRs) and improved OS. As a result, efforts have recently focused on evaluating the efficacy of CAR T therapies earlier in the disease course.

Three large phase III randomized studies evaluating axi-cel, tisa-genlecleucel, and liso-cel in the second line setting in LBCL against standard of care (SOC). Consisting of platinum-based chemoimmunotherapy followed by HDT and auto-HCT

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reported outcomes in 2021 [8–10] two of these studies namely, ZUMA-7 and TRANSFORM, on axi-cel and liso-cel, respectively, demonstrated superior outcomes vis-à-vis standard chemoimmunotherapy followed by auto-HCT [9,11], whereas a third study, namely, BELINDA, failed to show a therapeutic advantage of tisagenlecleucel in this setting [10].

We analyze the outcomes of the three studies and highlight the strengths and weaknesses associated with their rationale and trial design. We also extensively evaluate the reported outcomes and discuss future applicability.

## 2. Traditional approach to second line

Despite advances in the frontline treatment of LBCL, nearly 23% of patients are either primary R/R after an initial response [12]. Since the 1990s, the PARMA trial has established HDT followed by auto-HCT as second-line treatment for eligible patients with intermediate- and high-grade non-Hodgkin lymphoma (NHL) who had relapse after a first-line anthracycline-containing regimen [2]. A total of 215 patients were enrolled in the study. All patients received two cycles of dexamethasone, cisplatin, and cytarabine (DHAP) salvage chemotherapy. Patients who achieved CR or PR were randomized to auto-HCT or conventional treatment (CT) consisting of four additional cycles of DHAP. The overall response rate (ORR) to salvage DHAP was 64%, with only 21% ORR among patients with primary refractory disease. The remaining 109 patients were randomized to treatment arms. The trial showed a significant improvement in event-free survival (EFS) (46% vs 12%,  $P = 0.001$ ) and OS (53% vs 32%,  $P = 0.038$ ) in the auto-HCT arm compared to CT. None of the patients assigned to the CT arm died of toxic effects of treatment, whereas the death rate in the auto-HCT arm was approximately 6% [2]. This trial showed that auto-HCT significantly improves survival as second-line therapy in patients with LBCL and established its role in this setting.

The Collaborative Trial in Relapsed Aggressive Lymphoma trial is a phase III trial that compared three cycles of rituximab, ifosfamide, carboplatin, and etoposide (R-ICE) to three cycles of R-DHAP followed by auto-HCT in patients with LBCL who achieved CR or PR. All patients were refractory to or relapsing after a first-line anthracycline-containing regimen [1]. The ORR to salvage chemotherapy was similar in both arms, 63.5% (R-ICE) vs 62.8% (R-DHAP). The 3-year EFS and OS were not significantly different between the treatment arms, 26% and 35% ( $p = 0.6$ ) and 47% and 51% ( $p = 0.4$ ), with R-ICE and R-DHAP, respectively. The same rates of

febrile neutropenia occurred with both regimens (16%), while patients in the R-DHAP arm had a higher proportion of grade 4 renal toxicities [1]. A third regimen, gemcitabine, dexamethasone, and cisplatin (GDP) was compared to salvage DHAP in the LY.12 trial [13]. GDP was non-inferior to DHAP, whereby both regimens had similar response rates (45.2% vs 44%,  $P = 0.005$ ). Moreover, comparable rates of auto-HCT, EFS, and OS were observed in both arms. GDP was associated with less toxicity, particularly a lower frequency of febrile neutropenia (9% vs 23%;  $p < 0.001$ ). Thus far, no regimen has proven to be clearly superior, and the choice of a particular regimen depends on patients' comorbidities and associated toxicity profiles.

Despite improved survival with HDT and auto-HCT, almost half of patients with LBCL do not respond to chemoimmunotherapy salvage treatments and, consequently, are not eligible for auto-HCT. Moreover, the outcome of patients who are primarily refractory to first-line chemoimmunotherapy is poor. In the SCHOLAR-1 trial, patients refractory to at least one line of treatment had poor ORR and CRR of 36% and 7%, respectively. The 2-year OS was only 20% before availability of CAR T [4]. These data highlight the need for novel treatments to improve patient outcomes, particularly in refractory cases.

## 3. CAR T-cell therapy

### – Efficacy of CAR T therapy beyond second line

Currently available CAR T products for the management of diffuse LBCL (DLBCL) and high-grade B-cell lymphoma are axi-cel, tisagenlecleucel, and liso-cel. All three products target CD-19. Axi-cel has a CD28 co-stimulatory domain, whereas both liso-cel and tisagenlecleucel have a 4-1BB co-stimulatory domain. Both co-stimulatory domains provide effective signaling following CD-19 binding to its receptors. Nevertheless, CD28 leads to a more brisk CAR T expansion but with relatively limited CAR T persistence. Alternatively, 4-1BB causes gradual expansion of the CAR T, leading to a purportedly longer CAR T persistence [14]. The liso-cel manufacturing process differs from two other products by selecting CD8+ and CD4+ during T-cell apheresis, followed by an independent manufacturing process for each T-cell subset [15].

