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RESEARCH ARTICLE

Different sites of extranodal involvement may affect the survival of patients with relapsed or refractory non-Hodgkin lymphoma after chimeric antigen receptor T cell therapy

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Abstract **Factors associated with complete and durable remissions after anti-CD19 chimeric antigen receptor T (CAR-T) cell immunotherapy for relapsed or refractory non-Hodgkin lymphoma (r/r NHL) have not been well characterized. In this study, we found that the different sites of extranodal involvement may affect response, overall survival (OS), and progression-free survival (PFS) in patients with r/r NHL treated with anti-CD19 CAR-T cells. In a cohort of 32 treated patients, 12 (37.5%) and 8 (25%) patients exhibited soft tissue lymphoma and bone marrow (BM) infiltrations, respectively, and 13 (41%) patients exhibited infiltration at other sites. The factors that may affect prognosis were identified through multivariable analysis. As an independent risk factor, soft tissue infiltration was the only factor significantly correlated with adverse prognosis ($P < 0.05$), whereas other factors did not reach statistical significance. Furthermore, the site of extranodal tumor infiltration significantly and negatively affected OS and PFS in patients with r/r NHL treated with anti-CD19 CAR-T cell therapy. PFS and OS in patients with BM involvement were not significantly different from those of patients with lymph node involvement alone. Thus, anti-CD19 CAR-T cell therapy may improve the prognosis of patients with BM infiltration.**

Keywords anti-CD19 chimeric antigen receptor T cell; soft tissue; bone marrow; relapsed or refractory non-Hodgkin lymphoma

Introduction

Anti-CD19 chimeric antigen receptor T (CAR-T) cell therapy is effective in a significant proportion of patients with $CD19^+$ B cell malignancies. Two CD19-directed CAR-modified T cell immunotherapies, namely, axicabtageneciloleucel and tisagenlecleucel, have been approved by the US Food and Drug Administration for the treatment of B cell non-Hodgkin lymphoma (NHL) after demonstrating high overall response rates (ORRs) of 83% and 52%, with complete remission (CR) rates of 58% and 40%, in pivotal trials, respectively [1,2]. Response rates after treatment with anti-CD19 CAR-T cells manufactured by academic centers have been similarly impressive [3–7]. However, the median progression-free survival (PFS) is only 2.9–5.9 months [1,2,8], and factors that are independently associated with favorable therapeutic effects and long-term remissions or adverse outcomes remain unknown [9].

Extranodal involvement, particularly in soft tissues and bone marrow (BM), is a poor prognostic factor in patients with NHL [10]. We observed that patients with relapsed or refractory (r/r) NHL treated with CD19 CAR-T cells with soft tissue infiltration demonstrated shorter survival times than those without soft tissue infiltration. However, patients with BM infiltration were doing well. Therefore, to examine these observations in detail and determine the prognostic effects of lymphoma in different locations, we analyzed the different patterns of tissue infiltration in patients with r/r NHL treated with anti-CD19 CAR-T cells.

Patients and methods

Patients

We performed analysis for relapsed or refractory $CD19^+$ B

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cell NHL patients treated with anti-CD19 CAR-T cell therapy and retrospectively researched our institutional database. Then, we identified patients treated between October 2015 and July 2018. Patients with r/r NHL were eligible if they had primary refractory disease after at least two lines of therapy, including chemoimmunotherapy, and relapsed disease, and not eligible for autologous hematopoietic stem cell transplantation (HSCT) or had relapsed after HSCT. Between leukapheresis and lymphodepletion chemotherapy, 11 (34%) of 32 patients required systemic bridging chemotherapy for the control of disease progression. All patients with r/r NHL in our study received lymphodepletion chemotherapy with fludarabine (25 mg/m^2, days −5, −4, and −3) and cyclophosphamide (300 mg/m^2, days −5, −4, and −3). A total of 3×10^6/kg anti-CD19 CAR-T cells were administered to each patient intravenously at day 0 in one infusion. The CAR used in this study included a murine anti-CD19 single-chain variable fragment, 4-1BB costimulatory domain, and CD3ζ T cell activation domain. The study was approved by the Institutional Review Board of Tongji Hospital of Tongji University and registered in clintrials.gov (NCT02537977).

