

Comparison of CAR-T19 and autologous stem cell transplantation for refractory/relapsed non-Hodgkin's lymphoma

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BACKGROUND. Autologous stem cell transplantation (ASCT) is the standard treatment for refractory/relapsed B cell non-Hodgkin's lymphoma (R/R B-NHL), whereas chimeric antigen receptor T (CAR-T) therapy targeting CD19 is emerging as an alternative strategy. Here, we report a comparative analysis of the 2 strategies in a single center.

METHODS. We performed a prospective, single-arm study of CAR-T therapy in 29 patients with R/R B-NHL and compared the outcomes with 27 contemporaneous patients who received ASCT. NHL was diagnosed by histopathologic assessments, and the safety and efficacy of treatments were compared.

RESULTS. The CAR-T group exhibited better rates of complete response (CR) (48.0% vs. 20.8%, $P = 0.046$) and 1-year overall survival (OS) (74.4% vs. 44.5%, $P = 0.044$) compared with the ASCT group. Subpopulation analysis showed that patients with International Prognostic Index scores of at least 3 achieved a significantly higher objective response rate and CR rate in the CAR-T group than in the ASCT group (ORR 72.0% vs. 10.0%, $P = 0.002$, and CR 38.9% vs. 0%, $P = 0.030$, respectively). The most common severe adverse events in the CAR-T group were cytokine release syndrome, neurotoxicity, and infection compared with cytopenia, gastrointestinal toxicity, and infection in the ASCT group. Additionally, the incidence of nonhematologic severe adverse events was markedly lower in the CAR-T group than in the ASCT group (20.7% vs. 48.1%, $P = 0.030$).

CONCLUSION. CAR-T therapy exhibited superior clinical outcomes in safety and efficacy over ASCT in patients with R/R B-NHL, suggesting that CAR-T may be a recommended alternative to ASCT.

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Introduction

Though response and survival rates have improved with the development of rituximab and combined chemotherapies in B cell non-Hodgkin's lymphoma, therapeutic strategies for refractory/relapsed B cell non-Hodgkin's lymphoma (R/R B-NHL) remain inadequate. Approximately 30% to 40% of patients with R/R B-NHL relapse after initial therapies, and another 10% develop treatment-refractory diseases, leading to dismal prognoses (1–3). Actually, a multicohort, retrospective non-Hodgkin's lymphoma research study (SCHOLAR-1) showed that the objective response rates (ORRs) and complete response (CR) rates for patients with R/R B-NHL were only 26% and 7%, respectively, with a median

overall survival (OS) of 6.3 months (4). Therefore, huge unmet medical needs exist in R/R B-NHL, calling for effective therapeutic strategies.

Autologous stem cell transplantation (ASCT) following high-dose chemotherapy has been used as a standard salvage treatment in the past 20 years in R/R B-NHL, with approximately 30% to 45% of patients remaining progression free 3 years after transplantation (5–9). However, several disadvantages limit the clinical benefits of ASCT in patients with R/R B-NHL. Nearly half of patients with R/R B-NHL are not eligible for this approach because of stem cell mobilization failure and severe complications. Meanwhile, patients who do not respond to salvage chemotherapies exhibit inferior clinical outcomes from ASCT, and the expected long-term progression-free survival (PFS) rates decrease to only 10% to 30% (10–12). Indeed, some studies showed that patients with primary refractory NHL had worse prognosis after ASCT compared with patients with relapsed disease, and there were almost no therapeutic options left for such a group of patients (10, 12–14). Last, post-ASCT relapse happened in about 60% of patients with R/R B-NHL, and hardly any of those patients remained disease free over a year after ASCT (15, 16).

Chimeric antigen receptor T cell targeting CD19 (CAR-T19) is a new immunotherapeutic strategy for B cell lineage malignancies with tremendous clinical efficacy in refractory or relapsed patients (17–22). Currently, second-generation CAR-T cells equipped with an extracellular anti-CD19 single-chain fragment variable domain fused to an intracellular domain consisting of a costimulatory region of 4-1BB or CD28 and a CD3- ζ region are the most common form in clinical use. Several clinical trials demonstrated dramatic outcomes of CAR-T19 in adult and pediatric patients with relapsed/refractory acute lymphoblastic leukemia (R/R ALL) with complete remission rates ranging from 67% to 90% (17–19). Additionally, high response rates were observed in adult patients with R/R B-NHL receiving CAR-T19 with ORRs ranging from 50% to 82% (20–23).

ASCT and CAR-T share a series of similarities, both of which involve autologous immune cell infusions with the hope of reconstitution of host immunologic surveillance and long-term remission. Nevertheless, CAR-T exhibits several merits in clinical feasibility over ASCT. For example, CAR-T uses peripheral blood mononuclear cells (PBMCs), which are abundant and easy to collect compared with stem cells used in ASCT. Though both therapies require preconditioning chemotherapies, CAR-T does not mandatorily require responsiveness to chemotherapy, and the doses are moderate, which thereby reduces the risk of complications. These facts indicate that CAR-T therapy may be an alternative strategy for patients with R/R B-NHL when ASCT is not available. Indeed, it is claimed that CAR-T may be a possible candidate for standard therapeutic strategy for R/R B-NHL besides ASCT (24, 25).

However, the differences in clinical efficacy and safety between CAR-T and ASCT have not been well investigated. To address this question, we compared the effectiveness and toxicities of CAR-T therapy versus ASCT and assessed whether CAR-T therapy resulted in better clinical benefits in patients with R/R B-NHL than ASCT.

