Background aims: The combination therapy of autologous hematopoietic stem cell transplantation (ASCT) and chimeric antigen receptor T-cell (CART) therapy has been employed to improve outcomes for relapsed or refractory (R/R) B-cell non-Hodgkin-lymphoma (B-NHL). The widely used conditioning regimen before ASCT plus CART therapy reported in the literature was carmustine, etoposide, cytarabine and melphalan (BEAM). However, whether adding fludarabine to the BEAM regimen (BEAMF) can improve the survival of patients with R/R B-NHL remains unknown.

Methods: In total, 39 and 19 patients with R/R B-NHL were enrolled to compare clinical outcomes in the BEAM and BEAMF regimens before ASCT plus CD19/22 CART therapy, respectively.

Results: The objective response (OR) rates at 3 months to BEAM and BEAMF regimens before ASCT plus CART therapy were 71.8% and 94.7%, respectively ($P = 0.093$). The BEAMF regimen showed a trend towards a superior duration of response compared with the BEAM regimen ($P = 0.09$). After a median follow-up of 28 months (range: 0.93–51.9 months), the BEAMF regimen demonstrated superior 2-year progression-free survival (PFS) (89.5% versus 63.9%, respectively; $P = 0.09$) and 2-year overall survival (OS) (100% vs 77.3%; $P = 0.035$) compared with the BEAM regimen. In the multivariable Cox regression analysis, OR at month 3 (responders) was remarkably correlated with better OS (hazard ratio: 0.112, $P = 0.005$) compared with OR (non-responders).

Conclusions: For patients with R/R B-NHL, the BEAMF regimen before ASCT plus CD19/22 CART therapy was correlated with superior PFS and OS than the BEAM regimen, and the BEAMF regimen is a promising alternative conditioning regimen for ASCT plus CAR-T therapy.

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Key Words: autologous stem cell transplantation, B-cell non-Hodgkin-lymphoma, chimeric antigen receptor T-cell conditioning regimen, relapsed or refractory

Introduction

Autologous stem cell transplantation (ASCT) is considered the standard therapeutic option for chemosensitive relapsed/refractory (R/R) B-cell non-Hodgkin lymphoma (B-NHL) following high-dose chemotherapy [1,2]. However, it is estimated that only 50% of patients with R/R B-NHL are considered suitable for ASCT, and about 50% of patients who undergo ASCT still eventually relapse. Nevertheless, patients resistant to chemotherapy in this cohort had a complete
response (CR) rate of only 15% [3–5]. For patients who relapsed within 12 months after ASCT, only 34% responded to remedial chemotherapy, with a median survival of only 6.2 months [6]. Thus, there is an urgent need to discover new treatment options for patients with R/R B-NHL who benefit less from standard ASCT.

Recently, chimeric antigen receptor T-cell (CART) therapy has shown tremendous success in patients with R/R B-NHL, with objective response (OR) rates of 52–82% and CR rates of 40–58% [7–10]. However, 35–60% of patients who achieved CR underwent relapse after CART therapy during the first 1–2 years [7,9,11]. To pursue a greater response rate and durable remission, combined CART with ASCT has been explored. In 2016, the City of Hope group reported that 16 patients with B-NHL received first- or second-generation central memory—derived CD19 CART therapy following ASCT. Thirteen of the 16 patients achieved partial remission (PR) or CR and had a 1-year progression-free survival (PFS) of 50% and 75%, respectively. The persistence of CART expansion was >28 days [12]. The MD Anderson Cancer Center group reported that six of the seven subjects, including various histologies and three who were negative on fluoro-deoxyglucose–positron emission tomography (FDG-PET) testing, remained in CR at a median follow-up exceeding 2 years following ASCT plus CART therapy [13]. Wang et al. [14] reported a sequential infusing ASC and CD19 CART study that the OR rate (ORR) was 78.57%, with a median duration of PFS of 14.82 months and with a PFS rate of 64.29% at 6 months. The main reason for the remarkable success of the combined ASC and CART therapy was myeloablative conditioning. The immunosuppressive microenvironment and tumor volume were reduced due to myeloablative conditioning. Furthermore, myeloablative conditioning could deeply deplete lymphocytes that inhibit CART function. Then, CART therapy during hematopoietic reconstruction could enhance the function of CART, thereby eradicating residual disease and reducing the relapse rate after ASCT [12,14]. Therefore, the conditioning regimen before ASCT plus CART therapy plays an important role in the high remission rate and durable remission. The commonly employed conditioning regimens before ASCT plus CART cell therapy reported in the literature are carbustine, etopo-side, cytarabine and melphalan (BEAM) [12,15,16]. Studies showed that fludarabine, a widely used lymphodepletion agent, increased CART accumulation and persistence, thereby increasing disease-free survival [17]. Whether fludarabine can be applied in the conditioning regimen before ASCT plus CART therapy to obtain the best CART amplification and efficacy requires further study.

