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# Bendamustine is a safe and effective lymphodepletion agent for axicabtagene ciloleucel in patients with refractory or relapsed large B-cell lymphoma

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## ABSTRACT

**Background** Fludarabine in combination with cyclophosphamide (FC) is the standard lymphodepletion regimen for CAR T-cell therapy (CAR T). A national fludarabine shortage in 2022 necessitated the exploration of alternative regimens with many centers employing single-agent bendamustine as lymphodepletion despite a lack of clinical safety and efficacy data. To fill this gap in the literature, we evaluated the safety, efficacy, and expansion kinetics of bendamustine as lymphodepletion prior to axicabtagene ciloleucel (axi-cel) therapy.

**Methods** 84 consecutive patients with relapsed or refractory large B-cell lymphoma treated with axi-cel and managed with a uniform toxicity management plan at Stanford University were studied. 27 patients received alternative lymphodepletion with bendamustine while 57 received FC.

**Results** Best complete response rates were similar (73.7% for FC and 74% for bendamustine, p=0.28) and there was no significant difference in 12-month progression-free survival or overall survival estimates (p=0.17 and p=0.62, respectively). The frequency of high-grade cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome was similar in both the cohorts. Bendamustine cohort experienced lower proportions of hematological toxicities and antibiotic use for neutropenic fever. Immune reconstitution, as measured by quantitative assessment of cellular immunity, was better in bendamustine cohort as compared with FC cohort. CAR T expansion as measured by peak expansion and area under the curve for expansion was comparable between cohorts.

**Conclusions** Bendamustine is a safe and effective alternative lymphodepletion conditioning for axi-cel with lower early hematological toxicity and favorable immune reconstitution.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Lymphodepletion chemotherapy is essential for the efficacy of CAR T cells by playing a critical role in the expansion and persistence of infused CAR T cells.

⇒ Fludarabine and cyclophosphamide (FC) is the standard lymphodepleting regimen prior to axicabtagene ciloleucel (axi-cel). However, there is paucity of data on lymphodepletion with alternative regimens.

### WHAT THIS STUDY ADDS

⇒ Bendamustine is a safe and equally effective alternative lymphodepleting agent prior to axi-cel.

⇒ Lower hematological toxicity and improved immune reconstitution is seen with bendamustine lymphodepletion.

⇒ Lower proinflammatory cytokines were seen on day 0 after bendamustine lymphodepletion when compared with FC cohort. However, there was no difference in cytokine levels at day 7 between the cohorts suggesting high cytokine production by the homeostatic and antigen-driven CAR T cell expansion.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our findings provide insights in the design of future clinical trials for evaluating alternative lymphodepletion with bendamustine. Bendamustine may help reduce infectious complication given excellent early cellular immune reconstitution.

## INTRODUCTION

Chimeric antigen receptor T (CAR T) cell therapies have significantly improved the outcomes of patients with relapsed/refractory large B-cell lymphoma (LBCL). Early studies have demonstrated that lymphodepletion (LD) regimen is essential for the efficacy of CAR T cells.[1] LD chemotherapy is known to play a critical role in the expansion and persistence of infused CAR T cells.[2] It has been proposed that LD functions by creating T-cell modulating cytokine milieu and enhancing antigen presenting cell activation. Additionally, LD results in the reduction

of endogenous lymphocytes and reprogramming of the marrow and nodal microenvironment to ensure optimal engraftment, homing, and long-term survival of CAR T cells.[3–5]

Fludarabine in combination with cyclophosphamide (FC) is currently the standard of care LD regimen prior to axicabtagene-ciloleucel (axi-cel).[6] Although essential, FC chemotherapy poses notable risks, particularly hematological toxicities, and susceptibility to infections.[7] Additionally, FC LD can initiate the mobilization of hematopoietic progenitor cells that differentiate into immunosuppressive myeloid cells, leading to a dramatic increase in peripheral myeloid-derived suppressor cells.[8] During a national shortage of fludarabine in 2022[9], bendamustine, a purine analog and alkylating chemotherapy agent[10], was commonly used as an alternative to FC LD for many CAR T products. Bendamustine was a favorable choice given its excellent tolerability and robust LD effect.[9] Relative to FC, bendamustine was found to have comparable efficacy when used as LD prior to tisagenlecleucel in adults with lymphoma in the third or later lines of therapy.[11]

However, there is a paucity of data on alternative LD regimens administered prior to axi-cel, the most used CAR T cell therapy in adults with lymphoma in the USA.[12 13] Alternative LD regimens are needed amidst the growing frequency of drug shortages in both the USA and globally.[14] Lastly, there is an ongoing need to study alternative LD regimens to improve safety while preserving efficacy, especially in patients who are frail and have pre-existing cytopenia prior to the initiation of LD. Here, we compare the safety, efficacy, CAR T expansion kinetics, and immune reconstitution following FC and bendamustine as LD agents prior to axi-cel treatment.

## METHODS

### Patients

84 consecutive patients with relapsed/refractory LBCL treated with axi-cel were included in this single-institution, retrospective cohort study.27 patients who received bendamustine LD (90 mg/m$^2$) on days −4 and −3 were compared with a historic control of 57 patients who received fludarabine (30 mg/m$^2$) and cyclophosphamide (300 mg/m$^2$) on day −5, day −4, and day −3. Patients were consented and enrolled on an IRB-approved data collection and biobanking protocol.

### CAR T toxicity management plan

To ensure the consistency in toxicity management, this study exclusively enrolled consecutive patients who underwent CAR T infusion after August 30, 2021, when changes were made to our institutional standard intervention protocol. A response to the fludarabine shortage led to the collective decision by our institution's panel to initiate bendamustine as an LD agent starting July 1, 2022. The shortage concluded on September 21, 2022, marking the conclusion of bendamustine administration for this study cohort. All 84 patients adhered to the same toxicity management plan throughout the study duration.