Axi-cel was evaluated in the ZUMA-1 phase II trial, including 101 patients with DLBCL, primary mediastinal large B-cell lymphoma, and DLBCL transformed from indolent NHL (t-iNHL). The ORR was 83%, with a CRR of 58%. The 2-year OS was

50.5%. For side effects, grade 3 or higher cytokine release syndrome (CRS) and neurological events (NE) were reported in 11% and 32% of patients, respectively [16]. Long-term data presented at the American Society of Hematology annual meeting 2021 showed that, after a median follow-up of 51.1 months, the median OS was 25.8 months, with 42.6% of the patients alive after 5 years. The median EFS was 5.7 months, with a 24-month EFS rate of 38% [17]. ZUMA-1 results granted axi-cel approval for treatment of R/R LBCL after two or more lines of treatment in October 2017.

Tisagenlecleucel was evaluated in the JULIET phase II trial in 111 patients with DLBCL not otherwise specified, DLBCL transformed from follicular lymphoma (t-FL), and double- or triple-hit B-cell lymphomas who received two or more prior lines of therapy. The ORR was 52%, and the CRR was 40%. At 1 year, OS was 49% in all patients and higher in patients who achieved CR with an estimated OS of approximately 80% [6]. With longer follow-up (median, 40.3 months), the ORR was 53% (95% confidence interval (CI), 43.5–62.4) with a CRR of 39%. The median PFS and OS were 2.9 and 11.1 months, respectively. In a post hoc analysis, PFS and OS were not reached for patients who achieved CR. In terms of safety profile, the most common grade 3–4 side effects were cytopenias, mainly anemia (39%) and neutropenia (34%). Grade 3–4 CRS and NE developed in 23% and 11% of patients, respectively. No treatment-related deaths were reported [18]. Moreover, 60% of patients had sustained response at 5 years [19]. The results of the JULIET study led to the approval of tisagenlecleucel for R/R LBCL after two or more lines of systemic therapy in May 2018.

Liso-cel was evaluated in the TRANSCEND-NHL-001 trial in 269 patients with DLBCL, primary mediastinal large B-cell lymphoma, DLBCL t-iNHL, and follicular lymphoma grade 3B. The ORR was 73%, and the CRR was 53% [7]. At a median follow-up of  $\geq 24$  months, the median OS, PFS, and duration of response (DoR) were 27.3, 6.8, and 26.1 months, respectively [20]. The most common grade 3 or higher toxicities were cytopenias (neutropenia, 60%; anemia, 42%; and thrombocytopenia, 27%). Moreover, grade 3 or more CRS or NE were noted in 2% and 10% of patients, respectively. Based on this study, liso-cel was the third CAR T product approved for the treatment of R/R LBCL beyond second line in November 2020.

All three trials used fludarabine and cyclophosphamide as the preferred regimen for lymphodepletion prior to CAR T infusion. The JULIET trial also allowed bendamustine as a lymphodepleting strategy in approximately 20% of patients [6].

Long-term efficacy and safety data confirm CAR T as an effective therapeutic option for patients with R/R LBCL [16–18]. Real-world data validated these findings. A retrospective study assessing the outcomes of patients with DLBCL treated with CAR T therapy compared to alternate therapies showed an improved PFS and OS with CAR T therapy. Moreover, the CRR was significantly improved with CAR T therapy (52% vs 22%;  $p < 0.001$ ) [21]. In a retrospective analysis, the outcomes of patients enrolled in the ZUMA-1 pivotal trial were compared to those in the retrospective SCHOLAR-1 study. The median OS survival was sixfold higher in patients in ZUMA-1 (31 months) compared to those in SCHOLAR-1 (5.4 months), with a 73% reduction in risk of death (hazard ratio (HR), 0.27; 95%CI, 0.00–0.38) [22]. Altogether, these results emphasize the efficacy of CAR T in the third line and beyond and provided the basis to explore CAR T in the second-line setting, especially in patients with primary refractory disease or early relapse who are expected to have poor outcomes even with HDT and auto-HCT.

