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

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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 contemporaneous 27 patients who received ASCT. NHL was diagnosed by histopathological assessments, and the safety and efficacy were compared.

RESULTS. The CAR-T group exhibited better rates of CR (48.0% vs. 20.8%, P=0.046) and one-year OS (74.4% vs. 44.5%, P=0.044) compared with the ASCT group. Subpopulation analysis showed that patients with IPI scores ≥ 3 achieved significantly higher ORR and CR rates in the CAR-T group than in the ASCT group (ORR: 72.0% vs. 10.0%, P=0.002; 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 non-hematologic severe adverse events (SAEs) was markedly lower in the CAR-T group than in the ASCT […]

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RUNNING TITLE

Auto-transplantation versus CAR-T in lymphoma treatment

AUTHOR

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Authorship note: D. W. and L. Y. are co–senior authors.

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MANUSCRIPT

Abstract

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CONCLUSION. CAR-T therapy exhibited superior clinical outcomes in safety and efficacy over ASCT in patients with R/R B-NHL, suggesting 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-NHL, therapeutic strategies for R/R B-NHL remain inadequate. Approximately 30% to 40% R/R B-NHL patients relapse after initial therapies, and another 10% develop treatment-refractory diseases, leading to dismal prognosis (1-3). Actually, a multicohort retrospective non-Hodgkin lymphoma research (SCHOLAR-1) showed that the objective and complete response rates for R/R B-NHL patients were only 26% and 7%, respectively, with a median overall survival 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 utilized as a standard salvage treatment in the past 20 years in R/R B-NHL with approximately 30% to 45% patients remaining progression-free 3 years after transplantation (5-9). However, several disadvantages limit the clinical benefits of ASCT in R/R B-NHL patients. Nearly half of R/R B-NHL patients 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 to ASCT and the expected long-term 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). Lastly, post-ASCT relapse happened in about 60% R/R B-NHL patients, and those patients hardly 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-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 (scFv)

domain fused to intracellular domain consisting of a co-stimulatory regions of 41BB or CD28 and a CD3zeta region are the most common form in clinical use. Several clinical trials demonstrated dramatic outcomes of CAR-T19 in adult and pediatric patients of relapsed or 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 of R/R B-NHL receiving CAR-T19 with objective response rates 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 reconstitutions of host immunological surveillance and long-term remissions. Nevertheless, CAR-T exhibited 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 pre-conditioning 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 R/R B-NHL patients when ASCT is not available. Indeed, it is claimed that CAR-T may be a possible candidate of standard therapeutic strategy for R/R B-NHL besides ASCT.

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 R/R B-NHL patients than ASCT.

Results

Patient Characteristics. Between March 2017 and September 2018, a total of 56 patients were treated and analyzed, including 29 in the CAR-T group and 27 in the ASCT group. Patients' baseline characteristics are shown in Table 1. Disease assessments for both groups immediately before treatments revealed that 82.8% and

48.1% patients were assessed as 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 between the two groups. CAR-T group showed a tendency of more patients with advanced ages (≥60), high IPI scores (baseline characteristics of patients with IPI scores≥ 3 were shown in Supplemental Table 1), poor prognosis from prior treatments and advanced disease stages (stage 3 or 4). Additionally, 5 patients in the CAR-T group were post-hematopoietic stem cell transplantation (HSCT) relapsed, including four relapsed after ASCT and one after allogeneic hematopoietic stem cell transplantation (allo-HSCT). The five patients of 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 the 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 patients expired before reaching the primary efficacy endpoint or lost to follow-up. Complete responses were achieved in 12 (48.0%) of 25 patients in the CAR-T group compared with 5 (20.8%) of 24 patients in the ASCT group (P=0.046; Table 2). Objective response 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 ORR and CR rates in the CAR-T group than in the ASCT group were observed in subgroup analysis of patients with IPI scores ≥ 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 achieving PR till the latest follow-up, while the rest 4/6 PR patients experienced disease progression in a median time of 5.3 months. In contrast, in the ASCT group, five patients achieved CR , and 4 of 5 maintained in remission and the rest one patients died from multiple organ dysfunction syndrome. Disease progressions were observed in 9/24 patients in ASCT, including 3 of 6 patients who had PR and another

6 patients who had SD with a median duration of 2.7 months (individual durations of remission were shown in Supplemental Figure 2). These results suggested that a higher proportion of patients in the CAR-T group achieved complete responses, 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 rest one patient achieved PR. The only one patient with prior allo-HSCT achieved PR after CAR-T treatment, and died from cerebral hemorrhage because of thrombocytopenia on Month 2 after CAR-T infusion. These results indicated that CAR-T might work as an salvage therapy for patients relapsing after stem cell transplantation with comparable efficacy to regular R/R B-NHL patients.