Herein, we compared the prognostic differences between BEAM (n = 39) and carbustine, etopo-side, cytarabine, melphalan and fludarabine (BEAMF) (n = 19) conditioning regimens before ASCT plus CD19/22 CART therapy in 58 patients with R/R B-NHL. Furthermore, we also analyzed the patients’ adverse events, responses and survival. Our results revealed that the BEAMF regimen demonstrated superior PFS and overall survival (OS) compared with the BEAM regimen.

Methods

Patients and study design

We retrospectively analyzed the patients with R/R B-NHL treated with CD19/22 CART immunotherapy following ASCT with BEAM or BEAMF conditioning regimen at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology from March 31, 2019, to July 12, 2022. Eligible patients were R/R to their previous treatments (therapy lines ≥2). All patients were diagnosed with B-NHL based on the World Health Organization classification of tumors of hematopoietic and lymphoid tissues. The positive dual expression of CD19 and CD22 in the malignancy was confirmed via flow cytometry or immunohistochemistry. The cut-off value for CD19 and CD22 positive expression was 80% or the mean fluorescence intensity was greater than 5000. The conditioning regimen included the BEAM and BEAMF protocols. Inclusion criteria were good Eastern Cooperative Oncology Group performance status (≤2), measurable disease, essentially normal organ function and a life expectancy of 12 weeks or longer. Patients with uncontrollable infections were excluded. Informed consent was obtained by eligible participants according to the Declaration of Helsinki. This clinical study (ChiCTR-OPN-16009847) was approved by the institutional review board of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. The authors obtained consent from the participants to publish the individual patient data.

All enrolled subjects underwent two separate apheresis procedures, including the collection of autologous stem cells induced by granulocyte colony-stimulating factor and lymphocyte apheresis for CART manufacturing. The third-generation CAR used in our study consists of a single-chain variable fragment derived from mouse monoclonal antibodies against human CD19 or CD22, two co-stimulatory domains from CD28 and 4-1BB and the CD3ζ chain as the activation domain. The quality control and analysis related to CART manufacturing were done by Wuhan Bio-Raid Biotechnology Co., Ltd., as previously mentioned [18]. After the successful production of CART, a standard BEAM or BEAMF conditioning regimen was given before the infusion of autologous stem cells. The BEAM regimen consisted of carmustine 300 mg/(m2·d) for day –7, etoposide 200 mg/(m2·d) from days –6 to –3, cytarabine 400 mg/(m2·d) from days –6 to –3 and melphalan 140 mg/(m2·d) for days –2. The BEAMF regimen was based on the BEAM regimen with the addition of fludarabine 25 mg/(m2·d) from days –5 to –3. According to our previous clinical study with the sequential infusion of CD19/22 CART cells following ASCT (ChiCTR-OPN-16009847), we have chosen to use cell doses ranging from (1.0 to 10) × 10^6/kg CD19 CART T cells and (1.0 to 10) × 10^6/kg CD22 CART T cells, that have been determined to be safe and effective in patients with B-NHL. The CD19/22 CART cells were infused within the range of 2 to 6 days (d2 to d6) after autologous stem cell infusion (d0). CD22 CART infusion was typically administered 1 day earlier than CD19 CART infusion, given patient tolerability.

Treatment response and toxicity assessment

Neutrophil engraftment was defined as the absolute neutrophil count >0.5 × 10^9/L for 3 consecutive days. Platelet engraftment was defined as a platelet count >20 × 10^9/L for 7 consecutive days without transfusion support. The response assessments were defined according to the US National Comprehensive Cancer Network guidelines and the Lugano Treatment Response Criteria [19]. Computed tomography or FDG-PET at baseline (pre-ASCT) and post-ASCT at 3, 6, 12 and 24 months were performed to confirm the treatment response. The grades of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) were evaluated based on the American Society of Transplantation and Cellular Therapy [20]. Adverse events were assessed according to the US National Cancer Institute Common Terminology Criteria for Adverse Events v5.0. The expansion of CART in vivo was determined by droplet digital polymerase chain reaction technology at multiple time points after CART infusion [21]. Lymphocyte subsets, including B cells (CD19+), Th cells (CD3+CD4+), Ts cells (CD3+CD8+), natural killer cells (CD3−CD16+56+), NK cells (CD3+CD16+56+) and regulatory T cells (CD4+CD25+CD127−) were detected and quantified using flow cytometry. Absolute counts of various cells in lymphocyte subsets were calculated by multiplying the percentage by the total number of lymphocytes. The level of ferritin in the serum samples was detected by immunoturbidimetry. The chemiluminescence method was used to detect the expression level of interleukin-6 in the serum samples. The cutoff date for data collection was June 30, 2023.
Statistical analysis