Clinical response, relapse, and toxicity assessment

Patients were assessed using computerized tomography (CT) scans before lymphodepletion chemotherapy and 4 weeks and 2, 3, 6, and 12 months after CAR-T cell infusion and by positron emission tomography/CT (PET/CT) before lymphodepletion chemotherapy and 3 months after CAR-T cell infusion, as clinically indicated. BM aspiration and biopsy samples were obtained before lymphodepletion and 4 weeks after the administration of CAR-T cells from patients with BM disease on initial staging. The best responses in the absence of additional antitumor therapy were reported according to the Lugano criteria [11]. Relapse and/or progression were defined according to clinical, radiological, and/or biopsy evaluations.

Statistical analyses

Statistical analysis was performed using SPSS (Version 13.0). We performed a survival analysis that compared primary extranodal sites of the disease. PFS was defined as the time from CAR-T cell infusion to disease progression or death. Overall survival (OS) was defined as the time from CAR-T cell infusion to the date of death from any cause. Kaplan–Meier analyses were used in estimating PFS and OS (median and 95% CIs). Differences in PFS and OS between groups were assessed using the log-rank test. The associations of factors with PFS and/or OS were evaluated with a multivariable Cox regression model. The comparison of the means between the groups was performed using the Student's *t*-test. Statistical significance was defined as a P value of less than 0.05.

Results

Patient characteristics

A total of 32 patients (median age, 55 years; range, 16–71 years) with B cell r/r NHL received lymphodepletion and anti-CD19 CAR-T cell infusion. The patients had diffuse large B cell lymphoma (DLBCL), high-grade B cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements, and other histologic subtypes. They had received a median of nine previous lines of treatment regimens (range, 3–31) and 12.5% (n = 4) had failed previous autologous HSCT (Table 1).

Durable response in patients with r/r NHL achieving CR

All 32 patients were evaluated for response. The best ORR without additional therapy after CAR-T cell infusion was 78% (25/32), and 34% (11/32) of the patients achieved CR. At a median follow-up of 6 months (range, 1–33), 20 patients (62.5%) suffered relapse or disease progression, and 11 patients (34%) died because of disease relapse or progression. The 1 year OS and PFS rates were 51% and 29%, respectively. The median time to the best response was 1 month (range, 1–3 months). Among the histology subtypes, patients with DLBCL had ORR and CR rates of 75% (18/24) and 38% (9/24), respectively. The median OS of patients who achieved CR was 12 months (95% CI, 6.0–22.0), and that of patients who did not achieve CR was 3 months (95% CI, 2.5–8.3 months). The median PFS was 8.0 months (95% CI, 6–22 months) and 2 months (95% CI, 1.0–3.0 months) for those who achieved and did not achieve CR, respectively. A significant difference was observed between the two groups for OS (P = 0.007) and PFS ($P < 0.001$, Fig. 1).

Relationship between prognosis and the invasive sites of tumors

Patients with BM involvement had ORR and CR rates of 87.5% (7/8) and 62.5% (5/8), respectively. Patients with soft tissue involvement had ORR and CR rates of 75% (9/12) and 8% (1/12), respectively. This result indicates a low rate of CR in patients with soft tissue involvement. The percentage reduction rate of sum of the perpendicular diameters (SPD) in patients with soft tissue involvement was 80.4% ± 8.5% (mean ± SD), and the obtained CR or PR was 89.4% ± 2.8% in patients with lymph node involvement. A significant difference was observed between the two groups (P = 0.02).