Survival. PFS and OS were analyzed and compared between the two groups with a median follow-up time of 5.0 months (CAR-T group, 5.2 months [range 0–12]; ASCT group, 4.7 months [range 0–12]). The CAR-T group exhibited a higher one-year overall survival (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 $\geq$ 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 including all 29 and 27 patients in the CAR-T and ASCT groups, respectively. 48.1% patients in the CAR-T group and 20.7% in the ASCT group developed grade 3 or higher treatment-related adverse events (referred to as severe adverse events, or SAEs), respectively. AEs of special interest were summarized in Table 3, and all AEs were shown in Supplemental Table 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 non-hematologic 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).

Infection. Infections were observed in both CAR-T and ASCT groups as a shared type of AE. Four (13.8%) patients in the CAR-T group and 11 (40.7%) in the ASCT group developed 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, while two patients died in the ASCT group (one 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 hematological toxicities in the form of myelosuppression-related AEs. Most patients had hematopoietic reconstitutions and the toxicities resolved over time except that two patients died before the recoveries of absolute neutrophil count (ANC) and platelet (PLT). During the myelosuppressive periods, bleeding occurred in three patients (one patient had hematemesis, one had bloody stools, and one had hemoptysis) and 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 $\geq 20 \times 10^9$/L without platelet support) was 12 days (range 9–25).

Cytokine release syndrome and neurotoxicity. CRS and neurotoxicity were CART-specific adverse events. CRS occurred in 23/29 patients (79.3%) of 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 adverse events 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 resolutions was 4 days. Seven of 23 patients received tocilizumab and 3 of 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 grade 3 or higher neurotoxicity. The most common neurologic events were confusion (in 10.3%) and aphasia (in 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 the rest one died from unrelated reason (due to 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), infections and other complications (4 patients). Early deaths occurred within 1 month in the two groups were mostly relapse unrelated due to irreversible severe complications such as CRS, infection and/or organ dysfunction. The major causes of death switched to disease progressions or relapses beyond 1 month in both groups, which also constituted the main portion of mortality of the whole studies.

Multivariate Analysis. Cox models with forward variable selection were constructed for PFS and OS, including all clinical characteristics shown in Table 1. The only factors significantly associated with PFS were 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 3). Analytic results with no statistical significance were shown in Supplementary Table 2. Furthermore, a binary logistic regression analysis also confirmed that receiving CAR-T rather than ASCT was an independent favorable impact factors on 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 two groups using multivariate analyses (data not shown).

Discussion

Patients with primary refractory or relapsed non-Hodgkin lymphoma were accompanied by 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 never achieved CR before ASCT was 26% (11). Southwest Oncology Group Trials reported that the OS and PFS were 29% and 22% 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' attentions. James N 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 CART-19 trial which enrolled 111 patients with B-cell lymphoma reported objective and complete response rates of 82% and 54%, respectively (23). All these results indicated that CAR-T therapy might be a competitive, if not superior, therapeutic strategy with ASCT for salvage treatments of patients with R/R B-NHL.

Independent reports revealed the respective clinical responses and adverse events of CAR-T and ASCT against NHL (16,21,22,24,25). However, no direct comparison between the two therapies was performed in a clinically equivalent condition. We hypothesized that CD19 CAR-T would achieve similar clinical efficacy as ASCT in

patient of R/R B-NHL with better feasibility and safety profiles. 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 contemporaneous patients who received HSCT at our institution. A total of 56 patients were analyzed for 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% of CR rates and 74.4% versus 44.5% of one-year OS rates in the CAR-T and ASCT groups, respectively. Moreover, CAR-T therapy displayed more sustained durations 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 subpopulation analysis of patients with IPI scores ≥ 3. Previous studies revealed that the International Prognostic Index (IPI) score was an unfavorable factor of prognosis associated with poor survival for NHL patients (26-28). 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 one-year OS). The differences in efficacy were more pronounced in this subpopulation of patients with IPI scores ≥ 3than in total population. The mechanism for these differences was not fully understood. Possible reasons of the poor outcome for patients with high IPI scores in the ASCT group include: (1) High IPI scores often associate with bone marrow involvements of the diseases, an adverse prognostic factor of ASCT proposed by Gugliemi et al. (29), while CAR-T therapy is seemingly less influenced by bone marrow involvements. (2) Patients with high IPI scores often exhibit lower response rates to salvage chemotherapies leading to