#### – CAR T in the second-line setting

Based on the positive results demonstrated in the third line and beyond, CD19 CAR T were evaluated in the second-line setting in three distinctive phase III trials. All three studies included patients potentially eligible for auto-HCT and who have chemorefractory disease, defined as primary refractory or relapsing within 12 months after first-line treatment (Table 1). ZUMA-7 (NCT03391466) used axi-cel, TRANSFORM (NCT03575351) used liso-cel, and BELINDA (NCT03570892) used tisagenlecleucel. The primary endpoint of these three trials was EFS, albeit with slightly different definitions. Stable disease (SD) was considered an event at week 21 in ZUMA-7, while TRANSFORM and BELINDA considered it as an event even earlier, at weeks 12 and 9, respectively. These differences in EFS definition might have affected the final results as ZUMA-7 allowed in theory more time for CAR T to demonstrate efficacy, whereas BELINDA might have had an earlier decision timepoint for defining lack of CAR T efficacy, by week 9. The long-term follow-up of the JULIET trial showed that five of eight patients who achieved SD by 3 months converted to CR after 6, 9, and 12 months showing the continued efficacy of CAR T beyond week 9 [18].

In the ZUMA-7 trial, 180 patients were enrolled in the axi-cel arm, of whom 170 received the product and 179 patients were included in SOC arm [11]. The ORR was significantly higher in the CAR T arm (83% vs SOC 50%,  $p < 0.001$ ), and a higher CRR was



Table 1. Characteristics of patients enrolled in second-line CAR T-cell therapy trials.

Patients characteristics	ZUMA-7 [8]	TRANSFORM [9]	BELINDA [10]
	N = 180	N = 184	N = 322
CAR T product	Axicabtagene ciloleucel	Lisocabtagene maraleucel	Tisagenlecleucel
Median age, years (range)	58 (21–80)	58 (26–75)	59.5 (19–79)
Patients >65 years, %	30%	N/A	31.10%
Patients who received TT			
CAR T arm, %	94%	97%	96%
SOC arm, %	36%	46%	32%
HGBCL/double/triple hit (CART/SOC), %	17/15%	23/24%	19.8/11.9%
ABC subtype (CAR T/SOC), %	9/5%	N/A	32/26.2%
Stage III/IV, %	79%	N/A	64%
Primary refractory	74%	73%	66%
Relapse within 12 months of first-line treatment	26%	27%	34%
Progressive disease at the time of CAR T cell therapy	1%	N/A	26%
Bridging therapy allowed	No	Yes	Optional
Bridging therapy	Glucocorticoid only	RDHAP, RICE, and RGDP ×1 cycle	RDHAP, RICE, RGenOx, and RGDP
%	36%	63%	83%
Median time from leukapheresis to CAR T cell infusion, days	29	31	54
Crossover	Not allowed	Allowed	Allowed
Patients who did not respond to SOC received CAR T			
Primary endpoints	EFS	EFS	EFS
EFS, start time point	Randomization	Randomization	Randomization
EFS definition	1) Disease progression 2) Death from any cause 3) Start of new therapy 4) SD as best response within 150 days from randomization	1) Disease progression 2) Death from any cause 3) Start of new therapy 4) Not achieving CR/PR by 9 weeks	1) SD or PD at or after week 12 2) Death (any time)

Abbreviations: N, number; N/A, not available; CAR, chimeric antigen receptor; TT, treatment; SOC, standard of care; HGBCL, high-grade B cell lymphoma; EFS, event-free survival; RDHAP: rituximab, dexamethasone, cisplatin, cytarabine; RICE, rituximab, ifosfamide, carboplatin, etoposide; RGDP, rituximab, gemcitabine, dexamethasone, cisplatin; RGenOx, Rituximab, gemcitabine, oxaliplatin; CR, complete remission; PR, partial response; SD, stable disease; PD, progressive disease.

observed in the axi-cel arm (65%) compared to the SOC arm (32%). After a median follow-up of 24.9 months, median and 24-month EFS were significantly improved in the axi-cel arm (8.3 months and 41%) compared to the SOC arm (4.5 months and 16%), respectively (HR, 0.40; 95% confidence interval (CI), 0.31–0.51,  $p < 0.001$ ). A trend toward improved OS was observed in the axi-cel group (not reached) versus the SOC group (35.1 months) (HR, 0.73; 95%CI, 0.53–1.01,  $P = 0.54$ ) (Table 2). This trial led to the approval of axi-cel for the treatment of patients with LBCL refractory to first-line chemotherapy or relapsed within 12 months of the end of first-line treatment on April 1, 2022.

The TRANSFORM trial included 184 patients. ORR and CRR were significantly higher in the

CAR T compared to the SOC arm (79% and 61% vs 44% and 36%,  $p < 0.0001$ ). Moreover, second-line CAR T significantly prolonged EFS compared to SOC (median, 10.1 vs 2.3 months, respectively,  $p < 0.0001$ ). OS was not reached in the CAR T arm and was 16.4 months in the SOC group ( $p = 0.0257$ ) (Table 2).