All statistical analyses were performed with SPSS 25.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 8.0 (San Diego, CA, USA). Continuous variables were described as medians and ranges, and categorical variables as frequencies and percentages. χ² or Fisher exact test was used to assess categorical variables, and a nonparametric test or independent two-sample t-test was used to evaluate continuous variables between subgroups. PFS was defined as the time from the first CART infusion to disease progression or death. OS was defined as the time from the first CART infusion to death. PFS and OS were censored at the final follow-up. Kaplan–Meier curves were used to estimate the probability rates of PFS and OS, and between-group comparisons were determined by the log-rank test. Univariate Cox regression analysis included only variables with P < 0.1. P values < 0.05 were treated as statistically significant.

Results

Patient characteristics

A total of 61 patients with R/R B-NHL were treated with CD19/22 CART immunotherapy following ASCT with BEAM or BEAMF conditioning regimen in Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology from 2019 to 2022. Three patients with severe infection were excluded from this study. Therefore, 58 patients with R/R B-NHL were enrolled in this study: 39 patients treated with the BEAM conditioning regimen before ASCT plus CD19/22 CART cell therapy (Figure 1).

There were no significant differences in baseline clinical characteristics between BEAM and BEAMF regimen groups (Table 1). The median age of the BEAM and BEAMF groups was 44 years (range: 19–63 years) and 41 years (range: 27–62 years), respectively (P = 0.285). In total, 89.7% and 78.9% of the BEAM and BEAMF groups were of advanced stage, respectively (P = 0.476). Extra-nodal organ involvement was detected in 84.6% and 63.2% of the BEAM and BEAMF groups, respectively. TP53 mutation or chromosome 17p deletion [del(17p)] was seen in 20.5% and 31.6% of the BEAM and BEAMF groups, respectively. In total, 76.9% and 89.5% of the BEAM and BEAMF groups underwent four or more previous lines of therapy, respectively. In addition, 92.3% and 89.5% of the BEAM and BEAMF groups of patients remained stable disease/progressive disease before undergoing ASCT, respectively. No significant difference in the median dose of infused CD22 and CD19 CART was observed between the BEAM and BEAMF groups. The median number of infused CD34+ cells was 4.95 × 10⁶/kg (range, 1.2–27.6 × 10⁶/kg) and 4.43 × 10⁶/kg (range, 1.78–12.19 × 10⁶/kg) in the BEAM and BEAMF groups, respectively (P = 0.855). The median number of days it took to achieve platelet engraftment was 14 days (range, 8–28 days) and 15 days (range, 11–23 days) in the BEAM and BEAMF groups, respectively (P = 0.205). The median number of days it took to achieve platelet engraftment was 14 days (range: 7–62 days) and 14 days (range: 7–41 days) in BEAM and BEAMF groups, respectively (P = 0.816). The median red blood cell transfusions in the BEAM and BEAMF groups was 2 (range: 0–11) units (P = 0.34). The median platelet transfusions in the BEAM and BEAMF groups were 4 (range: 2–12) and 5 (range: 3–15) apheresis platelets, respectively (P = 0.338). The median hospitalization for the BEAM and BEAMF groups were 28 (range: 21–50) and 28 (range: 22–54) days, respectively (P = 0.584).

Toxicity

All adverse events occurring within 30 days of ASCT plus CD19/22 CART therapy were graded (Table 2). All patients had grade 3 or 4 cytopenia due to the myeloablative conditioning regimen. The most common grade 1 or 2 toxicities among all patients were fever (n = 51; 87.9%), hypalbuminemia (n = 44; 75.9%), hypocalcemia (n = 37; 63.8%), diarrhea (n = 35; 60.3%), hyponatremia (n = 33; 56.9%), prolonged activated partial thromboplastin time (n = 31; 53.5%), hypoxia (n = 30; 51.7%), decreased appetite (n = 27; 46.6%), hypokalemia (n = 26; 44.8%) and nausea (n = 25; 43.1%). The grade 3
At 3 months' post-treatment, 46 of the 58 patients obtained the OR to ASCT plus CD19/22 CART therapy, including 34 patients with CR and 12 patients with PR. The ORRs (CR + PR) at 3 months for the BEAM and BEAMF groups were 71.8% and 94.7%, respectively ($P = 0.093$, Figure 2C). At the last follow-up, four cases in CR status experienced disease progression. Furthermore, 32 patients sustained remission for more than 20 months. A total of six patients died of disease progression, and one patient died of severe infection. The median follow-up time was 28 months (range: 0.93–51.9 months) and the 2-year OS and PFS for all patients were 85.5% and 72.7%, respectively (Figure 2A,B). In addition, the median duration of response (DOR), PFS and OS for the BEAM and BEAMF groups had not been reached at the cut-off date of June 30, 2023 (Figure 2D–F).