281 significantly negative impacts on subsequent ASCT, while the efficacy of CAR-T is
282 much less dependent on the responsiveness of pre-conditioning chemotherapies.
283 Moskowitz et al. have reported lymphoma patients with IPI scores of 3 and 4 had worse
284 efficacy than those with IPI scores of2 and 3 (26). Actually, the clinical efficacies of
285 both ASCT and CAR-T in our study decreased in patients of IPI score ≥ 3, while the
286 drop in the ASCT group was more dramatic than in the CAR-T group leading to an
287 apparent enlargement of the differences in efficacy between the two therapies.
288 Additionally, we also observed that the patients with high IPI scores who received
289 CART therapy had less AEs and SAEs than those receiving ASCT.

290 We also observed CAR-T therapy was effective in patients who relapsed post
291 HSCT. Schuster SJ revealed that CAR-T therapy was effective to patients who
292 relapsed after HSCT (30). Similarly, in our study, 3 of 5 patients of post-HSCT
293 achieved CR and 2/5 patients achieved PR. All three patients who achieved CR
294 maintained in remission till the most recent follow-up. Of the 2 PR patients, 1 died from
295 disease progression and the other died from intracranial hemorrhage caused by aplastic
296 anemia. CAR-T demonstrated good efficacy for patients relapsed after HSCT, a very
297 challenging subgroup of patients as reported by other groups.

298 Our data indicated that toxicities associated with CAR-T were relatively moderate
299 and manageable. The incidence of severe (grade 3 or higher) adverse events was
300 markedly lower in the CAR-T group than in the ASCT group indicating a generally
301 mild toxicity pattern and improved safety profile of CAR-T therapy. Infection was a
302 shared adverse event associated with both therapies which could be life-threatening in
303 certain circumstances. Our data demonstrated that the infection rate was much lower
304 in the CAR-T group under similar nursing and supportive treatment conditions. The
305 reason for this difference in infection rate may be related to higher rates of neutropenia
306 in ASCT induced by pre-conditioning chemotherapy and subsequent disturbance to
307 host immune system, which is consistent with previous reports of CD19 CAR-T in ALL
308 (31). Lastly, hematological toxicities and CRS/neurotoxicity are disease-specific AEs

of importance of ASCT and CAR-T, respectively. The management of these AEs partially determined the clinical feasibilities of the two therapies and usually required special medical interventions. And the comparison showed a lower incidence of disease-specific AEs in the CAR-T group than in the ASCT group.

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. Firstly, 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. Secondly, CAR-T is expected to induce higher response rates than ASCT in certain patient subgroups, such as those with high IPI scores or were anticipated to be unresponsive to pre-conditioning chemotherapy. Lastly, CAR-T therapy demonstrates better clinical feasibility which can be performed in regular hematological wards or even outpatient, which may shorten hospital stay and reduce cost.

Our study has several limitations. B cell non-Hodgkin lymphoma 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 details in our study. Additionally, the disease exhibit multi-refractory nature after prior therapies, and abnormalities in genomics, immunomics and epigenomics are not fully assessed in our study, such as tumor heterogenicity, microenvironment and other factors which may impact clinical efficacy of either or both therapies. And some types of bias may exist considering that we are comparing patients in a CAR-T trial with contemporaneous ones receiving ASCT as standard therapies rather than a two-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 or refractory leukemia patients who had short durations of remission and early relapses after CAR-T

treatment (32,33). 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 high 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 provided 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 group of patients. The results indicated that CAR-T therapy would be a competitive, if not superior, therapeutic strategy to ASCT for salvage treatments of patients with R/R B-NHL with expectations of better safety, efficacy and less 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 size 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 period, patients who experienced HSCT at our institution were used as controls. All the patients from either CART or ASCT group were treated consecutively, and all eligible R/R NHL patients from March 2017 to September 2018 (29 in the CAR-T group, 27 in the ASCT group) were analysed. Patients were diagnosed based on histopathological examinations and scored according to the international lymphoma prognostic index (IPI), and the clinical stages were defined according to the Ann Arbor clinical staging and Oncology Group (ECOG) performance status (PS) 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 (34).