Results

Patient characteristics. Between March 2017 and September 2018, 56 patients were treated and analyzed, including 29 in the CAR-T group and 27 in the ASCT group (Figure 1). Patients' baseline characteristics are shown in Table 1. Disease assessments for both groups immediately before treatments revealed that 82.8% and 48.1% of patients were assessed as having either SD or PD ($P = 0.006$), and 17.2% (all PR) and 51.9% (40.7% PR, 11.2% CR) were in remission in the CAR-T and ASCT groups, respectively. Patients had similar baseline characteristics in the 2 groups. The CAR-T group showed a tendency toward more patients with advanced ages (≥ 60), high International Prognostic Index (IPI) scores (baseline characteristics of patients with IPI scores of at least 3 are shown in Supplemental Table 1; supplemental material available online with this article; <https://doi.org/10.1172/jci.insight.130195DS1>), poor prognosis after prior treatments, and advanced disease stages (stage 3 or 4). Additionally, 5 patients in the CAR-T group had relapsed after hematopoietic stem cell transplantation (HSCT), including 4 who had relapsed after ASCT and 1 after allogeneic HSCT (allo-HSCT). The 5 patients with post-HSCT relapses were treated similarly as the other patients in the CAR-T group, except that the patient who relapsed after allo-HSCT accepted donor-derived CAR-T cells.

Response assessment and duration. There were 25/29 and 24/27 efficacy-evaluable patients in the CAR-T group and the ASCT group, respectively. The rest of the patients died before reaching the primary efficacy endpoint or were lost to follow-up. CRs were achieved in 12 of 25 patients (48.0%) in the CAR-T group compared with 5 of 24 patients (20.8%) in the ASCT group ($P = 0.046$; Table 2 and Supplemental Figure 3).

Table 1. Baseline characteristics of the patients

	CAR-T <i>n</i> = 29	ASCT <i>n</i> = 27	<i>P</i> value
Age and sex			
≥60	13, 44.8%	4, 14.8%	0.015
Male	17, 58.6%	15, 55.6%	0.817
Female	12, 41.4%	12, 44.4%	
ECOG performance status			
0–1	25, 86.2%	26, 96.3%	0.186
≥2	4, 13.8%	1, 3.7%	
Ann Arbor clinical stage			
II	0, 0%	4, 14.8%	0.031
III	5, 17.2%	4, 14.8%	
IV	24, 82.8%	19, 70.4%	
LDH higher than ULN	17, 58.6%	16, 59.3%	0.961
Disease type			
DLBCL	21, 72.5%	20, 74.1%	0.889
Transformed DLBCL	2, 6.9%	1, 3.7%	0.596
MCL	2, 6.9%	4, 14.8%	0.338
BL	2, 6.9%	0, 0%	0.165
MZL	1, 3.4%	0, 0%	0.330
CLL	1, 3.4%	0, 0%	0.330
FL	0, 0%	2, 7.4%	0.136
IPI risk group			
Low (0 or 1 factor)	3, 10.3%	8, 29.6%	0.034
Low/intermediate (2 factors)	6, 20.7%	8, 29.6%	
Intermediate/high (3 factors)	10, 34.5%	9, 33.3%	
High (4 or 5 factors)	10, 34.5%	2, 7.4%	
Prior therapies			
≥3 prior lines of therapies	17, 58.6%	12, 44.4%	0.289
Primary refractory	8, 27.6%	8, 29.6%	0.866
Prior disease status			
CR	0, 0%	3, 11.1%	0.060
PR	5, 17.2%	11, 40.7%	
SD	8, 27.6%	6, 22.2%	
PD	16, 55.2	7, 25.9%	

The median age of patients in the CAR-T group was 62 (range 27–70); the median age of patients in the ASCT group was 52 (range 22–64); $P = 0.015$. ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; ULN, upper limit of normal; DLBCL, diffuse large B cell; MCL, mantle cell lymphoma, BL, Burkitt lymphoma; MZL, marginal zone lymphoma; CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; IPI, International Prognostic Index; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Objective responses were achieved in 18 of 25 patients (72.0%) in the CAR-T group versus 12 of 24 (50.0%) in the ASCT group ($P = 0.114$). Similarly, higher ORRs and CR rates in the CAR-T group than in the ASCT group were observed in a subgroup analysis of patients with IPI scores of at least 3 (ORR: 72.2% vs. 10.0%, $P = 0.004$; CR: 38.9% vs. 0%, $P = 0.030$; respectively). Among all patients with objective responses in the CAR-T group, remission was sustained in all 12 patients achieving CR, and 2/6 achieved PR till the latest follow-up, while the remaining 4/6 PR patients experienced disease progression in a median time of 5.3 months. In contrast, in the ASCT group, 5 patients achieved CR, 4/5 maintained in remission, and the remaining patient died from multiple organ dysfunction syndrome. Disease progressions were observed in 9/24 patients in the ASCT group, including 3/6 patients who had PR and another 6 patients who had SD with a median duration of 2.7 months (individual durations of remission are shown in Supplemental Figure 2). These results suggested that a higher proportion of patients in the CAR-T group achieved CRs, overall responses, and long-term remission than those in the ASCT group.