Patients in the BEAMF group demonstrated better OS than those in the BEAM group (hazard ratio [HR]: 0.195; 95% confidence interval [CI]: 0.048–0.762; $P = 0.048$, Figure 2E). There was a trend toward a superior DOR in the BEAMF group compared with the BEAM group (HR: 0.199; 95% CI: 0.052–0.762; $P = 0.09$, Figure 2D). To determine the risk factors influencing PFS and OS in patients with R/R B-NHL treated with ASCT plus CD19/22 CART therapy, we performed univariate and multivariate Cox regression analysis of clinical characteristics, including conditioning regimen, gender, age, Ann Arbor stage, International Prognostic Index score, Eastern Cooperative Oncology Group score, tumor mass, extra-nodal organ

toxicities among all patients were oral mucositis ($n = 13$; 22.4%), hypotension ($n = 10$; 17.2%), y-gamma-glutamyl transferase increase ($n = 8$; 13.8%), and fever ($n = 5$; 8.6%). Except for cytopenia, no grade 4 adverse events occurred. Grade 2 hypoxia ($n = 6$ [31.6%] versus 3 [7.7%]; $P = 0.02$), peripheral edema ($n = 5$ [26.3%] versus 2 [5.1%]; $P = 0.025$) and aspartate transaminase (AST) increase ($n = 7$ [36.8%] versus 3 [7.7%]; $P = 0.035$) were more common in the BEAMF group compared with the BEAM group. Furthermore, 47 of 58 patients experienced grade 1 or 2 CRS, whereas 10 (17.2%) developed grade 3 CRS. Eight patients (13.8%) suffered from ICANS, of which 12.1% were grade 1–2 and 1.7% were grade 3. No significant difference in the incidence rates of CRS and ICANS was observed between the BEAM and BEAMF groups. Methylprednisolone and tocilizumab were administered to patients with grade 2–3 CRS and ICANS. No patient died of non reversible severe CRS/ICANS. No significant differences in the peak concentrations of serum interleukin-6 and ferritin were observed between the BEAM and BEAMF groups (supplementary Figure 1A,B).

Response and survival

At 3 months' post-treatment, 46 of the 58 patients obtained the OR to ASCT plus CD19/22 CART therapy, including 34 patients with CR and 12 patients with PR. The ORRs (CR + PR) at 3 months for the BEAM and BEAMF groups were 71.8% and 94.7%, respectively ($P = 0.093$, Figure 2C). At the last follow-up, four cases in CR status experienced disease progression. Furthermore, 32 patients sustained remission for more than 20 months. A total of six patients died of disease progression, and one patient died of severe infection. The median follow-up time was 28 months (range: 0.93–51.9 months) and the 2-year OS and PFS for all patients were 85.5% and 72.7%, respectively (Figure 2A,B). In addition, the median duration of response (DOR), PFS and OS for the BEAM and BEAMF groups had not been reached at the cut-off date of June 30, 2023 (Figure 2D–F). Patients in the BEAMF group demonstrated better OS than those in the BEAM group (hazard ratio [HR]: 0.195; 95% confidence interval [CI]: 0.043–0.895; $P = 0.035$, Figure 2F). The estimated 2-year PFS rate for patients in the BEAM and BEAMF groups was 63.9% (95% CI: 45.7–76.6%) and 89.5% (95% CI: 64.1–96.7%), respectively ($P = 0.048$, Figure 2E). There was a trend toward a superior DOR in the BEAMF group compared with the BEAM group (HR: 0.199; 95% CI: 0.052–0.762; $P = 0.09$, Figure 2D).