### Disease response assessments and toxicity grading

Response was assessed using the Lugano 2014 criteria.[15] Two of the study investigators initially conducted the response assessments. Their evaluations were later confirmed by independent blinded investigators to ensure the accuracy of the results. This approach was implemented to minimize potential biases and to strengthen the validity and robustness of our findings regarding treatment response. CAR T-related toxicities were evaluated using the American Society for Transplantation and Cellular Therapy consensus grading.[16]

### Measurement of absolute lymphocyte count, absolute neutrophil count and cytokine measurement

The clinical laboratory does not calculate the differential counts for samples with a white cell count (WCC) below 0.6k/µL. In such cases, the reported value is 0. To address this in our study, we calculated the nadir absolute lymphocyte count (ALC)/absolute neutrophil count (ANC) by averaging the last ALC/ANC measurement before the WCC dropped below 0.5 k/µL and the first ALC/ANC measurement after the WCC recovered. This calculation was performed for all patients who had a WCC count below 0.6 k/µL on the day of ALC/ANC nadir in both groups. 48 cytokines via the 48-plex Luminex platform were used to measure cytokines at pre-LD, day 0, and day +7.[17] Cytokines were quantified using mean fluorescence indices (MFIs).

### Measurement of CAR T expansion

CD19 CAR T expansion was measured by real-time flow cytometry with anti-idiotype-FMC63 conjugated to Dylight 650.[18 19] The expansion of CAR T cells was calculated by taking the log10-transformed sum of the absolute values of CD4 and CD8 CAR T cells at days +7 and +14. The linear trapezoidal method was used to calculate the area under the curve (AUC) of CAR T cell expansion for the first 28 days following CAR T infusion. AUCs were calculated for the CD4 and CD8 components individually and for the two components combined using raw values with the results then being transformed using a log base 10 transformation. Patients were required to have three time points for an accurate AUC to be calculated: both time points surrounding peak expansion (days 7 and 14) and at least one time point following peak expansion (days 21 or 28). A total of seven patients, six from the FC treatment group and one from the bendamustine treatment group, did not have both time points surrounding peak expansion.

### Statistical considerations

As this was a retrospective, non-randomized study, a propensity score weighting approach was implemented to reduce confounding bias. A covariate balancing propensity score weighting scheme was used to balance patient characteristics between treatment groups by weighting

**Table 1** Baseline characteristics of the study population before application of IPTW, stratified by treatment group

| | FC n=57 (%) | Benda n=27 (%) | P value |
|---|---|---|---|
| Age (median IQR) | 63.00 (56.00–71.00) | 67.00 (57.00–70.00) | 0.755 |
| Sex | | | 0.512 |
| Female | 27 (47.4) | 10 (37.0) | |
| Male | 30 (52.6) | 17 (63.0) | |
| ECOG performance status | | | 0.201 |
| 0 | 6 (10.5) | 3 (11.1) | |
| 1 | 46 (80.7) | 17 (63.0) | |
| 2 | 4 (7.0) | 6 (22.2) | |
| 3 | 1 (1.8) | 1 (3.7) | |
| R-IPI at apheresis | | | 0.514 |
| Very good (0) | 24 (42.1) | 11 (40.7) | |
| Good (1–2) | 17 (29.8) | 11 (40.7) | |
| Poor (3–5) | 16 (28.1) | 5 (18.5) | |
| Disease type: | | | 0.983 |
| DLBCL | 46 (80.7) | 21 (77.8) | |
| TFL | 11 (19.3) | 6 (22.2) | |
| Double expressor | 30 (52.6) | 17 (63.0) | 0.410 |
| Double hit | 11 (19.3) | 5 (18.5) | 0.427 |
| GCB cell of origin | 27 (47.4) | 12 (44.4) | 0.521 |
| Bulky disease | 8 (14.0) | 4 (14.8) | 1.000 |
| Pre LD LDH (median IQR) | 261.00 (209.00–338.00) | 218.00 (203.50–316.50) | 0.353 |
| HEMATOTOX | | | 0.99 |
| Low (0–1) | 22 (38.6%) | 10 (37.0%) | |
| High (>2) | 35 (61.4%) | 17 (63.0%) | |
| Prior lines of therapy: | | 6 (22.2) | 0.983 |
| 1 | 8 (14.0) | | |
| 2 | 24 (42.1) | | |
| 3+ | 25 (43.9) | 11 (40.7) | |
| Prior auto transplant | 11 (19.3) | 5 (18.5) | 1.000 |
| Bridging therapy | 32 (56.1) | 11 (40.7) | 0.278 |
| Pre LD ALC (median IQR) | 0.64 (0.38–1.10) | 1.02 (0.65–1.35) | 0.036 |
| Pre LD ANC (median IQR) | 3.56 (2.68–5.14) | 3.39 (1.83–4.23) | 0.149 |

ALC, absolute lymphocyte count; ANC, absolute neutrophil count; DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; FC, fludarabine and cyclophosphamide; IPTW,inverse probability of treatment weighting ; LD, lymphodepletion; LDH, lactate dehydrogenase; R-IPI, Revised International Prognostic Index; TFL, transformed follicular lymphoma.

each patient in the analytical dataset by the inverse probability of receiving their actual treatment (IPTW).[20] IPTW is an effective means of covariate balancing across treatment groups[21] and has superior performance over propensity score matching for low sample sizes as all cases are considered in the analysis.[22] Covariates included in the propensity score calculation were age at time of CAR T infusion, sex, bridging therapy utilization, number of prior treatment lines, International Prognostic Index at time of apheresis, ALC at leukapheresis, prior auto stem cell transplant, and pre-LD LDH, ALC, ANC, and platelet count. Standardized mean difference (SMD) between treatment groups was used to assess balance before and after IPTW. Continuous outcomes were compared using the Mann-Whitney U test, categorical outcomes using $\chi$ square tests (or Fisher's exact test, where applicable), and time-to-event outcomes using the Kaplan-Meier estimator and log-rank test, all weighted in the IPTW sample. All summary statistics, tables, and figures are presented in their raw, unweighted form while p values associated with inferential testing were calculated in the IPTW sample; statistical significance was considered when $p<0.05$. All analyses were conducted in R, V.4.3.1.

