Received: 5 February 2024 | Accepted: 11 April 2024

DOI: 10.1002/hem3.86

HemaSphere EHA EUROPEAN HEMATOLOGY ASSOCIATION

ARTICLE

Efficacy and safety of bendamustine-containing bridging therapy in R/R LBCL patients receiving CD19 CAR T-cells

Gloria Iacoboni[1,2,3] | **Mario A. Sánchez-Salinas**[1,2,3] | **Kai Rejeski**[4,5,6] | **Ana Á. Martín-López**[7,8] | **Mi Kwon**[9,10] | **Víctor Navarro**[11] | **Katarzyna A. Jalowiec**[12,13] | **Rafael Hernani**[14,15] | **Juan L. Reguera-Ortega**[16] | **Laura Gallur**[1,2,3] | **Viktoria Blumenberg**[4,5] | **María Herrero-García**[17] | **Claire Roddie**[12] | **Ana Benzaquén**[14,15] | **Javier Delgado-Serrano**[16] | **Rebeca Bailén**[9,10] | **Cecilia Carpio**[1,2,3] | **Paula Amat**[14,15] | **Lucia López-Corral**[7,8] | **Lourdes Martín-Martín**[17] | **Mariana Bastos**[9,10] | **Marion Subklewe**[4,5] | **Maeve O'Reilly**[12] | **Pere Barba**[1,2,3]

Correspondence: Gloria Iacoboni (giacoboni@vhio.net); Pere Barba (pbarba@vhio.net)

Abstract

Bridging therapy (BT) after leukapheresis is required in most relapsed/refractory (R/R) large B-cell lymphoma (LBCL) patients receiving chimeric antigen receptor (CAR) T cells. Bendamustine-containing regimens are a potential BT option. We aimed to assess if this agent had a negative impact on CAR-T outcomes when it was administered as BT. We included R/R LBCL patients from six centers who received systemic BT after leukapheresis from February 2019 to September 2022; patients who only received steroids or had pre-apheresis bendamustine exposure were excluded. Patients were divided into two BT groups, with and without bendamustine. Separate safety and efficacy analyses were carried out for axi-cel and tisa-cel. Of 243 patients who received BT, bendamustine (benda) was included in 62 (26%). There was a higher rate of BT progressors in the non-benda group (62% vs. 45%, $p = 0.02$). Concerning CAR-T efficacy, complete responses were comparable for benda versus non-benda BT cohorts with axi-cel (70% vs. 53%, $p = 0.12$) and tisa-cel (44% vs. 36%, $p = 0.70$). Also, 12-month progression-free and overall survival were not significantly different between BT groups with axi-cel (56% vs. 43% and 71% vs. 63%) and tisa-cel (25% vs. 26% and 52% vs. 48%); there were no differences when BT response was considered. CAR T-cell expansion for each construct was similar between BT groups. Regarding safety, CRS G ≥3 (6% vs. 6%, $p = 0.79$), ICANS G ≥3 (15% vs. 17%, $p = 0.68$), severe infections, and neutropenia post-infusion were comparable among BT regimens. BT with bendamustine-containing regimens is safe for patients requiring disease control during CAR T-cell manufacturing.

[1]Department of Hematology, University Hospital Vall d'Hebron, Barcelona, Spain
[2]Experimental Hematology, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain
[3]Department of Medicine, Universitat Autònoma de Barcelona, Bellaterra, Spain
[4]Department of Medicine III, University Hospital, LMU Munich, Munich, Germany
[5]Laboratory for Translational Cancer Immunology, Gene Center of the LMU Munich, Munich, Germany
[6]Adult BMT and Cellular Therapy Service, Memorial Sloan Kettering Cancer Center, New York, New York, USA
[7]Hematology Department, Hospital Clínico Universitario de Salamanca, IBSAL, CIBERONC, Salamanca, Spain
[8]Centro de Investigación del Cáncer-IBMCC, Salamanca, Spain
[9]Department of Hematology, Hospital General Universitario Gregorio Marañón, Madrid, Spain
[10]Gregorio Marañón Health Research Institute (IiSGM), Madrid, Spain
[11]Oncology Data Science (ODySey) Group, Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain
[12]Hematology Department, University College London Cancer Institute, London, United Kingdom
[13]Department of Hematology and Central Hematology Laboratory, University Hospital of Bern, Bern, Switzerland