To determine the risk factors influencing PFS and OS in patients with R/R B-NHL treated with ASCT plus CD19/22 CART therapy, we performed univariate and multivariate Cox regression analysis of clinical characteristics, including conditioning regimen, gender, age, Ann Arbor stage, International Prognostic Index score, Eastern Cooperative Oncology Group score, tumor mass, extra-nodal organ
involved, TP53 alterations, previous treatment lines, lactate dehydrogenase level, ICANS and CRS grade. Only variables with \( P < 0.1 \) were included in the multivariable Cox regression analysis. The results indicated that lactate dehydrogenase level was significantly associated with PFS of RR B-NHL in univariate analysis (supplementary Figure 2A). However, no clinical factors influenced PFS in the multivariable Cox regression analysis (supplementary Figure 2B). OS was remarkably correlated with maintained OR at month 3 (R3m) (responders) in univariate and multivariate analysis (supplementary Figure 2C). Thus, R3m was an independent risk factor closely associated with the OS of RR B-NHL. To further explore the impact of ASCT plus CD19/22 CART therapy on RR B-NHL, we evaluated the survival differences among subgroups. Patients with age greater than 65 years demonstrated superior PFS and OS than the patients with age \( \leq 65 \) years \( (P = 0.035) \) (Figure 3A,B). In addition, patients with R3m (responders) displayed extended PFS and OS than the patients who failed to achieve R3m (non-responders) \( (P < 0.05) \) (Figure 3C).

**CART kinetics and lymphocyte subsets**

The median peak copies of expansion for CD19 and CD22 CART in the blood were \( 5436 \) (range: \( 63–59215 \)) and \( 9254 \) (range: \( 523–219194 \)) copies/\( \mu \)g DNA, respectively. The median times to reach peak expansion for CD19 and CD22 CART in the blood were 7 (range: \( 1–18 \)) and 7.5 (range: \( 1–22 \)) days, respectively. The lentivirus copies in BEAM and BEAMF groups are presented in Figure 4A–F. The median peak levels of CD19 CART and CD22 CART lentivirus copies were \( 5508 \) (range: \( 63–59215 \)) and \( 4828 \) (range: \( 523–92419 \)) copies/\( \mu \)g DNA, respectively, in BEAM group, whereas it was \( 9551 \) (range: \( 640–48224 \)) and \( 8957 \) (range: \( 650–219194 \)) copies/\( \mu \)g DNA, respectively, in BEAMF group (Figure 4A,B). The kinetics and expansion of CD19 and CD22 CART were similar in the BEAM and BEAMF groups (Figure 4C–F). Three months after CART infusion, CD19 and CD22 CART were still detectable in 15 (25.9%) and 9 (15.5%) patients, respectively.

Lymphocyte subsets in peripheral blood showed a trend towards higher Tregs in the BEAM group compared with the BEAMF group at 2 months after CART infusion \( (P = 0.065, \text{Figure 5A, supplementary Figure 3C}) \). The ratio of Tregs to Th cells was greater in the BEAM group compared with the BEAMF group at 2 months after CART infusion \( (P = 0.044, \text{Figure 5B}) \). Th and natural killer T-cell counts at 3 months after CART infusion were greater in the BEAMF group than in the BEAM group \( (P < 0.05) \) (supplementary Figure 3A,B). Ts, natural killer and B-cell counts were not different in the BEAM and BMEAF groups within 6 months after CART infusion (supplementary Figure 3D–F).

**Discussion**

This study retrospectively analyzed the prognostic differences between BEAM and BEAMF conditioning regimens before ASCT plus CD19/22 CART therapy in 58 patients with RR B-NHL. The results indicated a trend towards a greater ORR at 3 months and superior DOR in the BEAMF group than the BEAM groups. The BEAMF group showed superior 2-year PFS (89.5% versus 63.9%; \( P = 0.048 \)) and 2-year OS (100% versus 77.3%; \( P = 0.035 \)) than the BEAM group. The

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**Table 2** Summary of adverse events in the first month after CART infusion.