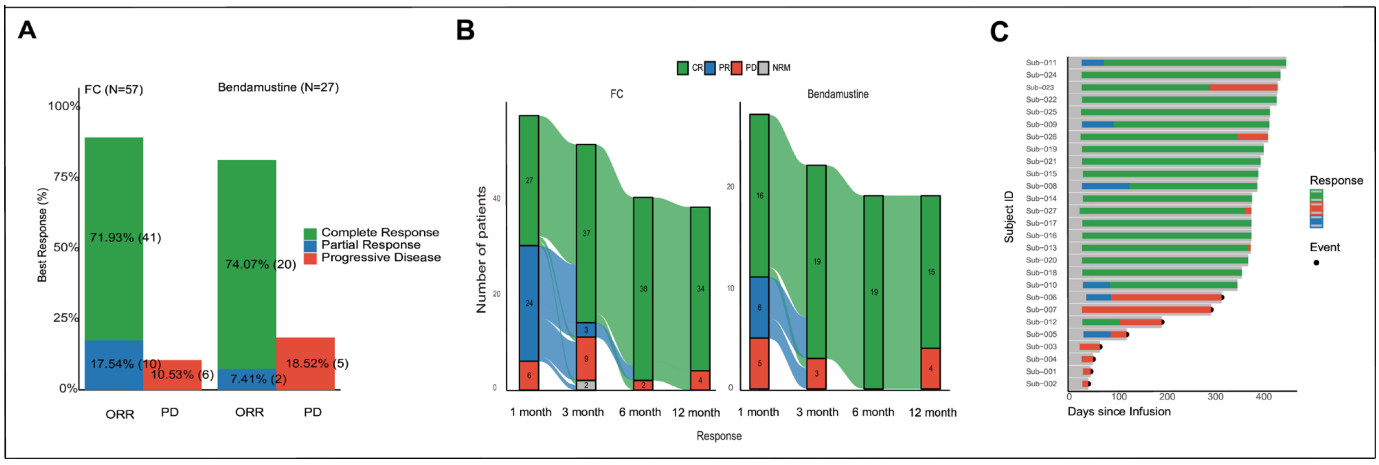


**Figure 1** Clinical response after axi-cel infusion according to lymphodepleting regimen given. (A) Best overall response rate at 6 months stratified by lymphodepletion regimen administered after axi-cel infusion. (B) Sankey flow diagram of patient response at 1, 3, 6 and 9 months post-axi-cel infusion, stratified by lymphodepleting regimen administered. Patients who have progressive disease are not included in subsequent response points (eg, a patient with progressive disease at the 1-month response assessment is not included in the patient counts at either the 3-month or 6-month response assessments). (C) Swimmer plot for the bendamustine lymphodepletion regimen cohort, showing each patient's response follow axi-cel infusion. Patients have been ordered in terms of their follow-up time, with shorter bars indicating a shorter window of follow-up. A black circle indicates patient death (n=8). Initial response was conducted 1 month following infusion. CR, complete response; ORR, overall response rate; PD, progressive disease; PR, partial response; NRM, non-relapse mortality.

## RESULTS

This is a summary of the patient demographics and clinical characteristics before application of IPTW, stratified by treatment group. Continuous variables were summarized using medians and IQRs while categorical variables were summaLDrized with counts and percentages, with missingness reported as its own category. Mann-Whitney U tests were conducted for continuous variables and $\chi^2$ tests (or Fisher's exact test, when appliable) were conducted for categorical variables. Prior to covariate balancing propensity score weighting, only pre-LD ALC was significantly different between treatment groups (p=0.036).

### Baseline characteristics

57 patients who received FC were compared with 27 patients who received bendamustine LD. Baseline characteristics, prior to application of IPTW, are summarized in table 1. In the FC and bendamustine cohort, median age at CAR T infusion was 63 (median IQR 56–71) years and 67 (median IQR 57–70) years. 25 (43.9%) and 11 (40.7%) received ≥3 prior lines of therapy, 11 (19.3%) and 5 (18.5%) received prior autologous stem cell transplantation in the FC and bendamustine cohort, respectively. Median ALC prior to LD was 0.64 k/µL (median IQR 0.38–1.10) in FC group compared with 1.02 k/µL (median IQR of 0.65–1.35) in the bendamustine group (p=0.03). Median pre-LD HEMATOTOX score[23 24] was 1 (median IQR 1–2) for both the FC and bendamustine cohorts; the distribution of low versus high HEMATOTOX scores was, identical between treatment groups (p>0.99). The SMD plot (online supplemental figure 3) generally shows a sufficient level of balance, with nearly all covariates having a post-IPTW SMD<0.1.

### Efficacy

The median follow-up time for the FC cohort was 18.5 months (IQR: 15.7–21.7) and for bendamustine, 12.9 months (IQR: 12.5–13.7). There was no significant difference in the best complete response (CR) rate and best overall response rate (ORR) between the two cohorts, with a CR rate of 73.7% for FC and 74% for bendamustine (p=0.28), and ORR of 89.4% for FC and 81.5% for bendamustine (p=0.50) (figure 1).

For the FC cohort, 12-month progression-free survival (PFS) and overall survival (OS) were 61.4% (95% CI 47.5% to 72.6%) and 77.2% (95% CI 63.9% to 86.0%), respectively. For the bendamustine cohort, 12-month PFS and OS point estimates were 58.8% (95% CI 37.9% to 74.7%) and 70.4% (95% CI 49.4% to 83.9%), respectively (figure 2). No significant difference was found between the cohorts in either 12 months PFS (p=0.65) or 12 months OS (p=0.78). Median PFS and OS were not achieved for either cohort.

Late progression is defined as disease relapse noted beyond 6 months after CAR T infusion. Compared with 19% (4/22) late progressors in FC cohort, the bendamustine cohort had a higher proportion with 33% (4/12) of late progressors (p=0.08) (figure 1).

Of the patients who did not have progressive disease, 26.5% (9/34) FC patients and 7.1% (1/14) bendamustine experienced a grade3 or 4 infection. 14 FC patients and 8 bendamustine patients died during the study's observation period, with the cause of death for all bendamustine patients being progressive disease while 11/14 of the FC patients' cause of death was progressive disease and 3/14 died due to infections.