The BELINDA trial failed to show a benefit of CAR T, namely, tisagenlecleucel. It included 322 patients. The ORR and CRR were similar in both arms (46% and 28.4% with CAR T vs 42% and 27.5% with SOC, respectively). The study did not meet its primary endpoint as EFS was comparable in both arms (HR, 1.07; 95% CI, 0.82–1.40,  $p = 0.61$ ) (Table 2).

In terms of safety data, CRS was more frequent in the ZUMA-7 trial, as noted in 92% of cases. This was

Table 2. Summary of responses and adverse events in the ZUMA-7, TRANSFORM, and BELINDA trials.

	ZUMA-7 [8]				TRANSFORM [9]				BELINDA [10]						
	CAR T arm (N = 180)	SOC arm (N = 179)	HR	95% CI	P-value	CAR T arm (N = 92)	SOC arm (N = 92)	HR	95% CI	P-value	CAR T arm (N = 162)	SOC arm (N = 160)	HR	95% CI	P-value
Median follow-up, months	25					6.2					10				
ORR	83%	50%			<0.001	86%	48%			<0.0001	46%	42%			
CR rate	65%	32%				66%	39%			<0.0001	28%	28%			
mEFS, months	8.3	2	0.4	0.31–0.51	<0.001	10.1	2.3	0.349	N/A	<0.0001	3	3	1.07	0.82–1.40	0.61
2-year OS, %	61%	52%				N/A					Not reached				
mOS, months	NR	32.1	0.73	0.53–1.01	0.054	NR	16.4	0.509	0.258–1.004	P = 0.0257	NR	NR			
CRS, any grade	92%					49%					61.30%				
CRS, grade 3–4	6%					1%*					5.20%				
NE, any grade	60%	20%				12%					10.30%				
NE, grade 3–4	21%	1%				4%					1.90%				

Abbreviations: CAR, chimeric antigen receptor; SOC, standard of care; CI, confidence interval; ORR, overall response rate; CR, complete response; EFS, event-free survival; OS, overall survival; CRS, cytokine release syndrome; NE, neurologic events.

\*Estimated as original manuscript reported incidence in one patient.

followed by 61% in the BELINDA trial and 49% in the TRANSFORM trial. Grade >2 CRS showed a low incidence in all three clinical trials (Table 2).

ZUMA-7 had the highest rate of NE (60%) with 21% grade 3–4 NE, followed by TRANSFORM (12%, with 4% grade 3–4) and BELINDA (10.3%, with 2% grade 3–4). Overall, adverse events were similar to those observed in previous CD19 CAR T pivotal trials [17,18,20]. The safety profile favored the experimental arm in all three trials, particularly pertaining to incidence of febrile neutropenia, thrombocytopenia, and gastrointestinal toxicity. Furthermore, the quality of life (QoL) and patient reported outcomes (PRO) were significantly better in the CAR T arms in ZUMA-7 and TRANSFORM trials. Axi-cel resulted in significantly improved QoL and PRO in terms of physical functioning, global health status, and visual analog scale by day 100 ( $p < 0.0001$ ) [8]. Similarly, the TRANSFORM trial showed that PRO, mainly cognitive function and quality of life, was more improved compared to SOC in patients who received liso-cel. QoL was either improved or maintained after liso-cel [23].

ZUMA-7 and TRANSFORM trials showed positive results with significantly improved EFS compared to SOC. This was not the case for the BELINDA trial as tisagenlecleucel failed to show a similar benefit of CAR T therapy in the second line compared to auto-HCT.

The three aforementioned trials differed in design, allowance of bridging therapy, follow-up duration, permitting crossover, and some endpoints definitions, which are summarized in Table 1. An important difference worth mentioning among these trials is allowing (or not) bridging therapy. ZUMA-7 did not allow bridging chemotherapy, which might have led to excluding patients with rapidly progressing or bulky disease, whereas TRANSFORM allowed one cycle and BELINDA allowed several rounds of bridging chemotherapy. Consequently, the latter trials might have included more patients with rapidly progressing or bulky disease. Patients who progressed after randomization did not receive axi-cel in the ZUMA-7 trial, whereas BELINDA included 26% of patients who had progressive disease at randomization. Additionally, the median time from leukapheresis to CAR T infusion was shorter in the ZUMA-7 and TRANSFORM trials (29 and 31 days, respectively) and almost twofold longer in the BELINDA trial (54 days). In fact, data have shown that patients who require bridging chemotherapy tend to have shorter PFS (3.4 months) compared to patients who do not (7.3 months) ( $p = 0.01$ ) [24]. We speculate that these differences in design could have possibly affected