Table 1 Patient characteristics

Characteristic	No. of patients, *n* (%)
Disease type	
DLBCL	24 (75%)
Non-germinal center origin (GCB)	10 (31%)
Mantle cell lymphoma (MCL)	4 (12.5%)
Follicular lymphoma IIIb	1 (3.1%)
Other aggressive lymphomas	3 (9.4%)
Age	
⩾65	10 (31%)
<65	22 (69%)
Sex	
Male	20 (62.5%)
Female	12 (37.5%)
LDH pre-lymphodepletion above the upper limit of normal	19 (59%)
International prognostic index (IPI)	
0–1	16 (50%)
2	8 (25%)
3	4 (12.5%)
4–5	4 (12.5%)
Disease stage	
I or II	8 (25%)
III or IV	24 (75%)
Extranodal disease	
Bone marrow	8 (25%)
Soft tissue	12 (37.5%)
Others (gastrointestinal tract, pleura, adrenal gland, others)	13 (41%)
Previous therapy	
Nine or more previous lines of therapy	20 (62.5%)
Previous autologous HSCT	
Y	4 (12.5%)
N	28 (87.5%)

As indicated in Table 2, patients with soft tissue involvement exhibited worse prognoses than those with BM, lymph node, or other site involvement. A statistically significant difference was found in OS and PFS between patients with different site involvements ($P = 0.005$ and $P = 0.037$, respectively; Fig. 1). Therefore, a multivariable analysis was performed to examine prognostic factors in more detail.

Multivariable analysis of factors affecting PFS and OS in r/r NHL

In the multivariable Cox regression analysis of clinical and treatment characteristics, the probabilities of OS and PFS were shorter in patients with soft tissue infiltration than in patients without soft tissue infiltration (Table 3). As an independent risk factor, soft tissue infiltration was the only factor significantly correlated with adverse prognosis ($P < 0.05$). Other factors did not reach statistical significance, including LDH level, BM infiltration, CD19 expression in peripheral blood, lymph node infiltration, other site infiltration, and SPD for target lesions.

The expression of CD19 in peripheral blood was analyzed. A total of 18 patients with CD19 lower than 0.1% in peripheral blood had ORR and CR rates of 72% (13/18) and 28% (5/18), respectively. Patients with CD19 higher than 0.1% in peripheral blood had ORR and CR rates of 86% (12/14) and 36% (5/14), respectively. The median OS and PFS of patients with CD19 lower than 0.1% were 6.5 months (95% CI, 3.0–9.0 months) and 3 months (95% CI, 1.5–3.5 months), respectively. The median OS and PFS of patients with CD19 higher than 0.1% in peripheral blood were 5 months (95% CI, 2.2–10.5 months) and 3.5 months (95% CI, 2–6 months), respectively. No significant difference in CD19 expression was found between the two groups for OS ($P = 0.208$) and PFS ($P = 0.945$, Fig. 2). Therefore, the levels of CD19 expression might not influence the prognosis.

Discussion

This report describes the clinical and laboratory factors that can influence response and long-term outcomes in patients with $CD19^+$ B cell r/r NHL treated with anti-CD19 CAR-T therapy. In summary, 32% of the patients achieved CR, which was associated with prolonged PFS. However, we have observed that patients with BM involvement had better OS and PFS than patients with soft tissue involvement, who generally had a poorer prognosis.

An initial soft tissue presentation of a lymphoproliferative neoplasm occurs rarely. The soft tissue involvement commonly occurs through the direct extension from lymph nodes or other extranodal structures or by hematogenic

Table 2 OS and PFS of invasive tumor sites

	OS (95% CI, month)	PFS (95% CI, month)
Bone marrow	13 (4.5–29.0)	8 (1.7–28.7)
Other sites	6 (3.3–19.6)	5 (2.6–19.2)
Lymph node	6 (4.6–13.0)	3 (2.3–10.9)
Soft tissue	3 (2.3–6.8)	2 (1.3–4.0)