Refractory disease was defined as not achieving at least a partial response after chemotherapy (>4 cycles of the first-line or >2 cycles of later-lines of therapies), or disease relapse within 1 year of autologous stem cell transplant (4,14).

Inclusion and Exclusion criteria. Patients in the ASCT groups were from regular clinical practice according to the consensus on hematopoietic stem cell transplantation (40). All patients treated between March 2017 and September 2018 were included. Patients in the CAR-T groups were selected according to a series of inclusion and exclusion criteria. The inclusion criteria were: (1) Patients with biopsy-confirmed relapsed or refractory B-cell lymphoma; (2) Age from 18 to 70; (3) ≥ Two prior lines of therapies; (4) No severe organ dysfunction (heart, lung, liver, kidney, etc.); (5) CBC results: Hb ≥ 80g/L; NE ≥1 × 10^9/L; PLT ≥50 × 10^9/L; (6) Expected survival of more than 3 months; (7) measurable lesions with long diameters ≥ 1.5 cm. The exclusion criteria were: (1) Uncontrolled active infection; (2) Active HIV, HBV or HCV infection; (3) Previous histories of malignancies other than NHL; (4) Pregnant or lactating females. Additionally, patients in ASCT group need to collect ≥ 2×10^6/kg of CD34-positive stem cells 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-T 19 cells, and follow-up. Patients underwent leukapheresis to obtain PBMCs for ex vivo CAR-T manufacture, and then received conditioning chemotherapy of fludarabine (30 mg/m^2 x 3 days) and cyclophosphamide (300 mg/m^2 x 3 day) on Day −5, −4, −3. CAR-T19 were administrated intravenously in doses ranging from 5.0-10.0x10^6 CAR-T cells per kilogram of body weight. (Treatment protocols was shown in Supplemental Figure 1A)

Treatment protocol in the ASCT group has been previously published (35-37). Briefly, the sources of hematopoietic progenitor cells were autologous peripheral blood of each patient. Key regimens for stem cell collection were disease-specific chemotherapies plus granulocyte colony-stimulating factor (G-CSF), and the conditioning regimens included BEAM and BU/CY treatment. Stem cell collections

were performed for 30 patients and 27 of them were successful, and the number of collected CD34-positive cells ranged from 2.2–7.9 $\times 10^6$/ kg (median 2.9 $\times 10^6$/kg). Treatment protocols was 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 catalog 130-097-043). T cells were then stimulated with anti-CD3 (Miltenyi catalog 170-076-116) and anti-CD28 (Miltenyi catalog 170-076-117) monoclonal antibodies, and transduced with lentiviral vectors encoding CD19-specific CAR with 4-1BB and CD3zeta intracellular domains. CAR-T cells were cultured in AIM-V media (Gibco) supplemented with 10% autologous human serum, 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 (CT) or positron emission tomography (PET) and evaluated according to 2007 Revised Response Criteria for Malignant Lymphoma (38). Bone marrow biopsies were performed in patients with bone marrow infiltrations. Overall response rate was defined as complete response (CR) plus partial response (PR) of the best response achieved after CAR-T or ASCT. Progression-free survival (PFS) was defined as the duration from the administration of CAR-T or ASCT to disease progression, relapse, or death (whichever occurs first). Overall survival (OS) was defined as the duration from the administration of CAR-T or ASCT to death due to any reason.

Safety: Adverse events (AEs) were collected from the first day of pre-conditioning chemotherapy to 30 days after CAR-T or ASCT treatment. AEs were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v 5.0. Two CAR-T-related AEs, neurotoxicity and CRS, were evaluated using Penn scale (39). Deaths and possible causes were recorded and therapy-related deaths were further analyzed.

Hematopoietic engraftment: Neutrophil engraftment was defined as an ANC ≥ 0.5

$\times 10^9$/L on the first day of three consecutive days with no subsequent decline. Platelet engraftment was defined as a PLTc $\geq 20 \times 10^9$/L on the first day of three consecutive days without the support of platelet transfusion.