A subgroup analysis was performed for the 5 patients with post-HSCT relapses in the CAR-T group. Three of 4 patients with prior ASCT achieved CR and maintained in remission, and the remaining patient

Table 2. Clinical response in the 2 groups

	CAR-T	ASCT	P value
Total (CAR-T group n = 25; ASCT group n = 24) ^A			
CR	12 (48.0)	5 (20.8)	0.046
PR	6 (24.0)	7 (29.2)	0.682
NR	7 (28.0)	12 (50.0)	0.114
ORR	18 (72.0)	12 (50.0)	0.114
IPI scores ≥ 3 (CAR-T group n = 18; ASCT group n = 10) ^B			
CR	7 (38.9)	0 (0)	0.030
PR	6 (33.3)	1 (10.0)	0.364
NR	5 (27.8)	9 (90.0)	0.004
ORR	13 (72.2)	1 (10.0)	0.004

Group values presented as n (percentage). ^AUsing the χ^2 test. ^BUsing Fisher's exact test. NR, no response.

achieved PR. The 1 patient with prior allo-HSCT achieved PR after CAR-T treatment and died from cerebral hemorrhage because of thrombocytopenia in month 2 after CAR-T infusion. These results indicated that CAR-T might work as a salvage therapy for patients relapsing after stem cell transplantation with comparable efficacy to the standard therapy for patients with R/R B-NHL.

Survival. PFS and OS were analyzed and compared between the 2 groups with a median follow-up time of 5.0 months (CAR-T group, 5.2 months and range 0–12; ASCT group, 4.7 months and range 0–12). The CAR-T group exhibited a higher 1-year OS rate than the ASCT group (74.4% vs. 44.5%, $P = 0.044$, Figure 2A) but not PFS (53.5% vs. 38.4%, $P = 0.225$, Figure 2B). When analyzing the survival rates in patients who responded to CAR-T or ASCT, the OS rates were 84.8% and 70.1% ($P = 0.386$), and the PFS rates were 59.2% and 70.7% ($P = 0.777$), respectively. Subgroup analysis of patients with IPI score of at least 3 revealed higher PFS and OS rates in the CAR-T group than in the ASCT group (OS: 75.0% vs. 13.3%, $P = 0.001$; PFS: 46.6% vs. 13.3%, $P = 0.020$; Figure 2, C and D).

Adverse events. The safety analysis included all 29 and 27 patients in the CAR-T and ASCT groups, respectively. Grade 3 or higher treatment-related adverse events (AEs; referred to as severe adverse events, or SAEs) developed in 48.1% of patients in the CAR-T group and in 20.7% in the ASCT group. AEs of special interest are summarized in Table 3, and all AEs are shown in Supplemental Tables 3 and 4. The most common therapy-associated SAEs in the CAR-T group were cytokine release syndrome (CRS) of grade 3 or higher (20.7%), infection (13.8%), and neurotoxicity (10.3%). In contrast, in the ASCT group, the most common therapy-associated SAEs were cytopenia (100%), gastrointestinal toxicity (48.1%), and infection (40.7%). Additionally, organ damages were rare and mild in both groups. Most toxicities resolved after supportive care in both groups. In summary, the incidence of nonhematologic SAEs was markedly lower in the CAR-T group than in the ASCT group (20.7% for CAR-T, 48.1% for ASCT, $P = 0.030$).

Infections. Infections were observed in both CAR-T and ASCT groups as a shared type of AEs. Four (13.8%) patients in the CAR-T group and 11 (40.7%) in the ASCT group developed an infection. Infection incidence in the ASCT group was higher than in the CAR-T group ($P = 0.023$). Pulmonary infections were the most common infections in both treatment groups. No patient died from infection in the CAR-T group, whereas 2 patients died in the ASCT group (1 died from sepsis and the other died from toxic myocarditis due to pulmonary infection). It suggested that under similar nursing and supportive treatment conditions, the infection rate in the CAR-T group was lower than in the ASCT group.

Hematologic toxicities. Hematologic toxicities were ASCT-specific AEs of importance. Twenty-seven (100%) patients in the ASCT group experienced grade 3 or higher hematologic toxicities in the form of myelosuppression-related AEs. Most patients had hematopoietic reconstitutions and the toxicities resolved over time. However, 2 patients died before the recovery of absolute neutrophil count (ANC) and platelet (PLT) count. During the myelosuppression periods, bleeding occurred in 3 patients (1 patient had hematemesis, 1 had bloody stools, and 1 had hemoptysis) and was resolved by supportive care. The median time from ASCT to neutrophil engraftment (ANC $\geq 0.5 \times 10^9/l$) was 10 days (range 8–15) and to platelet engraftment (PLT count $\geq 20 \times 10^9/l$ without PLT support) was 12 days (range 9–25).