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Grade 1, n (%)</th>
<th>Grade 2, n (%)</th>
<th>Grade 3, n (%)</th>
<th>Grade 4, n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRS</td>
<td>24 (61.5)</td>
<td>9 (23.1)</td>
<td>5 (12.8)</td>
<td>0 (0)</td>
<td>0.391</td>
</tr>
<tr>
<td>ICANS</td>
<td>3 (7.7)</td>
<td>1 (2.6)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0.73</td>
</tr>
<tr>
<td>Fever</td>
<td>22 (56.4)</td>
<td>12 (30.8)</td>
<td>4 (10.3)</td>
<td>0 (0)</td>
<td>0.888</td>
</tr>
<tr>
<td>Hypertension</td>
<td>11 (28.2)</td>
<td>4 (10.3)</td>
<td>5 (12.8)</td>
<td>0 (0)</td>
<td>0.606</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>13 (33.3)</td>
<td>3 (7.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.023</td>
</tr>
<tr>
<td>Tachycardia</td>
<td>13 (33.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.514</td>
</tr>
<tr>
<td>Tremor</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.328</td>
</tr>
<tr>
<td>Seizure</td>
<td>0 (0)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Headache</td>
<td>3 (7.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Chills</td>
<td>9 (23.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.059</td>
</tr>
<tr>
<td>Nausea</td>
<td>6 (15.4)</td>
<td>11 (28.2)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.444</td>
</tr>
<tr>
<td>Vomiting</td>
<td>7 (17.9)</td>
<td>8 (20.5)</td>
<td>3 (7.7)</td>
<td>0 (0)</td>
<td>1</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>15 (38.5)</td>
<td>4 (10.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.025*</td>
</tr>
<tr>
<td>Peripheral edema</td>
<td>10 (25.6)</td>
<td>2 (5.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.348</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>7 (17.9)</td>
<td>19 (48.7)</td>
<td>3 (7.7)</td>
<td>0 (0)</td>
<td>0.071</td>
</tr>
<tr>
<td>Dermatitis</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0.1</td>
</tr>
<tr>
<td>Oral mucositis</td>
<td>1 (2.6)</td>
<td>6 (15.4)</td>
<td>12 (30.8)</td>
<td>0 (0)</td>
<td>0.071</td>
</tr>
<tr>
<td>Heart failure</td>
<td>8 (20.5)</td>
<td>4 (10.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.388</td>
</tr>
<tr>
<td>Lung infection</td>
<td>0 (0)</td>
<td>3 (7.7)</td>
<td>2 (5.1)</td>
<td>0 (0)</td>
<td>0.615</td>
</tr>
<tr>
<td>Prolonged APTT</td>
<td>21 (53.8)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.723</td>
</tr>
<tr>
<td>Decreased fibrinogen</td>
<td>4 (10.3)</td>
<td>2 (5.1)</td>
<td>3 (7.7)</td>
<td>0 (0)</td>
<td>0.894</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>39 (100)</td>
<td>1</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>39 (100)</td>
<td>1</td>
</tr>
<tr>
<td>Anemia</td>
<td>0 (0)</td>
<td>1 (2.6)</td>
<td>7 (17.9)</td>
<td>31 (79.5)</td>
<td>1</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>39 (100)</td>
<td>1</td>
</tr>
<tr>
<td>ALT increase</td>
<td>8 (20.5)</td>
<td>2 (5.1)</td>
<td>0 (0)</td>
<td>39 (100)</td>
<td>0.095</td>
</tr>
<tr>
<td>AST increase</td>
<td>10 (25.6)</td>
<td>3 (7.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.035*</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>5 (12.8)</td>
<td>2 (5.1)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0.893</td>
</tr>
<tr>
<td>Hypalbuminemia</td>
<td>16 (41)</td>
<td>13 (33.3)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.079</td>
</tr>
<tr>
<td>ALP increase</td>
<td>5 (12.8)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.071</td>
</tr>
<tr>
<td>γ-GT increase</td>
<td>12 (30.8)</td>
<td>1 (2.6)</td>
<td>3 (7.7)</td>
<td>0 (0)</td>
<td>0.071</td>
</tr>
<tr>
<td>Creatinine increase</td>
<td>5 (12.8)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.1</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>15 (38.5)</td>
<td>2 (5.1)</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0.256</td>
</tr>
<tr>
<td>Hypocalcemia</td>
<td>21 (53.8)</td>
<td>2 (5.1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.087</td>
</tr>
<tr>
<td>Hypoanemia</td>
<td>19 (48.7)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>13 (33.3)</td>
<td>0.083</td>
</tr>
</tbody>
</table>

ALT, alanine transaminase; APPT, activated partial thromboplastin time; AST, aspartate transaminase; BEAM, carmustine, etoposide, cytarabine and melphalan; BEAMF, carmustine, etoposide, cytarabine, melphalan and fludarabine; CART, chimeric antigen receptor T-cell; CRS, cytokine release syndrome; GGT, gamma-glutamyl transferase; ICANS, immune effector cell-associated neurotoxicity syndrome.
incidence of adverse events such as grade 2 hypoxemia, peripheral edema and elevated AST in the BEMAF group was greater than that in the BEAM group. The incidence rate of grade 3 CRS and ICANS was 17.2% and 1.7%, respectively. No grade 4 CRS and ICANS was observed. There were no significant differences in the incidence of CRS and CRES and the peak concentration of serum interleukin-6 and ferritin between the BEAM and BEAMF groups. All adverse events were reversible. Furthermore, R3m was an independent risk factor affecting the OS of R/R B-NHL by univariate and multivariable analysis. Patients with age < median and R3m (responders) presented better PFS and OS in R/R B-NHL. Finally, the kinetics and expansion of CD19 and CD22 CART were similar in the BEAM and BEAMF groups. The ratio of Tregs to Th cells and the Tregs count were greater in the BEAM group compared with the BEAMF group at 2 months after CART infusion.