Statistics. Demographic and other baseline data were presented as frequencies and percentages. Proportions were compared using chi-squared 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 two groups were compared using the chi-squared test. All quoted P values were two-sided, and $P < 0.05$ were considered statistically significant. All statistical analyses were conducted using SPSS Version 24.0 (SPSS Inc, Chicago, IL).

Study approvals. 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 consents.

Author contributions

C. L., Y. Z., C. Z. and X. L. designed protocol and analyzed data; C. L., Y. Z., J. C., X. C., J. Z., Z. Y., X. Z., P. W., T. X., C. Q., H. H., Z. J., and D. W. participated in the treatment of the patient; C. L., Y. Z., C. Z., X.L. wrote and edited the manuscript; D. W., and L. Y. contributed equally to this study. All authors read and approved the manuscript.

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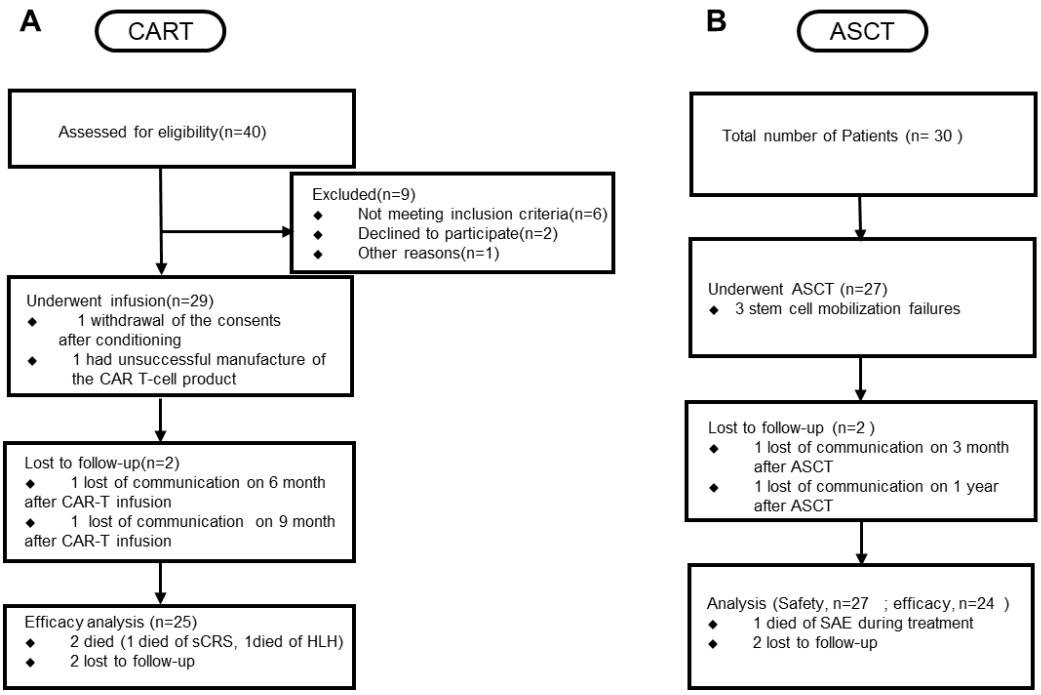
567 **Figure legends**