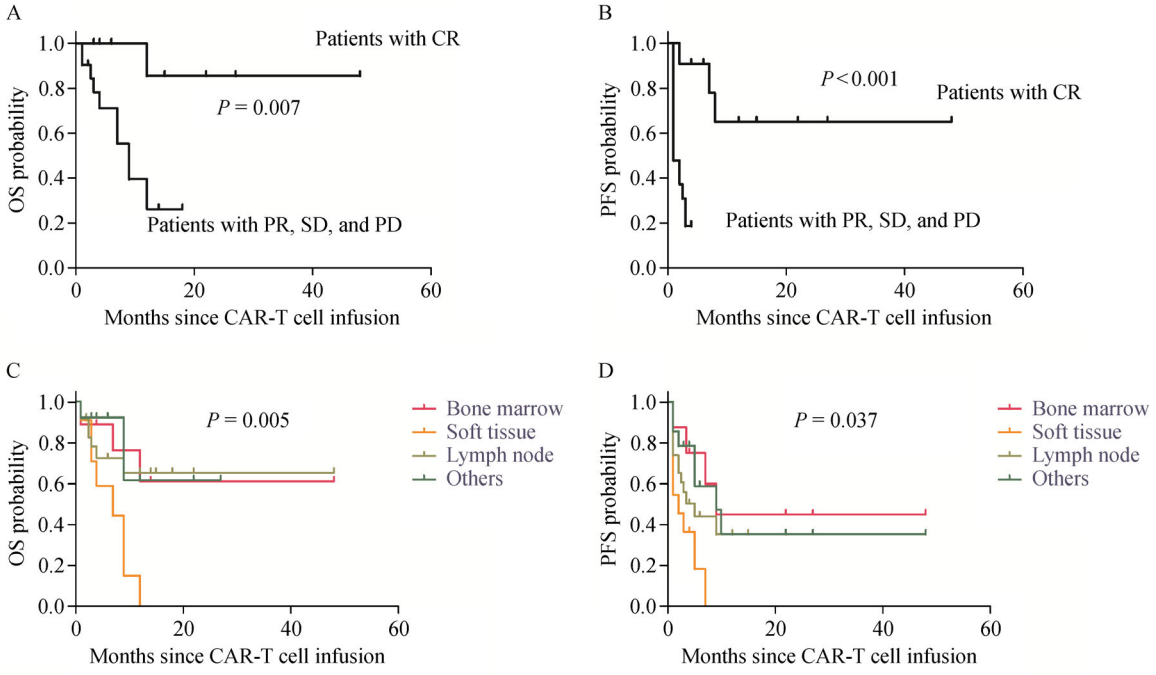

Fig. 1 PFS and OS in patients with r/r NHL after anti-CD19 CAR-T cell therapy. Kaplan–Meier estimates of OS (A) and PFS (B) in patients with NHL who achieved CR and did not achieve CR. The Kaplan–Meier estimates of OS (C) and PFS (D) in patients with bone marrow, soft tissue, lymph node, and others involvement.

Table 3 Multivariable analysis of factors affecting OS and PFS in patients with r/r NHL

Variable	OS			PFS		
	P	Hazard ratio	95% CI	*P*	Hazard ratio	95% CI
LDH*	0.807	1.164	0.344–3.942	0.606	1.254	0.531–2.961
Soft tissue	0.004	13.788	2.344–81.106	0.006	5.006	1.584–15.823
Bone marrow	0.352	0.522	0.133–2.051	0.532	0.683	0.207–2.256
Lymph node	0.748	0.751	0.131–4.297	0.302	1.804	0.588–5.533
Other sites	0.303	0.501	0.135–1.865	0.327	0.613	0.230–1.631
SPD	0.507	1.227	0.671–2.243	0.278	1.299	0.810–2.083

* Pre-lymphodepletion.