For patients with R/R B-NHL, those who were insensitive to chemotherapy or relapsed after ASCT had a dismal prognosis. Recently, several clinical trials, including ZUMA-7, BELINDA, and TRANSFORM, have evaluated whether CART cell therapy can replace salvage ASCT as a second-line treatment for R/R large B-cell lymphoma. The ZUMA-7 trial indicated that the OR and CR rates were significantly greater in the axicabtagene ciloleucel (axi-cel) arm (83% and 65%) compared with the ASCT arm (48% and 39%) [22]. The axi-cel and liso-cel have been recommended as second-line therapy by the National Comprehensive Cancer Network guidelines. However, the result of the BELINDA trial was that the OR and CR rates were not different between the tisagenlecleucel (46% and 28%) and ASCT arms (43% and 28%) [24]. Recently, one study reported that in patients with relapsed DLBCL in a PR status, ASCT therapy achieved superior 2-year OS (69% versus 47%), and displayed a lower relapse rate at 2 years (40% versus 53%) than CART therapy [25]. Moreover, only 30–40% of patients obtained long-term durability of response after CD19 CART therapy [7,9,26]. To enhance anti-tumor activity and durable remission, several studies have been conducted on CART following ASCT therapy [12–16]. Because prognostic factors, including bulky disease, International Prognostic Index score, extra-nodal organ involvement, duration of remission, response to salvage therapy and FDG-PET status, varied across studies, these results varied widely across studies. In our study, 58 patients with R/R B-NHL were treated with CD19/22 CART following ASCT, of whom 53 (91.4%) had stable disease or progressive disease before ASCT after salvage treatment. The ORR at 3 months was 79.3%. After a median follow-up of 28 (range: 0.93–51.9) months, the 2-year OS and PFS in all patients were 85.5% and 72.7%, respectively. CD19/22 CART rapidly expanded to maximal levels within approximately 7 days and subsequently maintained persistence for over
1-month post-infusion. Relapse in some patients could be partially attributed to the inhibitory effects of the complex tumor immunosuppressive microenvironment on the expansion and persistence of CART therapy. Treatment failure might also arise from the rapid progression of tumors to multiple sites before CART infusion.

The powerful efficacy and good safety of ASCT plus CART therapy can be attributed to a high-dose conditioning regimen and hematopoietic stem cell transplant. Previous studies showed that the ORR of CD19 CART in acute lymphoblastic leukemia (ALL) was greater than that in lymphoma [27,28]. Due to the characteristics of NHL between leukemia and solid tumors, the more prominent tumor immunosuppressive microenvironment in lymphoma might prevent anti-tumor T cells from proliferating, infiltrating and killing tumors, thus reducing the response rate to adoptive cellular immunotherapy. A myeloablative-conditioning regimen could diminish the lymphoma immunosuppressive microenvironment and endogenous “cytokine sinks,” such as Treg cells, myeloid-derived suppressor cells and tumor-associated macrophages, and facilitate the expansion, function

Figure 3. The PFS and OS analysis were based on age (A, B) and R3m (C, D) for patients with ASCT plus CD19/22 CART therapy.

Figure 4. Cellular kinetics of CD19/22 CART transgenes in peripheral blood. No significant difference was observed between CD19 CART Cmax (A) and CD22 CART Cmax (B) in BEAM or BEAMF conditioning regimen. Copies of CD19 CART (C) and CD22 CART (D) transgenes, respectively, in patients with BEAM regimen. Copies of CD19 CART (E) and CD22 CART (F) transgenes, respectively, in patients with BEAMF regimen. The red dashed lines showed the lower limit of quantitation (50 copies/µg).
and persistence of CART [29]. Furthermore, previous studies reported that intensive lymphodepletion inhibited anti-CAR immune responses against the murine single-chain variable fragments, and the myeloablative conditioning regimen might have the same effect [17,30]. Meanwhile, the conditioning regimen promoted the expression of major histocompatibility complex–peptide complexes and enhanced the pool of peptides available for presentation and the number of various co-stimulatory molecules, thereby facilitating better tumor recognition and killing by adoptive cells [31]. Finally, the myeloablative conditioning regimen reduced tumor volume and exacerbated leukopenia, leading to reductions in interleukin–1, interleukin-6 and granulocyte-macrophage colony-stimulating factor, thereby alleviating severe CRS and ICANS [32–37]. Our results suggested that most patients in this study developed grade 1 or 2 CRS and ICANS, possibly due to the reduction of cytokines by myeloablative chemotherapy. Transplantation of hematopoietic stem cells also significantly increased the expansion and anti-tumor effect of adoptively transferred self/tumor antigen–reactive T cells [38].