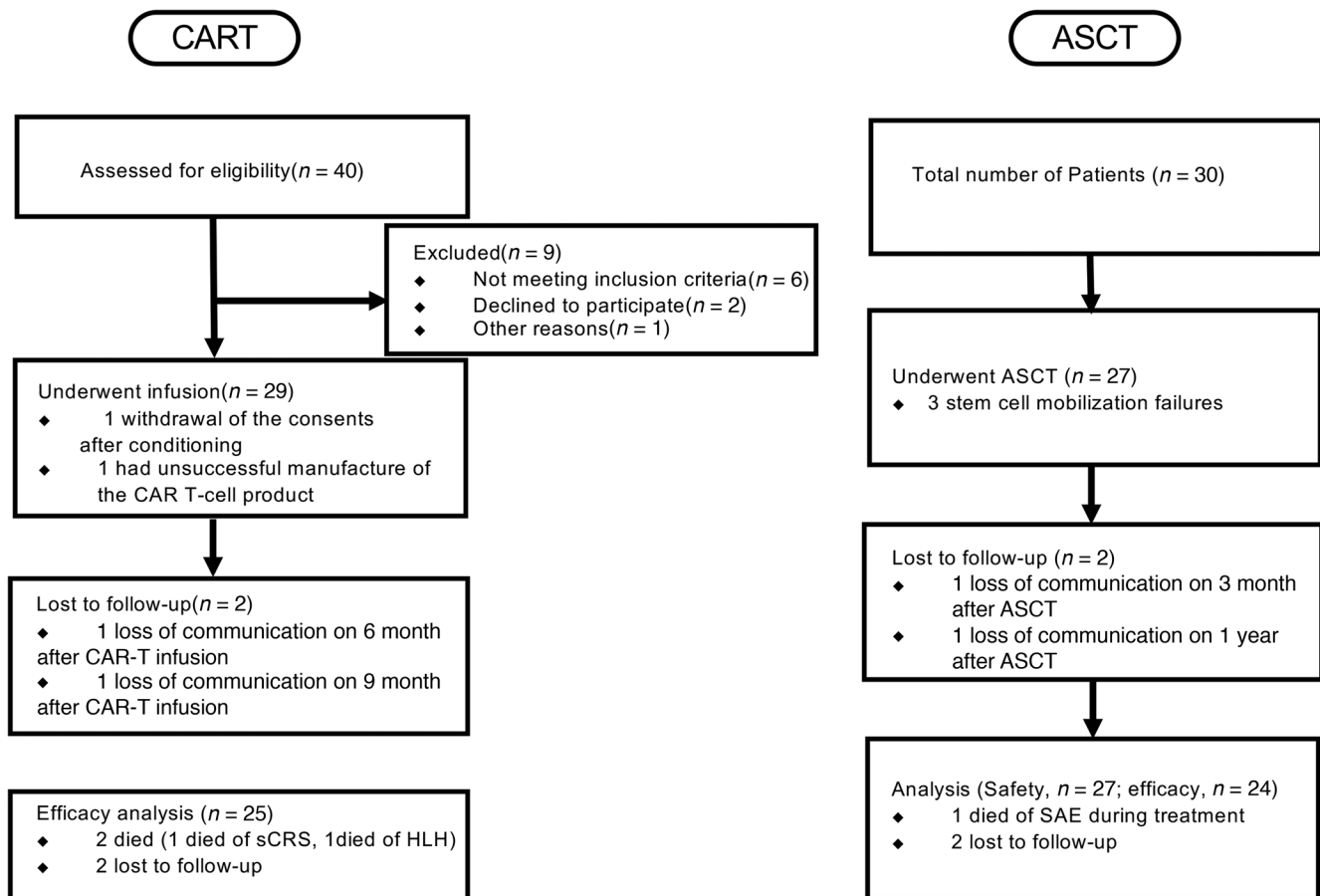


Figure 1. Flow diagrams of the patients. Status of enrolled patients in the CAR-T group and ASCT group. HLH, Hemophagocytic Lymphohistiocytosis.

CRS and neurotoxicity. CRS and neurotoxicity were CAR-T-specific AEs. CRS occurred in 23/29 patients (79.3%) in the CAR-T group, including 17/29 (58.6%) patients assessed as grade 1 or 2 and 6/29 (20.7%) as grade 3 or higher. The most common AEs related to severe CRS were pyrexia (20.7%), hypotension (13.8%), and hypoxia (10.3%). The median time from the first infusions of CAR-T cells to CRS was 3 days (range 1–20), and the median time to resolution was 4 days. Seven of 23 patients received tocilizumab and 3/23 received glucocorticoids for management of CRS. Most CRS cases ameliorated gradually within 2 weeks after supportive care and tocilizumab or glucocorticoids. One patient died from irreversible, severe CRS.

Neurologic events occurred in 3 patients (10.3%) in the CAR-T group; all 3 patients were assessed as having grade 3 or higher neurotoxicity. The most common neurologic events were confusion (10.3%) and aphasia (6.8%). The median time from the first infusions of CAR-T cells to neurotoxicity was 12.5 days (range 9–19). Two of 3 patients' neurotoxic events resolved within 1 week with no treatment, and remaining patient died from an unrelated reason (CRS-associated heart dysfunction).

Death. Nineteen deaths occurred in both treatment groups. Six deaths (20.7%) occurred in the CAR-T group, and the causes were disease relapses and progressions (3 patients), severe CRS (1 patient), tumor lysis syndrome (1 patient), and cerebral hemorrhage because of thrombocytopenia (1 patient). Thirteen deaths (48.1%) occurred in the ASCT group, and the causes were disease progressions (9 patients) and infections and other complications (4 patients). Early deaths that occurred within 1 month in the 2 groups were mostly relapse unrelated and due to irreversible, severe complications, such as CRS, infection, and organ dysfunction. The major causes of death switched to disease progressions or relapses beyond 1 month in both groups, which also constituted the main cause of mortality of the whole study.