the results of the studies as requirement for bridging therapy may be considered an indicator for a more aggressive disease. This could represent a selection bias in the ZUMA-7 trial, which might have selected patients with less aggressive disease that were probably destined to have better outcomes. Moreover, Bommier et al. did a reconstruction of individual patient data from published figures, EFS curves showed that the BELINDA trial perhaps included patients with more aggressive disease in the CAR T arm [25]. Furthermore, less than half of patients included in the SOC arm received auto-HCT, being only 32% in BELINDA, 36% in ZUMA-7, and 42% in TRANSFORM. This further shows the difference in the patient population among the three trials and suggests that the population ultimately assigned to the CAR T arm in the BELINDA trial might have had a more aggressive disease.

The median follow-up duration was longer in the ZUMA-7 study. There was a trend toward improvement in OS; however, it was not statistically significant. A longer follow-up is needed to better understand the impact of second-line axi-cel on OS.

Most patients included in the three trials were of white race, denoting the underrepresentation of minorities. This certainly limits, in theory, the ability to generalize these results to the minority population.

Recently, a phase II, single-arm, multicenter trial, ZUMA-12, showed the benefit of axi-cel in what was described as “first-line” setting [26]. The trial included 40 patients with high-risk DLBCL, defined as double- or triple-hit lymphoma or with an international prognostic index of three or above, who remained positron emission tomography positive (PET+) following two cycles of chemotherapy with anthracycline-containing regimen. The ORR was 89% (33/37), and CRR was 78% (29/37). The median DoR, EFS, and PFS were not reached. The 1-year EFS and OS were 72.5% and 90.6%, respectively. Safety data were in line with previous axi-cel studies with 8% and 23% incidence of grade  $\geq 3$  CRS and NE, respectively [26]. We question whether this therapeutic strategy can be defined as “first-line” or is perhaps more of a second line; nevertheless, these data are definitely promising, further emphasizing the efficacy of CAR T in patients with high-risk LBCL.

#### 4. Conclusions and future directions

With the results of these trials, the main question on the future management of second-line LBCL with the addition of CAR T into this space remains.

Future directions will likely be influenced by a better understanding of the intricacies of the results of these phase III clinical trials, specifically given the differences in EFS definitions, bridging therapies, and crossover designs as previously discussed. Moreover, it will be important to discern outcomes based on remission status attained prior to CAR T. A recent observational registry study from the Center for International Blood and Marrow Transplant Research demonstrated the benefit of auto-HCT versus CAR T therapy in patients in PR [27].

An important consideration pertains to time to administer CAR T therapy. The timeline from identifying patients with disease indication to CAR T treatment can be influenced by several factors, including manufacturing time and aggressive disease biology, which may require bridging therapy. This is also important given the variation in time from enrollment to CAR T infusion in all three phase III studies and ZUMA-7 clinical trial not allowing bridging therapy nor crossover, while TRANSFORM and BELINDA allowed both bridging therapy and crossover [9–11].

One strategy is to reduce time to CAR T infusion centers around manufacturing. Novartis is currently studying autologous CD-19 CAR T product YTB323 (NCT03960840), produced via an enhanced manufacturing process. Similar enhanced manufacturing platforms are being investigated by Bristol Myers Squibb using the NEX-T platform for both CD-19 and BCMA-directed autologous CAR T products (NCT04231747, NCT04394650).

Efforts to improve efficacy of autologous CAR T therapies are evaluating a multitargeted approach in addition to CD19. Some targets being evaluated in clinical trials include CD22 and CD79b, among others (NCT04723914, NCT04877080, NCT05388695, NCT04429438). We anticipate that if they demonstrate added efficacy vis-à-vis CD19 CAR Ts in the R/R setting, they may eventually make their way into being evaluated in the second-line setting.

The development of allogeneic or “off the shelf” CAR T (allo-CAR) products is also an important strategy to limit time to CAR T infusion [28]. Allo-CAR is being developed using T lymphocytes collected from healthy donors. Allo-CAR would provide a more readily access to the product, circumventing delays in securing apheresis and avoiding the long manufacturing time associated with autologous CAR T production. Infusion of allo-donor T-cell products raises concerns for the development of graft-versus-host disease (GVHD) and rapid elimination of T cells by host immune system, potentially limiting persistence and efficacy. These concerns have been addressed through the

use of gene editing, which aims to disrupt or eliminate the  $\alpha\beta$  T-cell receptor, thus abrogating GVHD risk. Common examples of this include disrupting the gene encoding for the T-cell receptor constant  $\alpha$  chain (TRAC) [29]; gene editing utilized to enhance CAR T persistence, including CD52 knockout [30]; disruption of major histocompatibility complex class I molecules (by knocking out the  $\beta_2$ -microglobulin gene [B2M]) [31]; and addition of a natural killer cell inhibitor [32]. Few studies in allo-CAR directed against CD-19 are ongoing in early-phase clinical trials, with results from these studies eagerly anticipated.