568

569 **Figure 1. Flow diagrams of the patients.**

570 Status of enrolled patients in the CAR-T group (**A**) and ASCT group (**B**).

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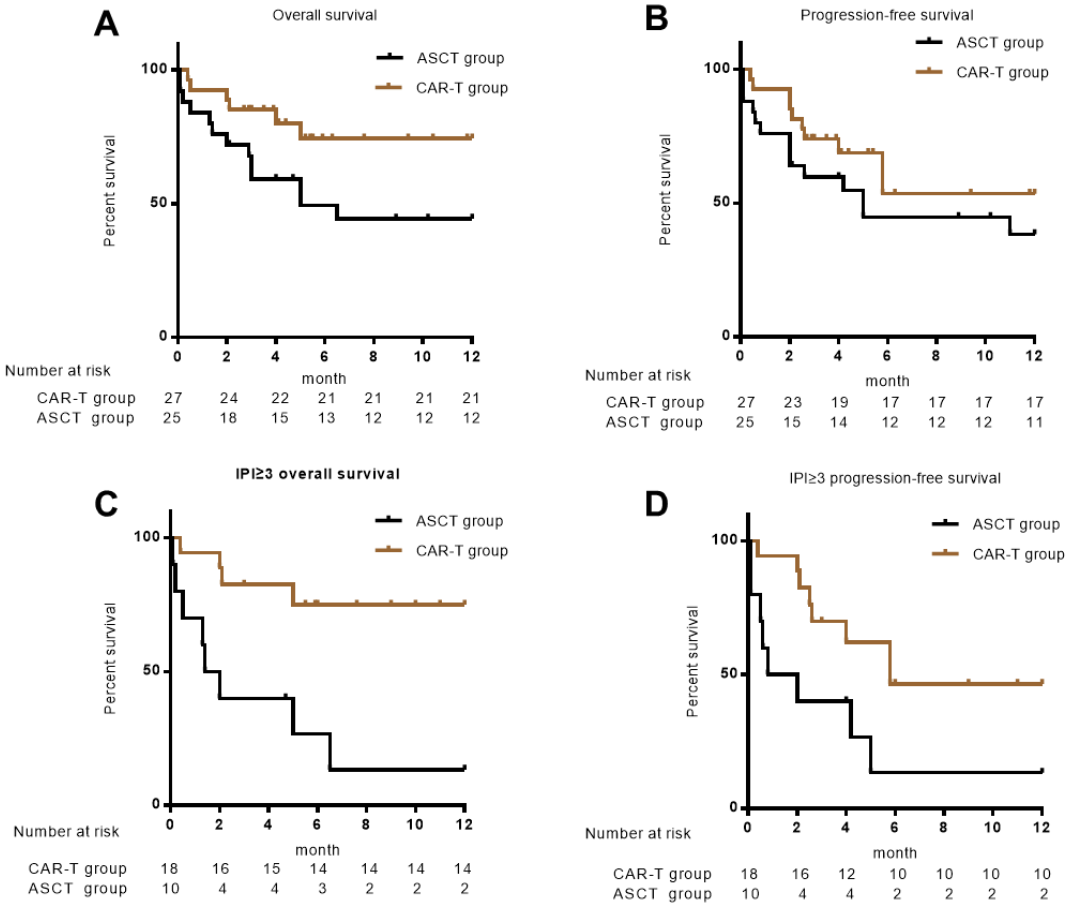

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573 **Figure 2. Kaplan–Meier estimates of the progression-free survival and overall**
574 **survival.**

575 The one-year overall survival (OS) and progression-free survival (PFS) in the two
576 groups (CAR-T group, n = 29; ASCT group, n = 27). (**A** and **B**) OS in CAR-T group
577 was higher than in ASCT group using the log-rank test (74.4% vs.44.5%, P=0.044),
578 while no statistic difference was achieved in PFS using the log-rank test (53.5% vs.
579 38.4% P=0.225). (**C** and **D**) OS and PFS results were shown in subpopulations of
580 patients with IPI scores ≥3 (CAR-T group, n = 20; ASCT group, n = 11). Significantly
581 higher OS and PFS were observed in CAR-T group than in ASCT group using the log-
582 rank test (OS: 75.0% vs.13.3%, P=0.001; PFS: 46.6% vs. 13.3% P=0.020).

583

584

Tables

Table 1. Baseline characteristics of the patients

	CAR-T		Auto-SCT		P Value
	n=29		n=27		
Age and Gender					
median (range)	62(27-70)		52(22-64)		0.015
≥60	13	44.8	4	14.8	
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 1factor)	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	

Abbreviations: FL, follicular lymphoma; DLBCL, diffuse large B-cell; Transformed DLBCL, Transformed diffuse large B-cell; MCL, mantle cell lymphoma,; BL, burkitt lymphoma; MZL, marginal zone lymphoma; CLL, chronic lymphocytic leukemia;CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; IPI, international prognostic index.

Table 2. Clinical response in the two groups

	CAR-T	ASCT	P value
Total (CART 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 (CART 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

[A] Using the chi-square test. [B] Using the Fisher exact test

Table 3. AEs of special interest

	CAR-T (n=29)		Auto-SCT (n=27)	
	Any grade	Grade ≥3	Any grade	Grade ≥3
Any AE, n (%)	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)	1(3.4)	0(0)	0(0)

Table 4. Multivariate analysis of overall survival risk factors

Variable	Relative risk of overall survival (95% Confidence interval)	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

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