Multivariate analysis. Cox models with forward variable selection were constructed for PFS and OS, including all clinical characteristics shown in Table 1. The only factor significantly associated with PFS was

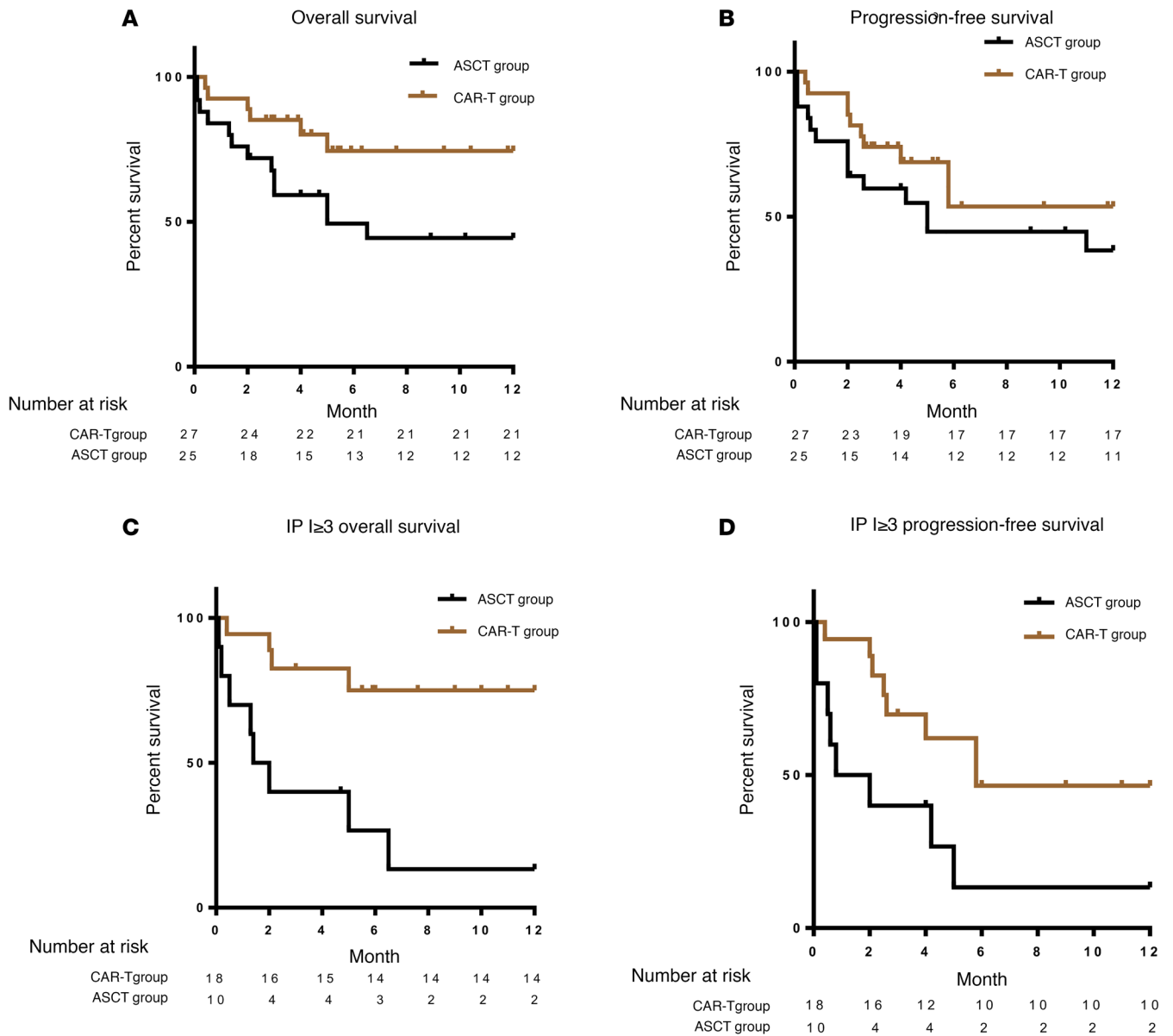


Figure 2. Kaplan-Meier estimates of the progression-free survival and OS. The 1-year overall survival (OS) and progression-free survival (PFS) in the 2 groups (CAR-T group, $n = 29$; ASCT group, $n = 27$). (A and B) OS in the CAR-T group was higher than in the ASCT group based on results of the log-rank test (74.4% vs. 44.5%, $P = 0.044$), while no statistically significant difference was achieved in PFS based on results of the log-rank test (53.5% vs. 38.4%, $P = 0.225$). (C and D) OS and PFS results are shown in subpopulations of patients with IPI scores of at least 3 (CAR-T group, $n = 20$; ASCT group, $n = 11$). Significantly higher OS and PFS were observed in the CAR-T group than in the ASCT group based on results of the log-rank test (OS: 75.0% vs. 13.3%, $P = 0.001$; PFS: 46.6% vs. 13.3%, $P = 0.020$).

elevated LDH level (95% CI 0.085–0.732; $P = 0.012$). Additionally, CAR-T therapy (95% CI 0.090–0.641; $P = 0.004$) was an independent favorable factor and elevated LDH level (95% CI 0.048–0.578; $P = 0.005$) was an independent unfavorable factor for OS (Table 4). Analysis results with no statistical significance are shown in Supplemental Table 2. Furthermore, a binary logistic regression analysis also confirmed that receiving CAR-T rather than ASCT was an independent favorable impact factor in CR (95% CI 0.052–0.870; $P = 0.031$). Patient baseline characteristics, prior lines of chemotherapy, and disease status had no significant impact on OS or CR in the 2 groups using multivariate analyses (data not shown).