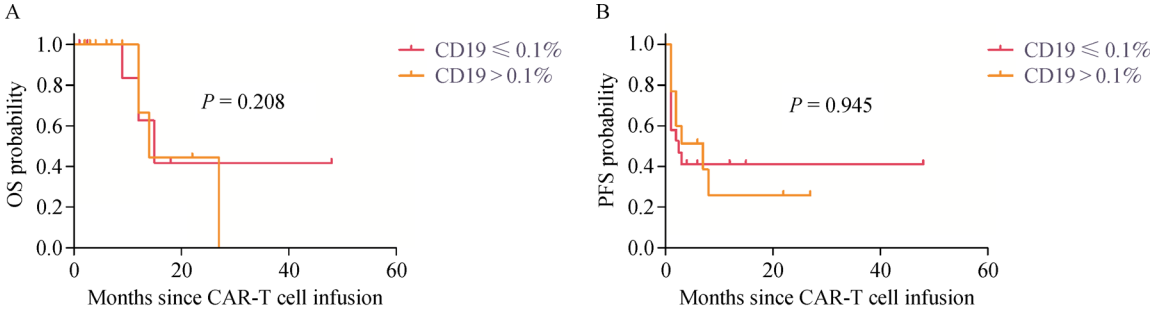

Fig. 2 Kaplan–Meier estimates of OS (A) and PFS (B) in patients with high and low expression of CD19 in peripheral blood.

dissemination [12]. The prognosis of patients with the DLBCL of soft tissues is generally poor, confirming the aggressiveness of the soft tissue clinical presentation, particularly during extranodal sites. In the meta-analysis by Derenzini *et al*. [13], 83 patients with primary NHL lymphoma with soft tissue involvement were analyzed. Actuarial OS and PFS rate were 44% and 47%, respectively. Basing on the outcome according to different histological subtypes, they observed a poor outcome for DLBCL. PFS and OS were 34% and 38%, respectively. In a study by Suárez *et al*. [14], the 3-year disease-specific survival of patients with leg tumors and multiple skin lesions were 43% and 39%, respectively. In our study of CAR-T cell therapy in r/r NHL, no patient with primary soft tissue B cell lymphoma was found. Specifically, 6 of 12 (50%) patients with soft tissue involvement had non-GCB DLBCL, and 2 of 12 patients (17%) had aggressive lymphoma. In summary, our study showed that 78% and 34% of the patients achieved ORR and CR, respectively. However, patients who developed soft tissue involvement had ORR and CR rates of 75% (9/12) and 8% (1/12), respectively. The CR rate was significantly low. Patients with extranodal site involvement and those with soft tissue involvement had similar response rates to CAR-T cell therapy, but only one patient achieved CR and relapsed within 6 months. Therefore, patients with soft tissue involvement may require additional treatment after CAR-T cell therapy or accept hematopoietic stem cell transplantation to improve outcome.

A study by Yao *et al*. [15] included 263 *de novo* DLBCL patients with positive BM involvement and 449 patients with negative BM. The data showed that in the rituximab era, concordant BM involvement demonstrates poorer OS and PFS compared with advanced DLBCL with negative BM. The 5-year OS of patients with concordant BM involvement is 42.2%, which was significantly worse than patients with DLBCL with negative BM (5-year OS: 67.7%) and advanced DLBCL with negative BM (5-year OS: 57.2%). In this study, we found that the survival rates of patients with BM infiltration were similar to those of patients with lymph node infiltration only and were significantly higher than those of patients with soft tissue infiltration. Therefore, CAR-T cell therapy may overcome adverse prognostic factors associated with BM infiltration. Kochenderfer *et al.* [16] detected the expression of CAR gene in the BM cells of one patient with CLL and one patient with FL, with 0.03% and 0.13% of the BM cells measured by qPCR, respectively. The patient with CLL exhibited the CAR gene 14 weeks after CAR-transduced T cell infusion, and the patient with FL showed the CAR gene 8 weeks after CAR-transduced T cell infusion. The best effect of the disease was PR in two patients after the infusion of CAR-T cells. However, given the small sample size in this study, additional data from larger clinical trials are required to further confirm our hypothesis.

In conclusion, our study provides the first evidence that the prognosis of patients with BM infiltration may be improved by the therapeutic effect of CAR-T cell therapy. However, soft tissue infiltration significantly and negatively affects OS and PFS in patients with r/r NHL treated with CAR-T cell therapy.

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Compliance with ethics guidelines

Lili Zhou, Ping Li, Shiguang Ye, Xiaochen Tang, Junbang Wang, Jie Liu, and Aibin Liang declare no conflict of interest. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the *Helsinki Declaration* of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

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