We strongly believe that future clinical trials should focus on enrolling a diverse population of patients to ensure a good representation and guarantee reproducibility in the real-life setting across various racial and ethnic groups. Efforts should also be focused on lowering the high cost of these CAR T products to make it affordable to patients in developing countries.

### Declaration of Competing Interest

The authors R.M, M.A.M, MA, and HM declare no relevant conflicts of interest in relation to the content of this manuscript. MAK-D declares research/grant from Novartis and Bristol Myers Squibb.

### References

- [1] Gisselbrecht C, Glass B, Mounier N, Singh Gill D, Linch DC, Trneny M, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol* 2010;28(27):4184–90. <https://doi.org/10.1200/JCO.2010.28.1618>. Epub 2010/07/28 PubMed PMID: 20660832; PubMed Central PMCID: PMC2664033.
- [2] Philip T, Guglielmi C, Hagenbeek A, Somers R, Van der Lelie H, Bron D, 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(23):1540–5. <https://doi.org/10.1056/NEJM199512073332305>. Epub 1995/12/07 PubMed PMID: 7477169.
- [3] Philip T, Armitage JO, Spitzer G, Chauvin F, Jagannath S, Cahn JY, et al. High-dose therapy and autologous bone marrow transplantation after failure of conventional chemotherapy in adults with intermediate-grade or high-grade non-Hodgkin's lymphoma. *N Engl J Med* 1987;316(24):1493–8. <https://doi.org/10.1056/NEJM198706113162401>. Epub 1987/06/11 PubMed PMID: 3295541.
- [4] Crump M, Neelapu SS, Farooq U, Van Den Neste E, Kuruvilla J, Westin J, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. *Blood* 2017;130(16):1800–8. <https://doi.org/10.1182/blood-2017-03-769620>. Epub 2017/08/05 PubMed PMID: 28774879; PubMed Central PMCID: PMC5649550.
- [5] Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med* 2017;377(26):2531–44. <https://doi.org/10.1056/NEJMoa1707447>. Epub 2017/12/12 PubMed PMID: 29226797; PubMed Central PMCID: PMC5882485.
- [6] Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med* 2019;380(1):45–56. <https://doi.org/10.1056/NEJMoa1804980>. Epub 2018/12/07 PubMed PMID: 30501490.
- [7] Abramson JS, Palomba ML, Gordon LJ, Lunning MA, Wang M, Arnason J, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet* 2020;396(10254):839–52. [https://doi.org/10.1016/S0140-6736\(20\)31366-0](https://doi.org/10.1016/S0140-6736(20)31366-0). Epub 2020/09/06 PubMed PMID: 32888407.
- [8] Elsayy M, Chavez JC, Avivi I, Larouche J-F, Wannesson L, Cwynarski K, et al. Patient-reported outcomes in a phase 3, randomized, open-label study evaluating the efficacy of axicabtagene ciloleucel (axi-cel) versus standard of care therapy in patients with relapsed/refractory large B-cell lymphoma (ZUMA-7). *Blood* 2021;138(Supplement 1):430. <https://doi.org/10.1182/blood-2021-147598>.
- [9] Kamdar M, Solomon SR, Arnason JE, Johnston PB, Glass B, Bachanova V, et al. Lisocabtagene maraleucel (liso-cel), a CD19-directed chimeric antigen receptor (CAR) T cell therapy, versus standard of care (SOC) with salvage chemotherapy (CT) followed by autologous stem cell transplantation (ASCT) as second-line (2L) treatment in patients (Pts) with relapsed or refractory (R/R) large B-cell lymphoma (LBCL): results from the randomized phase 3 transform study. *Blood* 2021;138(Supplement 1):91. <https://doi.org/10.1182/blood-2021-147913>.
- [10] Bishop MR, Dickinson M, Purtil D, Barba P, Santoro A, Hamad N, et al. Second-line tisagenlecleucel or standard care in aggressive B-cell lymphoma. *N Engl J Med* 2022;386(7):629–39. <https://doi.org/10.1056/NEJMoa2116596>. Epub 2021/12/15 PubMed PMID: 34904798.
- [11] Locke FL, Miklos DB, Jacobson CA, Perales MA, Kersten MJ, Oluwole OO, et al. Axicabtagene ciloleucel as second-line therapy for large B-cell lymphoma. *N Engl J Med* 2022;386(7):640–54. <https://doi.org/10.1056/NEJMoa2116133>. Epub 2021/12/11 PubMed PMID: 34891224.
- [12] Harrysson S, Eloranta S, Ekberg S, Enblad G, Jerkeman M, Wahlin BE, et al. Incidence of relapsed/refractory diffuse large B-cell lymphoma (DLBCL) including CNS relapse in a population-based cohort of 4243 patients in Sweden. *Blood Cancer J* 2021;11(1):9. <https://doi.org/10.1038/s41408-020-00403-1>. Epub 2021/01/09 PubMed PMID: 33414443; PubMed Central PMCID: PMC7791057.
- [13] Crump M, Kuruvilla J, Couban S, MacDonald DA, Kukreti V, Kouroukis CT, et al. Randomized comparison of gemcitabine, dexamethasone, and cisplatin versus dexamethasone, cytarabine, and cisplatin chemotherapy before autologous stem-cell transplantation for relapsed and refractory aggressive lymphomas: NCIC-CTG LY.12. *J Clin Oncol* 2014;32(31):3490–6. <https://doi.org/10.1200/JCO.2013.53.9593>. Epub 2014/10/01 PubMed PMID: 25267740.
- [14] Westin JR, Kersten MJ, Salles G, Abramson JS, Schuster SJ, Locke FL, et al. Efficacy and safety of CD19-directed CAR-T cell therapies in patients with relapsed/refractory aggressive B-cell lymphomas: observations from the JULIET, ZUMA-1, and TRANSCEND trials. *Am J Hematol* 2021;96(10):1295–312. <https://doi.org/10.1002/ajh.26301>. Epub 2021/07/27 PubMed PMID: 34310745.
- [15] Meng J, Wu X, Sun Z, Xun R, Liu M, Hu R, et al. Efficacy and safety of CAR-T cell products axicabtagene ciloleucel, tisagenlecleucel, and lisocabtagene maraleucel for the treatment of hematologic malignancies: a systematic review and meta-analysis. *Front Oncol* 2021;11:698607. <https://doi.org/10.3389/fonc.2021.698607>. Epub 2021/08/13 PubMed PMID: 34381720; PubMed Central PMCID: PMC8350577.
- [16] Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma



- (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. *Lancet Oncol* 2019;20(1):31–42. [https://doi.org/10.1016/S1470-2045\(18\)30864-7](https://doi.org/10.1016/S1470-2045(18)30864-7). Epub 2018/12/07 PubMed PMID: 30518502; PubMed Central PMCID: PMC6733402.
- [17] Jacobson C, Locke FL, Ghobadi A, Miklos DB, Lekakis LJ, Oluwole OO, et al. Long-term ( $\geq 4$  year and  $\geq 5$  year) overall survival (OS) by 12- and 24-month event-free survival (EFS): an updated analysis of ZUMA-1, the pivotal study of axicabtagene ciloleucel (axi-Cel) in patients (Pts) with refractory large B-cell lymphoma (LBCL). *Blood* 2021;138(Supplement 1):1764. <https://doi.org/10.1182/blood-2021-148078>.
- [18] Schuster SJ, Tam CS, Borchmann P, Worel N, McGuirk JP, Holte H, et al. Long-term clinical outcomes of tisagenlecleucel in patients with relapsed or refractory aggressive B-cell lymphomas (JULIET): a multicentre, open-label, single-arm, phase 2 study. *Lancet Oncol* 2021;22(10):1403–15. [https://doi.org/10.1016/S1470-2045\(21\)00375-2](https://doi.org/10.1016/S1470-2045(21)00375-2). Epub 2021/09/14 PubMed PMID: 34516954.
- [19] Chong EA, Ruella M, Schuster SJ. Lymphoma Program Investigators at the University of P. Five-year outcomes for refractory B-cell lymphomas with CAR T-cell therapy. *N Engl J Med* 2021;384(7):673–4. <https://doi.org/10.1056/NEJMc2030164>. Epub 2021/02/18 PubMed PMID: 33596362.
- [20] Abramson JS, Palomba ML, Gordon LI, Lunning MA, Wang M, Arnason JE, et al. Two-year follow-up of Transcend NHL 001, a multicenter phase 1 study of lisocabtagene maraleucel (liso-cel) in relapsed or refractory (R/R) large B-cell lymphomas (LBCL). *Blood* 2021;138(Supplement 1):2840. <https://doi.org/10.1182/blood-2021-148948>.
- [21] Sermer D, Batlevi C, Palomba ML, Shah G, Lin RJ, Perales MA, et al. Outcomes in patients with DLBCL treated with commercial CAR T cells compared with alternate therapies. *Blood Adv* 2020;4(19):4669–78. <https://doi.org/10.1182/bloodadvances.2020002118>. Epub 2020/10/02 PubMed PMID: 33002134; PubMed Central PMCID: PMC67556134.
- [22] Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Reagan PM, Miklos DB, et al. Comparison of 2-year outcomes with CAR T cells (ZUMA-1) vs salvage chemotherapy in refractory large B-cell lymphoma. *Blood Adv* 2021;5(20):4149–55. <https://doi.org/10.1182/bloodadvances.2020003848>. Epub 2021/09/04 PubMed PMID: 34478487; PubMed Central PMCID: PMC68945634.
- [23] Abramson JS, Solomon SR, Arnason JE, Johnston PB, Glass B, Crotta A, et al. Improved quality of life (QOL) with lisocabtagene maraleucel (liso-cel), a CD19-directed chimeric antigen receptor (CAR) T cell therapy, compared with standard of care (SOC) as second-line (2L) treatment in patients (Pts) with relapsed or refractory (R/R) large B-cell lymphoma (LBCL): results from the phase 3 transform study. *Blood* 2021;138(Supplement 1):3845. <https://doi.org/10.1182/blood-2021-151611>.