Discussion

Patients with primary R/R NHL had limited therapeutic options and poor prognosis. Although being a standard salvage therapy for R/R B-NHL, ASCT was not a universally satisfying strategy in clinical efficacy. Vose et al. reported that the CR rate in patients with diffuse aggressive NHL who had never achieved

Table 3. AEs of special interest

	CAR-T (n = 29)		ASCT (n = 27)	
	Any grade	Grade ≥3	Any grade	Grade ≥3
Any AE	25 (86.2)	6 (20.7)	25 (92.6)	13 (48.1)
Pyrexia	22 (75.8)	6 (20.7)	11 (40.7)	2 (7.4)
Fatigue	2 (6.9)	0 (0)	4 (14.8)	0 (0)
GI (vomiting)	3 (10.3)	0 (0)	7 (24.1)	5 (18.5)
GI (diarrhea)	2 (6.9)	0 (0)	13 (48.1)	9 (33.3)
GI (mucositis/stomatitis)	0 (0)	0 (0)	14 (51.9)	12 (44.4)
Hepatic (ALT/T-BIL)	3 (10.3)	1 (3.4)	9 (33.3)	(0)
Cr increased	7 (24.1)	2 (6.9)	4 (14.8)	(0)
Hypotension	5 (17.2)	4 (13.8)	3 (11.1)	3 (11.1)
Hypoxia	3 (10.3)	3(10.3)	3(11.1)	3 (11.1)
Epilepsy	1(3.4)	0(0)	1(3.7)	1(3.7)
Aphasia	1(3.4)	1(3.4)	0(0)	0(0)
Dysphonic disorder	1(3.4)	1(3.4)	0(0)	0(0)
Cognitive disturbance	1(3.4)	0(0)	0(0)	

Group values presented as *n* (percentage). GI, gastrointestinal; ALT/T, alanine aminotransferase; Cr, creatinine.

CR before ASCT was 26% (11). EBMT Lymphoma Working Party reported that the OS and PFS were 29% and 22%, respectively, for patients with chemotherapy-resistant diseases before ASCT (12). In contrast, excellent response rates of CAR-T19 therapy for R/R B-NHL reported in recent years attracted clinicians' attention. Kochenderfer et al. reported that the CR rate of autologous CAR-T cells targeting CD19 in patients with chemotherapy-refractory NHL was 53.3% (22). Moreover, the ZUMA-2 CAR-T19 trial, which enrolled 111 patients with B cell lymphoma, reported an ORR and CR rate of 82% and 54%, respectively (23). All these results indicated that CAR-T therapy might be a competitive therapeutic strategy with, if not superior than, ASCT for salvage treatment of patients with R/R B-NHL.

Independent reports revealed the respective clinical responses and AEs of CAR-T and ASCT against NHL (16, 21, 22, 26, 27). However, no direct comparison between the 2 therapies was performed in a clinically equivalent condition. We hypothesized that CD19-targeted CAR-T would achieve similar clinical efficacy as ASCT in patients with R/R B-NHL, with a better feasibility and safety profile. Based on this hypothesis, we performed a prospective, single-arm study of CAR-T therapy in patients with R/R B-NHL and compared the outcomes with patients who received contemporaneous HSCT at our institution. A total of 56 patients were analyzed for treatment efficacy and safety.

We demonstrated that CAR-T therapy exhibited improved CR and OS over ASCT in patients with statistically identical demographic characteristics. Indeed, we reported 48.0% versus 20.8% CR rate and 74.4% versus 44.5% 1-year OS rate in the CAR-T and ASCT groups, respectively. Moreover, CAR-T therapy displayed more sustained duration of remission and survival than ASCT in a long-term (>6 months) pattern. These results emphasized CAR-T therapy was a potentially more promising novel therapy and might be a better therapeutic option in some cases of R/R B-NHL than ASCT.

CAR-T also exhibited superior clinical efficacy over ASCT in a subpopulation analysis of patients with IPI scores of at least 3. Previous studies revealed that the IPI score was an unfavorable factor of prognosis associated with poor survival for patients with NHL (28–30). In our study, we demonstrated that IPI score was an independent unfavorable factor for OS and PFS in the ASCT group but not in the CAR-T group. Further analysis showed that the ASCT group exhibited lower response and survival rates than the CAR-T group (10.0% vs. 72.2% for ORR; 13.3% vs. 75% for 1-year OS). The differences in efficacy were more pronounced in this subpopulation of patients with IPI scores of at least 3 than in the total population. The mechanism for these differences was not fully understood. Possible reasons for the poor outcome for patients with high IPI scores in the ASCT group include (a) high IPI scores often associate with bone marrow involvements of the diseases, an adverse prognostic factor of ASCT Guglielmi et al. (31) proposed, whereas CAR-T therapy is seemingly less influenced by bone marrow involvements; and (b) patients with high IPI scores often exhibit lower response rates to salvage chemotherapies, leading to substantially negative impacts on subsequent

Table 4. Multivariate analysis of OS risk factors

Variable	Relative risk of OS (95% CI)	P value
Elevated LDH level	0.166 (0.048–0.578)	0.005
CAR-T vs. ASCT	0.241 (0.090–0.641)	0.004

ASCT, whereas the efficacy of CAR-T is much less dependent on the response to preconditioning chemotherapies. Moskowitz et al. have reported lymphoma patients with IPI scores of 3 and 4 had worse treatment efficacy than those with IPI scores of 2 and 3 (28). Actually, the clinical efficacies of both ASCT and CAR-T in our study decreased in patients with IPI score of at least 3, whereas the drop in the ASCT group was more dramatic than in the CAR-T group, leading to an apparent enlargement of the differences in efficacy between the 2 therapies. Additionally, we also observed that the patients with high IPI scores who received CAR-T therapy had fewer AEs and SAEs than those receiving ASCT.