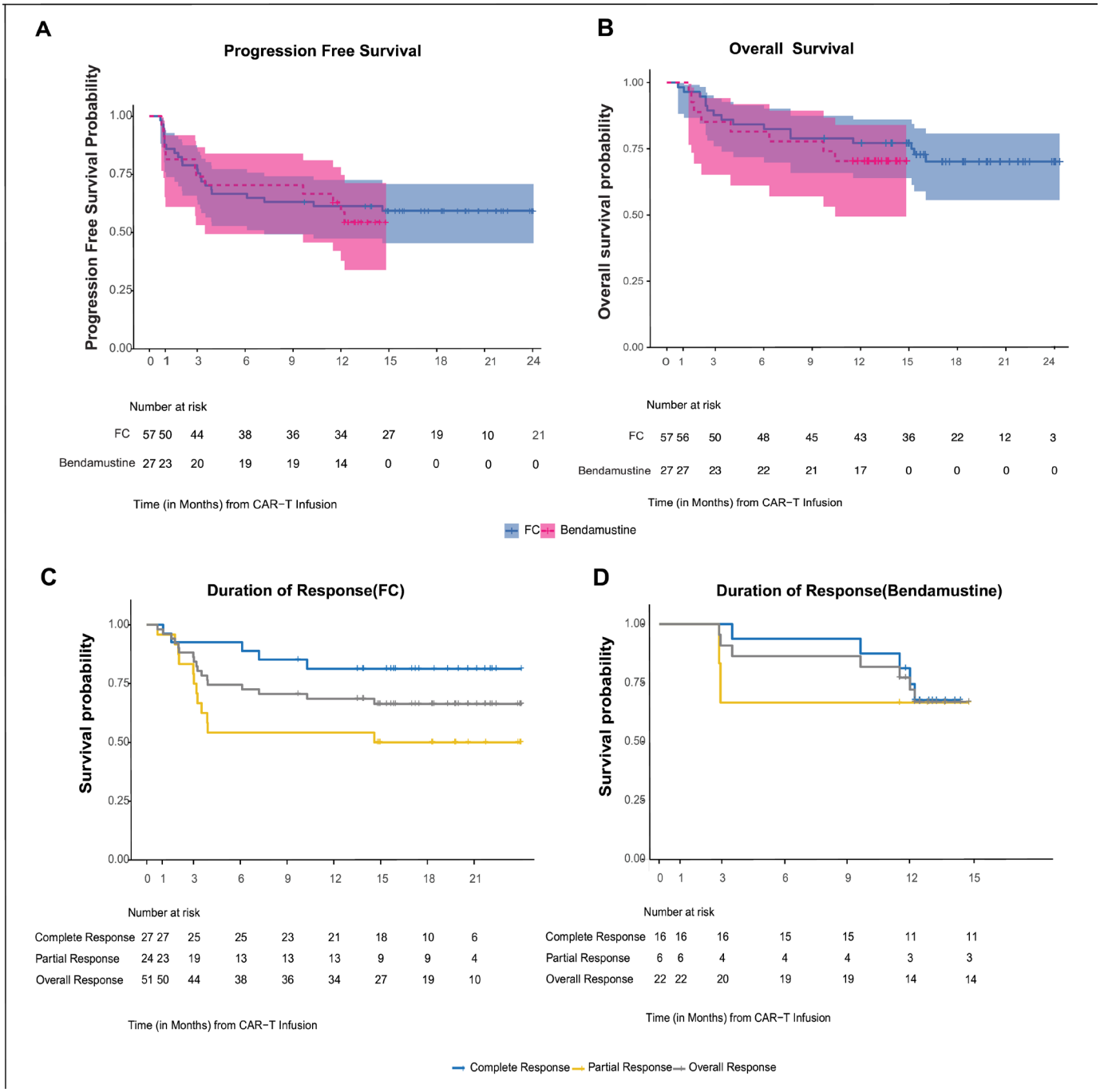


**Figure 2** (A) Progression-free survival (the time from axi-cel infusion to either disease progression or death), stratified by lymphodepletion regimen. Blue line represents FC-treated patients while pink line represents bendamustine-treated patients, with the shaded areas surrounding each line representing the 95% CI. Median progression-free survival was not achieved for either treatment cohort. (B) Overall survival (the time from axi-cel infusion to death) stratified by lymphodepletion regimen. Blue line represents FC-treated patients while pink line represents bendamustine-treated patients, with the shaded areas surrounding each line representing the 95% CI. Median overall survival was not achieved for either treatment cohort. (C) Duration of response (from time of axi-cel infusion to disease progression or death) for bendamustine-treated patients, stratified by 1-month response following infusion. Blue line represents complete response, yellow line represents partial response, and gray line represents overall response (complete+partial response). Median duration of response was not achieved for any of the strata. (D) Duration of response (from time of axi-cel infusion to disease progression or death) for FC-treated patients, stratified by 1-month response following infusion. Blue line represents complete response, yellow line represents partial response, and gray line represents overall response (complete+partial response). Median duration of response was not achieved for any of the strata. FC, fludarabine/cyclophosphamide lymphodepleting regimen.

Table 2 CAR-T-associated toxicity and healthcare utilization data of the patients within the first 30 days after CAR-T infusion

| | FC (n=57) | Benda (n=27) | P value |
|---|---|---|---|
| CRS all grades | 96.49% (n=55) | 81.48% (n=22) | 0.02 |
| CRS grade 3 and 4 | 0% (n=0) | 11.11% (n=3) | 0.08 |
| CRS time-to-onset | 4 days | 4 days | 0.64 |
| ICANS all grades | 49.12% (n=28) | 37.04% (n=10) | 0.20 |
| ICANS grade 3 and 4 | 12.28% (n=7) | 18.52% (n=5) | 0.25 |
| ICANS time-to-onset | 7 days | 7.5 days | 0.74 |
| Tocilizumab use | 73.68% (n=42) | 55.56% (n=15) | 0.17 |
| Anakinra use | 32.08% (n=17) | 30.77% (n=8) | 0.86 |
| Steroids use | 91.23% (n=52) | 81.48% (n=22) | 0.33 |
| Length of hospital stay | 13 days | 13 days | 0.65 |
| ICU utilization rate | 8.77% (n=5) | 11% (n=3) | 0.60 |
| Readmission rate | 26.3% | 15.4% | 0.16 |
| GCSF use within 14 days | 96.4% | 44.4% | <0.01 |
| Cryoprecipitate use within 14 days | 28.07% (n=16) | 33.33% (n=9) | 0.83 |
| PRBC transfusion within 14 days | 35.1% (n=20) | 37% (n=10) | 0.66 |
| Platelet transfusion within 14 days | 19.3% (n=11) | 14.8% (n=4) | 0.83 |

.Benda, bendamustine; CRS, cytokine release syndrome; G-CSF, granulocyte colony stimulating factor ; ICANS, immune effector cell-associated neurotoxicity syndrome; ICU, intensive care unit; PRBC, packed red blood cell.