It is well known that various conditioning regimens have been developed as alternatives to improve ASCT outcomes. Currently, the conditioning regimens before ASCT plus CART therapy for R/R NHL reported in the literature include BEAM, CEAC (semustine, cytarabine, etoposide and cyclophosphamide), GBC (gemcitabine, busulfan and cyclophosphamide) and thiopeta-based regimens [12,14,39,40]. Whether the development of a new conditioning regimen before ASCT plus CART therapy can improve the result of R/R B-NHL is unknown. Fludarabine, a purine nucleoside, induces cytotoxicity through various pathways, thereby inhibiting DNA synthesis. Because lymphocytes are abundant in deoxycytidine kinases, the activity of which is a limiting step in the inhibition of DNA synthesis, and the accumulation of deoxycytidine kinase by the metabolites of fludarabine is susceptible, so fludarabine has particularly potent lymphodepleting properties [41]. One literature reported that fludarabine added to the lymphodepleting regimen could improve the persistence of CART in melanoma [42]. Several studies found that the addition of fludarabine to cyclophosphamide as a lymphodepleting regimen significantly improved clinical CART expansion, persistence and event-free survival in children and adults with B-ALL/ B-NHL [17,30,43,44]. In addition, optimal fludarabine exposure was associated with markedly lower rates of relapse and improved survival after CD19 CART therapy in children and adults with B-ALL/ B-NHL [45–47]. The mechanism included increased availability of cytokines or improvement of the cytokine milieu, which enhanced CART expansion, potency and efficacy [48,49]. Therefore, we added fludarabine to the BEAM conditioning regimen before ASCT plus CART therapy to see whether it would improve R/R B-NHL outcomes. In our study, adding fludarabine in the ideal BEAM backbone demonstrated extended 2-year PFS and 2-year OS than the BEAM group. Although there were more grade 2 adverse events such as grade 2 hypoxemia, peripheral edema and elevated AST in the BEAMF group than in the BEAM group, they were reversible and acceptable. Previous studies have discovered a connection between delayed neurotoxicity in adult patients with leukemia treated with fludarabine [50,51]. Currently, no increase in delayed neurotoxicity was noted in our analysis. No significant difference in the incidence rates of CRS and ICANS was observed between the BEAM and BEAMF groups. Therefore, adding fludarabine to the BEAM regimen before ASCT plus CART therapy is a good alternative, which not only increases the efficacy but also does not significantly increase irreversible adverse events.

The mechanism by which the BEAMF regimen is superior to the BEAM regimen remains unclear. Our results indicated that the BEAMF group did not enhance the peak levels and duration of CD19/22 CART cells compared with the BEAM group. Lymphocyte subsets demonstrated that the ratio of Tregs to Th cells and the Tregs count (B) were greater in the BEAM group compared with the BEAMF group at 2 months after CART infusion. In our hematology center, transplantation-related mortality was very low or even not if patients with good physical condition were selected for ASCT plus CART therapy.

**Figure 5.** The ratio of Tregs to Th cells and the Tregs count in the lymphocyte subsets of the BEAM and BEAMF groups at 2 months after CART infusion. The ratio of Tregs to Th cells (A) and the Tregs count (B) were greater in the BEAM group compared with the BEAMF group at 2 months after CART infusion.
The limitation of this study was that it was a retrospective study. In addition, one cohort was small, with only 19 patients in the BEAMF regimen. Although the efficacy and safety of the BEAMF regimen before ASCT plus CART therapy are encouraging, a prospective study with a larger cohort is needed to provide a superior comparison of BEAM and BEAM regimen before ASCT plus CART therapy for patients with R/R B-NHL. Furthermore, the pharmacokinetics and pharmacodynamics of fludarabine should be studied to understand whether optimal fludarabine exposure would affect the outcome of ASCT plus CART therapy. Finally, another limitation of this study is that the conditioning regimen was not identified as an independent risk factor in both univariate and multivariate Cox regression analysis.

In summary, our findings indicate that the combination of ASCT and CD19/22 CART therapy can achieve greater response rates and durability of response in some R/R B-NHL cases and that the BEAMF conditioning regimen has shown superior PFS and OS than the BEAM regimen. We recommend the BEAMF regimen before ASCT plus CART therapy as salvage therapy for R/R B-NHL.

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Declaration of competing interest
The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

Author Contributions
Concept and design of the study: YZ, YC and XZ. Acquisition of data: XZ, YY, NW, JW, JX, JW, LH, MZ, LL, YX, FM, YC and YZ. Analysis and interpretation of data: XZ. Drafting or revising the manuscript: XZ. All authors have approved the final article.

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Data availability statement
The data supporting the current study will be made available by the authors, without reservation.

Supplementary materials
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References