We also observed CAR-T therapy was effective in patients who had relapsed after HSCT. Schuster et al. revealed that CAR-T therapy was effective in patients who had relapsed after HSCT (32). Similarly, in our study, 35 patients with post-HSCT relapse achieved CR and 2/5 patients achieved PR. All 3 patients who achieved CR maintained remission till the most recent follow-up. Of the 2 PR patients, 1 died from disease progression, and the other died from intracranial hemorrhage caused by aplastic anemia. CAR-T demonstrated good efficacy for patients who had relapsed after HSCT, a very challenging subgroup of patients as reported by other groups.

Our data indicated that toxicities associated with CAR-T were relatively moderate and manageable. The incidence of severe (grade 3 or higher) AEs was markedly lower in the CAR-T group than in the ASCT group, indicating a generally mild toxicity pattern and improved safety profile of CAR-T therapy. Infection was a shared AE associated with both therapies, which could be life-threatening in certain circumstances. Our data demonstrated that the infection rate was much lower in the CAR-T group under similar nursing and supportive treatment conditions. The reason for this difference in infection rate may be related to higher rates of neutropenia in ASCT induced by preconditioning chemotherapy and subsequent disturbance to the host immune system, which is consistent with previous reports of CD19-targeted CAR-T in ALL (33). Last, hematologic toxicities and CRS/neurotoxicity are disease-specific AEs of importance in ASCT and CAR-T, respectively. The management of these AEs partially determined the clinical feasibilities of the 2 therapies and usually required special medical interventions. Also, the comparison showed a lower incidence of disease-specific AEs in the CAR-T group than in the ASCT group.

Obtained with an aim to facilitate decision-making of therapeutic strategies in R/R B-NHL, our data exhibited several advantages of CAR-T over ASCT. First, CAR-T therapy is potentially applicable to a wider range of patients, including those with advanced age, stem cell mobilization failure, advanced disease stage, and relapse after prior HSCT. Second, CAR-T therapy is expected to induce higher response rates than ASCT in certain patient subgroups, such as those with high IPI scores or those who were expected to be unresponsive to preconditioning chemotherapy. Last, CAR-T therapy demonstrates better clinical feasibility and can be performed in regular hematologic wards or even as an outpatient, which may shorten hospital stay and reduce cost.

Our study has several limitations. B-NHL is a group of heterogeneous malignancies consisting of multiple subtypes with different clinical characteristics, prognosis, and responsiveness to certain treatments. Thus, results may vary among different subgroups, which is not fully demonstrated in detail in our study. Additionally, the disease exhibits a multirefractory nature after prior therapies, and abnormalities in genomics, immunomics, and epigenomics were not fully assessed in our study, such as tumor heterogeneity, microenvironment, and other factors, which may affect clinical efficacy of either or both therapies. Also, some types of bias may exist, considering that we are comparing patients in a CAR-T trial with contemporaneous ones receiving ASCT as standard therapy rather than a 2-cohort randomized controlled trial. Therefore, our findings need to be further validated by extended clinical trials with increased sample size and well-designed cohorts and subgroups. Furthermore, there are reports of subpopulations of relapsed/refractory leukemia patients who had short durations

of remission and early relapses after CAR-T treatment (34, 35). Although neither previous reports about CAR-T against lymphoma nor our study exhibited high early relapsed rates like those in leukemia, some patients, especially those with high tumor burdens and highly invasive lymphoma subtypes, progressed after CAR-T therapy in our study. It is worthwhile to characterize this subgroup of patients and study whether they need CAR-T/HSCT sequential therapy or other combinations of therapies to improve the long-term efficacy.

In summary, our data provide clinical evidence that CAR-T exhibited better clinical responses and safety patterns in treating R/R B-NHL compared with ASCT and thereby improved clinical benefits to such a group of patients. The results indicated that CAR-T therapy would be a competitive therapeutic strategy with, if not superior than, ASCT for salvage treatment of patients with R/R B-NHL with expectations of better safety and efficacy and fewer limitations of patient and hospital conditions, which might facilitate decision-making in the treatment of R/R B-NHL. Future multicenter clinical trials with larger sample sizes are warranted.

Methods

Patients

We performed a prospective, single-arm study of CAR-T therapy in patients with R/R B-NHL at the First Affiliated Hospital of Soochow University between March 2017 and September 2018. The study was registered on ClinicalTrials.gov (NCT03196830). At the same time, patients who had experienced HSCT at our institution were used as controls. All the patients from either the CAR-T or ASCT group were treated consecutively, and all eligible patients with R/R B-NHL from March 2017 to September 2018 (29 in the CAR-T group, 27 in the ASCT group) were analyzed. Patients were diagnosed based on histopathologic examinations and scored according to the IPI, and the clinical stages were defined according to the Ann Arbor clinical staging and ECOG performance status of 0–2. Relapse was defined as the appearance of any new lesion or increase by 50% in the size of previously involved sites after a CR (36). Refractory disease was defined as not achieving at least a PR after chemotherapy (>4 cycles of the first-line therapy or >2 cycles of later lines of therapies) or as disease relapse within 1 year of ASCT (4, 14).