### Toxicity

The overall incidence of cytokine release syndrome (CRS) was higher in the FC cohort compared with the bendamustine cohort (96.5% vs 81.5%, p=0.01), but grade 3 or higher CRS events were not statistically different (FC 0% n=0, bendamustine 11.1% n=3; p=0.08). The incidence and severity of immune effector cell-associated neurotoxicity syndrome (ICANS) were similar between the two cohorts. Healthcare utilization measures such as length of hospital stay, ICU admissions, tocilizumab, steroid and anakinra use were similar in both cohorts (table 2).

The FC cohort had a significantly lower median nadir ANC (0.00 K/µL; IQR: 0.00–0.29) in the first 30 days following CAR T infusion compared with the bendamustine cohort (1.25 K/µL; IQR: 0.85–2.16; p<0.01). In the FC cohort, 59.6% received antibiotics for neutropenic fever within the first 30 days post-CAR T, compared with 3.7% in the bendamustine cohort (p<0.01) (figure 3, table 2). Additionally, there was higher usage of G-CSF use within first 14 days of LD in FC group (96.4%) as opposed to bendamustine group (44.4%), p<0.01. There were no significant differences in ANC, hemoglobin, and platelets at months 3, 6 or 12 postinfusion.

The FC cohort had a significantly lower median ALC nadir of 0.03 K/µL (IQR: 0.01–0.06 p=0.01), occurring within 1 day after CAR T infusion (figure 3) in comparison to bendamustine group. However, bendamustine was effective in achieving LD, with ALC nadir of 0.11 K/µL (IQR: 0.04–0.17) occurring within 1 day after CAR T infusion.

### Immune reconstitution

Both cohorts showed a similar pattern of lymphocyte recovery over 1 month, at month 3 and month six post-CAR T therapy. After 12 months postinfusion, the FC cohort had a lower median ALC of 0.98 K/µL (IQR: 0.59–1.23) compared with 1.43 K/µL (IQR: 1.19–2.05) in the bendamustine cohort (p<0.01). At 12 months, non-progressors in the FC cohort had a median B-cell count of 0 (IQR: 0–12), whereas the bendamustine cohort had significantly higher levels with a median of 234.5 cells/µL (IQR: 190–375.5; p=0.02). Additionally, median of natural-killer cell count was 176 cells/µL (IQR: 85–215) for the FC cohort and 332 cells/µL (IQR: 190.5–445.5) for the bendamustine cohort (p<0.01). Furthermore, 80% of the bendamustine cohort had T-cell count of CD4>200 cells/µL compared with 52% of the FC cohort as early as 9–12 months post-CAR T infusion (p=0.02). Overall, the immune reconstitution, as measured by quantitative assessment of cellular immunity, was more robust in patients in bendamustine cohort when compared with FC cohort.

### CAR T cell expansion

CAR T cell expansion was measured on days +7, +14, +21, and +28 after infusion, and peak expansion was seen on day +7 in both cohorts (figure 4A). The median of absolute CAR T-cells at day +7 was 2.3 cells/µL (IQR: 0.2–12.8) in the FC cohort and 8 cells/µL (IQR: 2.5–23.7) in the bendamustine cohort (p=0.01) (figure 4B). At day +14, the median of absolute CAR T-cells in the FC cohort was 2.7 cells/µL (IQR: 1.1–9.8) and 5.8 cells/µL (IQR:

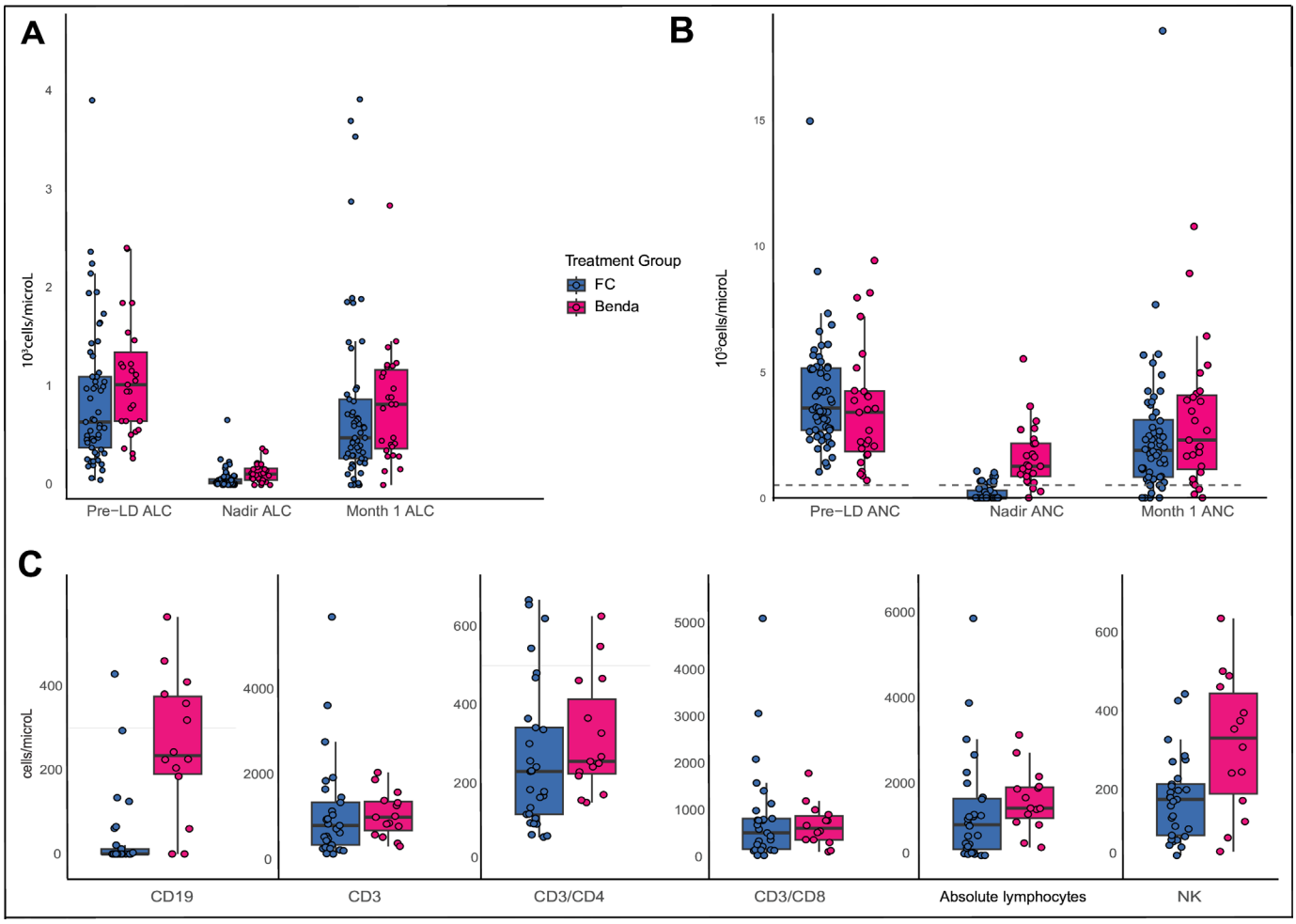


**Figure 3** (A) Boxplots of ALC in cells/microliter prelymphodepletion (left panel), at nadir (center panel), and 1-month post-axi-cel infusion (right panel). Blue represents FC-treated patients and pink represents bendamustine-treated patients. Each dot represents a patient-level value. (B) Boxplots of ANC in cells/microliter prelymphodepletion (left panel), at nadir (center panel), and 1-month post-axi-cel infusion (right panel). Blue represents FC-treated patients and pink represents bendamustine-treated patients. Each dot represents a patient-level value. The dashed line represents the count associated with severe neutropenia. (C) Boxplots of analysis of TBNK lymphocyte subsets in cells/microliter between FC (n=29) and Benda (n=15) in non-progressors. Blue represents FC-treated patients and pink represents bendamustine-treated patients. Each dot represents a patient-level value. TBNK was measured between 9 and 12 months following CAR-T infusion. ALC, absolute lymphocyte count; ANC, absolute neutrophil count.

2.1–8.1) for bendamustine (p=0.11). Comparable log-transformed AUC was observed between the cohorts, with the bendamustine cohort showing a median total CAR T expansion of 2.3 log10 cells/µL/day, having a marginally higher AUC than the FC cohort, 1.9 log10 cells/µL/day (p=0.01) (figure 4C).

### Cytokine analysis

Pre-LD cytokine levels were comparable within the group suggesting similar cytokine milieu within cohorts (online supplemental figure 1). After LD, day 0 MFIs of monocyte chemoattractant protein-1 (MCP-1), interleukin-15 (IL15) and IL-7 were significantly higher in the FC cohort compared with the bendamustine cohort (figure 5). Additionally, intrapatient change from pre-LD to day 0 in proinflammatory cytokines such as IL-6, IL-7, IL-15, and MCP-1 was more pronounced in the FC cohort as compared with the bendamustine cohort (online supplemental figure 1 and 2). There was no difference in proinflammatory cytokines (IL-6, IL-7, TNFa, IFNg and MCP-1) at day 7 presumably due to change in cytokine milieu due to homeostatic and antigen-driven CAR T expansion (online supplemental figure 1).

## DISCUSSION

LD chemotherapy plays a critical role in the expansion and persistence of infused CAR T-cells. While CAR T-cell therapy has revolutionized the treatment of non-Hodgkin's lymphoma, the incidence of CAR T-cell therapy-related toxicity is high and contributes to morbidity and mortality. In addition to drug shortage situations, there is a need to study alternative LD regimens to improve safety while preserving efficacy. Therefore, we analyzed the safety, efficacy, CAR T expansion kinetics, duration