- [24] Pinnix CC, Gunther JR, Dabaja BS, Strati P, Fang P, Hawkins MC, et al. Bridging therapy prior to axicabtagene ciloleucel for relapsed/refractory large B-cell lymphoma. *Blood Adv* 2020;4(13):2871–83. <https://doi.org/10.1182/bloodadvances.2020001837>. Epub 2020/06/27 PubMed PMID: 32589728; PubMed Central PMCID: PMC67362355.
- [25] Bommier C, Lambert J, Thieblemont C. Comparing apples and oranges: the ZUMA-7, TRANSFORM and BELINDA trials. *Hematol Oncol* 2022. <https://doi.org/10.1002/hon.3001>. Epub 2022/04/09 PubMed PMID: 35394082.
- [26] Neelapu SS, Dickinson M, Munoz J, Ulrickson ML, Thieblemont C, Oluwole OO, et al. Axicabtagene ciloleucel as first-line therapy in high-risk large B-cell lymphoma: the phase 2 ZUMA-12 trial. *Nat Med* 2022;28(4):735–42. <https://doi.org/10.1038/s41591-022-01731-4>. Epub 2022/03/23 PubMed PMID: 35314842; PubMed Central PMCID: PMC69018426.
- [27] Shadman M, Pasquini M, Ahn KW, Chen Y, Turtle CJ, Hematti P, et al. Autologous transplant vs chimeric antigen receptor T-cell therapy for relapsed DLBCL in partial remission. *Blood* 2022;139(9):1330–9. <https://doi.org/10.1182/blood.2021013289>. Epub 2021/09/28 PubMed PMID: 34570879; PubMed Central PMCID: PMC68900276.
- [28] Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. *Nat Rev Drug Discov* 2020;19(3):185–99. <https://doi.org/10.1038/s41573-019-0051-2>. Epub 2020/01/05 PubMed PMID: 31900462.
- [29] Torikai H, Reik A, Liu PQ, Zhou Y, Zhang L, Maiti S, et al. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. *Blood* 2012;119(24):5697–705. <https://doi.org/10.1182/blood-2012-01-405365>. Epub 2012/04/27 PubMed PMID: 22535661; PubMed Central PMCID: PMC3382929.
- [30] Poirot L, Philip B, Schiffer-Mannioui C, Le Clerre D, Chion-Sotinel I, Derniame S, et al. Multiplex genome-edited T-cell manufacturing platform for "off-the-shelf" adoptive T-cell immunotherapies. *Cancer Res* 2015;75(18):3853–64. <https://doi.org/10.1158/0008-5472.CAN-14-3321>. Epub 2015/07/18 PubMed PMID: 26183927.
- [31] Wang D, Quan Y, Yan Q, Morales JE, Wetsel RA. Targeted disruption of the beta2-microglobulin gene minimizes the immunogenicity of human embryonic stem cells. *Stem Cells Transl Med* 2015;4(10):1234–45. <https://doi.org/10.5966/sctm.2015-0049>. Epub 2015/08/20 PubMed PMID: 26285657; PubMed Central PMCID: PMC4572902.
- [32] Gornalusse GG, Hirata RK, Funk SE, Rioloobos L, Lopes VS, Manske G, et al. HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. *Nat Biotechnol* 2017;35(8):765–72. <https://doi.org/10.1038/nbt.3860>. Epub 2017/05/16 PubMed PMID: 28504668; PubMed Central PMCID: PMC65548598.