Inclusion and exclusion criteria

Patients in the ASCT groups were from regular clinical practice according to the consensus on HSCT (37). All patients treated between March 2017 and September 2018 were included. Patients in the CAR-T group were selected according to a series of inclusion and exclusion criteria. The inclusion criteria were (a) patients with biopsy-confirmed R/R B-NHL; (b) age from 18 to 70; (c) ≥ 2 prior lines of therapies; (d) no severe organ dysfunction (heart, lung, liver, kidney, etc.); (e) complete blood count results of hemoglobin ≥ 80 g/l, Neutrophilic Granulocyte (NE) $\geq 1 \times 10^9/l$, and PLTs $\geq 50 \times 10^9/l$; (f) expected survival of >3 months; and (g) measurable lesions with long diameters ≥ 1.5 cm. The exclusion criteria were (a) uncontrolled active infection; (b) active HIV, HBV, or HCV infection; (c) previous history of malignancies other than NHL; and (d) pregnant or lactating females. Additionally, patients in the ASCT group needed to have at least $2 \times 10^6/kg$ CD34⁺ stem cells collected from them, referred to as successful stem cell mobilizations.

Study design

The treatment procedure in the CAR-T group consisted of autologous leukapheresis, conditioning chemotherapy, infusions of CAR-T19 cells, and follow-up. Patients underwent leukapheresis to obtain PBMCs for ex vivo CAR-T manufacture and then received conditioning chemotherapy of fludarabine ($30 \text{ mg/m}^2 \times 3$ days) and cyclophosphamide ($300 \text{ mg/m}^2 \times 3$ days) on days -5 , -4 , and -3 . CAR-T19 cells were administrated intravenously in doses ranging from 5×10^6 to 10×10^6 cells/kg of body weight. (Treatment protocols are shown in Supplemental Figure 1A.)

The treatment protocol in the ASCT group has been previously published (38–40). Briefly, the source of hematopoietic progenitor cells was the autologous peripheral blood of each patient. Key regimens for stem cell collection were disease-specific chemotherapies plus granulocyte colony-stimulating factor, and the conditioning regimens included BEAM (carmustine, etoposide, cytarabine, and melphalan) and BU/CY (busulfan, cyclophosphamide) treatment. Stem cell collections were performed for 30 patients, and 27 of them were successful, and the number of collected CD34⁺ cells ranged from 2.2×10^6 to 7.9×10^6 cells/kg (median 2.9×10^6 cells/kg). Treatment protocols are shown in Supplemental Figure 1B.

CAR-T manufacture

Autologous T cells were isolated from apheresis blood by gradient centrifugation and enriched using anti-CD3 magnetic beads (Miltenyi Biotec catalog 130-097-043). T cells were then stimulated with anti-CD3 (Miltenyi Biotec catalog 170-076-116) and anti-CD28 (Miltenyi Biotec catalog 170-076-117) monoclonal antibodies and transduced with lentiviral vectors, manufactured by UniCar Therapy Ltd., and CD3- ζ intracellular domains. CAR-T cells were cultured in AIM-V media (Thermo Fisher Scientific) supplemented with 10% autologous human serum (UniCar Therapy Ltd.), 100 IU/ml IL-2 (PeproTech), 5 ng/ml IL-7 (PeproTech), and 5 ng/ml IL-15 (PeproTech) for 9–12 days.

Measurements of clinical endpoints

Efficacy. Responses were assessed by imaging via computed tomography or positron emission tomography and evaluated according to 2007 Revised Response Criteria for Malignant Lymphoma (41). Bone marrow biopsies were performed in patients with bone marrow infiltrations. ORR was defined as CR plus PR of the best response achieved after CAR-T or ASCT. PFS was defined as the duration from the administration of CAR-T or ASCT to disease progression, relapse, or death (whichever occurred first). OS was defined as the duration from the administration of CAR-T or ASCT to death due to any reason.

Safety. AE reports were collected from the first day of preconditioning chemotherapy to 30 days after CAR-T or ASCT treatment. AEs were graded according to the Common Terminology Criteria for Adverse Events v 5.0. Two CAR-T-related AEs, neurotoxicity and CRS, were evaluated using the Penn scale (42). Deaths and possible causes were recorded and therapy-related deaths were further analyzed.

Hematopoietic engraftment. Neutrophil engraftment was defined as an ANC of at least $0.5 \times 10^9/l$ on the first day of 3 consecutive days with no subsequent decline. PLT engraftment was defined as a PLT count of at least $20 \times 10^9/l$ on the first day of 3 consecutive days without the support of PLT transfusion.

Statistics

Demographic and other baseline data were presented as frequencies and percentages. Proportions were compared using χ^2 test or Fisher's exact test, and quantitative variables were compared using the Mann-Whitney U test. Logistic regression models were used to evaluate whether baseline factors of subpopulations influenced the clinical responses. The probabilities of OS and PFS were calculated by the Kaplan-Meier method and compared using a log-rank test. The Cox regression model was used to perform multivariate analyses on survival outcome variables. AEs in the 2 groups were compared using the χ^2 test. All quoted P values were 2 sided, and P values less than 0.05 were considered statistically significant. All statistical analyses were conducted using SPSS Version 24.0 (SPSS Inc).

Study approval

This study was conducted according to the principles of the Declaration of Helsinki and with the approval of the Institutional Ethics Committee of the First Affiliated Hospital of Soochow University. All participants provided written informed consent.

Author contributions

CL, YZ, CZ, and XL designed the protocol and analyzed data; CL, YZ, JC, XC, JZ, ZY, XZ, PW, TX, CQ, HH, ZJ, and DW participated in the treatment of the patient; CL, YZ, CZ, and XL wrote and edited the manuscript; DW and LY contributed equally to this study. CL, YZ, CZ, JC, XL, XC, LK, NX, ML, JT, XS, JZ, ZY, XZ, PW, TX, CQ, HH, ZJ, LY, and DW read and approved the manuscript.

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