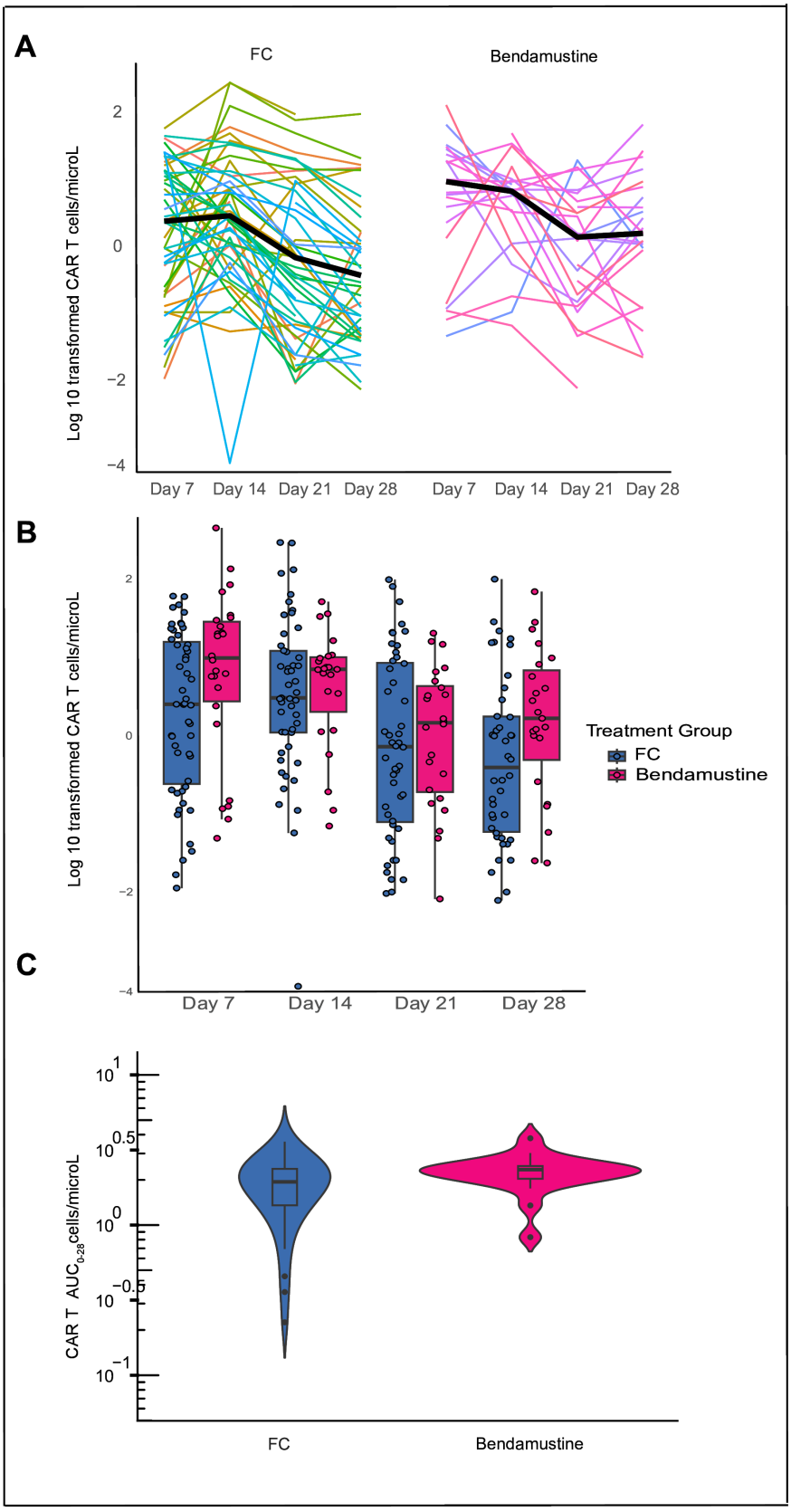


**Figure 4** CAR-T expansion in the first 28 days following axi-cel infusion. (A) Spaghetti plot of patient-level log10-transformed CAR-T expansion over 28 days, stratified. by FC and Bendamustine cohorts. Solid black line represents the median expansion. (B) Box plot of log10-transformed CAR-T expansion at days 7, day 14, day 21 and day 28. Blue represents FC-treated patients and pink represents Bendamustine-treated patients. Each dot represents a patient-level value. (C) Violin plot of area under the curve of log10-transformed CAR-T expansion within in first 28 days following CAR-T infusion using the linear trapezoidal method. Blue represents FC-treated patients and pink represents bendamustine-treated patients.

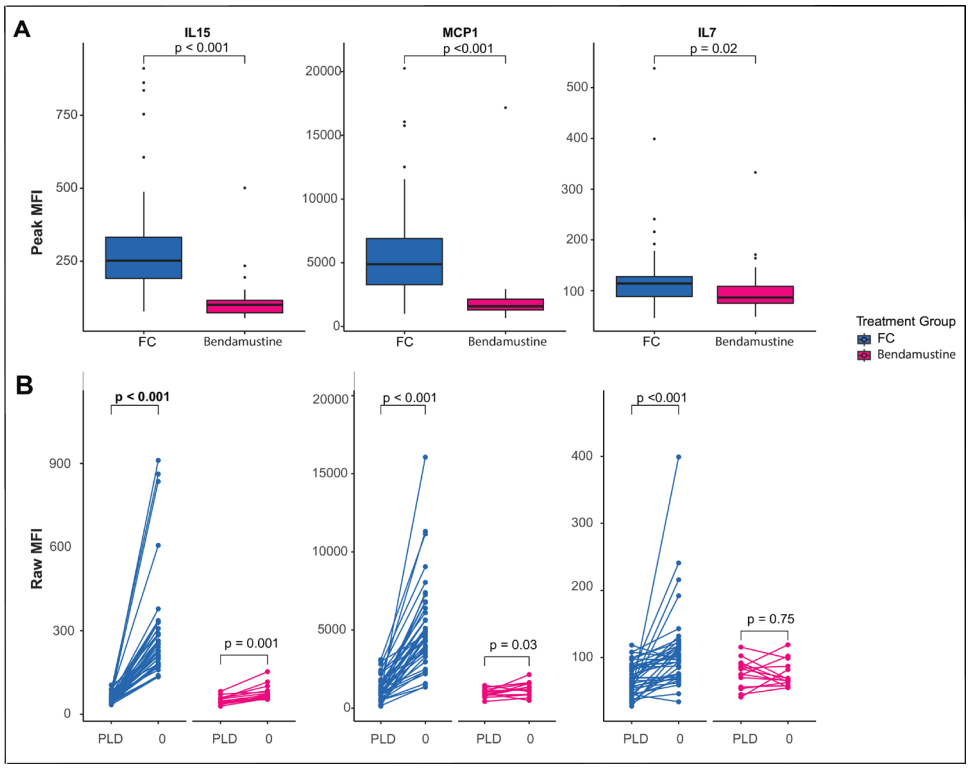


**Figure 5** Peak mean fluorescence intensity (MFI) (A) was compared using the unpaired Mann-Whitney U test (FC: n=56, bendamustine: n=26) (A) Shown are cytokine levels of IL15 (left), monocyte chemoattractant protein-1 (MCP1) (middle), and IL7 (right) at D0 comparing FC (blue) and bendamustine group(pink). (B) Raw MFIs at prelymphodepletion (PLD) and D0 were compared with the unpaired Mann-Whitney U test (FC (blue): n=41, bendamustine (pink): n=14). (B) Only patients who had both a PLD and D0 sample were included. Lines connect cytokine levels for individual patients.

of response, and immune reconstitution of bendamustine as LD. To limit bias and confounding, we studied consecutively treated patients with only LBCL histology, managed with the same toxicity management plan for axi-cel CAR T product. Lastly, a median follow-up of over a year in both FC and Bendamustine cohorts allows for an understanding of long-term efficacy and safety between the groups.

We found that bendamustine is a safe and effective alternative LD agent for axi-cel. The best CR and OR rates between the FC and bendamustine cohorts were similar. In addition, there was no significant difference in 12 month PFS or OS estimates. The efficacy endpoints observed in our cohort align closely with findings from various recent real-world analyses of CAR T in LBCL, affirming the absence of any center-specific bias.[25–27] While the bendamustine cohort represents a more proximally treated cohort and there were minor imbalances within the cohorts, the variables were balanced using a covariate balancing propensity score weighting scheme IPTW to balance patient characteristics between treatment cohorts. Lastly, compared with the FC cohort, we noted a non-statistically significant higher proportion of relapses beyond 6 months in the bendamustine cohort. This could be related to higher proportion of patients in the bendamustine cohort experiencing B cell recovery (surrogate for loss of CAR T persistence) as compared with the patients in FC cohort (figure 1C); however, larger studies with adequate sample size with longer follow-up are needed to further evaluate this non-statistically significant signal.

Bendamustine was effective in achieving LD. Nadir median ALC of 0.11 K/µL (IQR: 0.04–0.17) occurred at day+1. Bendamustine-treated patients experienced lower rates of hematological toxicities. The FC cohort had a significantly lower median nadir ANC (0.00 K/µL; IQR: 0.00–0.29) in the first 30 days following CAR T infusion compared with the bendamustine cohort (1.25 K/µL; IQR: 0.85–2.16; p<0.01). In the FC cohort, 59.6% received antibiotics for neutropenic fever within the first 30 days post-CAR T, compared with 3.7% in the bendamustine cohort. This finding highlights the better safety profile of bendamustine than FC as LD within 30 days post-CAR T infusion. Except for differences in hematological toxicity and the incidence of febrile neutropenia, our analysis revealed no significant differences in CAR T-mediated toxicity and overall healthcare utilization. Notably, there were no discernible variations in the occurrence of grade 3 or 4 CRS or ICANS, nor in the subsequent administration of tocilizumab, anakinra, and steroids. Furthermore, there was no discrepancy observed in the duration of hospitalization for CAR T therapy, the rate of ICU utilization, readmission rates, or the use of blood products. These consistent findings are likely attributable to the effective LD achieved by bendamustine, which spares severe neutropenia, facilitating sufficient CAR T

expansion and persistence. This ultimately resulted in comparable CAR-mediated toxicities but with lower incidence of hematological toxicity.

Previously, it has been shown that high levels of proinflammatory cytokines like MCP-1, IL-15 and IL-7 were essential for CAR T expansion.[28 29] In our study, pre-LD cytokine levels were similar between cohorts suggesting comparable patient population. However, after LD as noted on the day of CAR T infusion (day 0), MFIs of proinflammatory cytokines MCP-1, IL-15 and IL-7 were significantly higher in the FC cohort compared with the bendamustine cohort. Although lower levels of cytokines were noted in the bendamustine cohort compared with the FC cohort, peak CAR T expansion at day +7 was significantly higher in the bendamustine cohort. Total expansion as measured by AUC for the first 28 days of infusion was comparable among both cohorts. Additionally, there was no difference in cytokine levels at day 7 between the cohorts suggesting high cytokine production by the homeostatic and antigen-driven CAR T cell expansion. Impact of differential proinflammatory cytokine profile with the two LD regimens on tumor microenvironment, immune cells within the tumor microenviroment and CAR T tumor trafficking needs to be investigated further.

Several infectious complications are thought to be related to poor immune reconstitution after CAR T therapy. Our group has previously noted that less than 50% of patients had CD4 T-cell count <200 cells/µL by 18 months postinfusion in patients who received FC as LD regimen followed by axi-cel.[30] Prolonged lymphocyte recovery attributed to LD chemotherapy and direct CAR T effects. In our cohort, at 12 months, the bendamustine cohort had superior ALC recovery compared with the FC cohort. Furthermore, 80% of the bendamustine cohort had CD4 T-cells >200 cells/µL compared with 52% of the FC cohort as early as 9–12 months post-CAR T infusion (p=0.02). While the quantitative CD4 T cells recovery was better in bendamustine cohort, its impact on qualitative T cell functions needs to be studied. As such bendamustine LD could have a potential impact on future repeat CART therapy or T cell engager therapy on progression.[31] Further studies are needed to assess impact of LD on subsequent cell-based therapies.

Limitations of this study include the observational nature of this study with no randomization. While IPTW helps bridge the gap between observational and randomized controlled trials, it is imperfect in elucidating the causal relationship between treatment groups and patient outcomes. As such, adequately powered randomized study to compare FC with bendamustine is needed to establish causal relationship and non-inferiority of alternative LD with bendamustine. However, since the US national shortage of fludarabine was an unexpected event with resulting unplanned adoption of bendamustine served as a natural randomization experiment validating the findings observed in our study. Study limitations also include small sample size coming from a single institution, which may hinder the generalizability of the results to the wider patient population.

Lastly, given the increased frequency of generic drug shortages globally and the increasing use of cell and gene therapies, alternative lymphodepleting regimens need to be explored. While most investigations have focused on FC and bendamustine LD, limited experience with alternative agents has been reported.[32] Single-center experience with cladribine and cyclophosphamide showed comparable efficacy with FC and similar toxicity profile. Trials are underway to investigate role to total body irradiation and total lymphoid irradiation.[33 34] Cumulatively, these clinical investigations, including the current report, provide an alternative option for LD in therapeutic adoptive cell therapy.

In conclusion, bendamustine is a safe and comparably effective alternative lymphodepleting agent when administered before axi-cel. Bendamustine use is associated with a reduction in hematological toxicity and an improved immune constitution. Additionally, we demonstrate the differential impact of the FC versus bendamustine LD on cytokine profile at various time point expanding on the knowledge on interplay between dynamic cytokine milieu and CAR T expansion. Our findings support the prospective conduct of a randomized controlled trial to establish the safety and efficacy of bendamustine in comparison to FC for LD.

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**Contributors** SB, EL, DM and SD designed the study; SB, EL, AG and MPH, performed the data collection. SB, EL, AG, AJ, SD and HS analyzed the data and wrote the original manuscript. All authors performed critical review of the manuscript and had full access to all the data in the study. SB and SD are responsible for the overall content of the manuscript. All authors contributed and approved the final version of the manuscript.

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