INTRODUCTION

The advent of CD19-targeted chimeric antigen receptor (CAR) T-cell therapy has significantly improved the prognosis of patients with relapsed/refractory (R/R) large B-cell lymphoma (LBCL).[1–3] However, in the third or later line setting, only 30%–40% of patients will achieve durable responses.[2–11] Therefore, over 60% of patients will experience disease progression with dismal long-term outcomes.[12–14] Consequently, it is essential to optimize each aspect of the CAR T-cell journey, to increase the chances of benefiting from this T cell-engaging strategy.

A high tumor burden at the time of CAR T-cell treatment is associated with an increased risk of severe toxicity and lower efficacy after infusion.[15–19] Bridging therapy (BT) after leukapheresis, during CAR T-cell manufacturing, is a key step to achieve disease control prior to CAR T-cells. This is especially relevant when a prolonged turnaround is expected and/or the patient has an aggressive disease biology.[20–23] Across centers, BT regimens are heterogeneous and dependent on patient status and local reimbursement policies. Bendamustine-containing regimens are a potential BT option with increasing use after the Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval of rituximab-bendamustine-polatuzumab (RBP) for R/R LBCL.[24] Bendamustine is an alkylating agent with prolonged lymphotoxic capacity that has been discouraged prior to leukapheresis given its deleterious impact on T-cell fitness and CAR T-cell outcomes in the B-cell lymphoma setting.[25–28] However, its use as an alternative lymphodepleting chemotherapy regimen has shown a favorable toxicity profile and similar efficacy to the standard combination of fludarabine and cyclophosphamide (Flu/Cy).[29–31] Nonetheless, the potential benefit of treatment schemas including this agent in the bridging period is not well documented. The additive effect of bendamustine bridging followed by Flu/Cy lymphodepletion could lead to an increased risk of hematological toxicity and infections after CAR T-cell therapy, as reported when this agent was administered prior to leukapheresis.[27] Also, the deeper lymphodepletion provided by the sequential administration of both regimens could have an impact on endogenous non-CAR T-cells of the tumor microenvironment which contribute to CAR T-cell efficacy and immune reconstitution after infusion.[32–34]

In this multicenter study, we aimed to evaluate the impact of bendamustine-containing BT in CAR T-cell recipients in the third or later line setting who were not exposed to this agent before leukapheresis. Also, we compared the bridging success and CAR T-cell outcomes of patients receiving this BT approach with other systemic regimens.

METHODS

Patients

We conducted a retrospective, multicenter study including patients with R/R LBCL treated at six European sites with CD19-targeted commercial CAR T-cells in the third or later line of treatment from February 2019 until September 2022 (Table S1). Patients who had been exposed to bendamustine before leukapheresis were excluded, as were patients who did not receive any BT or who only received steroids and/or radiotherapy (Figure S1).

All included patients met label criteria for CAR T-cell therapy and received lymphodepleting chemotherapy (LDC) with fludarabine and cyclophosphamide. Grading of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) was performed following the American Society for Transplantation and Cellular Therapy criteria[35] and management followed local guidelines. Response evaluation was based on local PET/CT assessment, following Lugano criteria.[36] Each participant provided informed consent, and the study was approved by the ethics committee of the Vall d'Hebron Hospital Board (PR[AG]404/2020).

Definitions and endpoints

We analyzed pre-CAR T-cell therapy characteristics, response to BT, and CAR T-cell efficacy depending on the bridging strategy, focusing on bendamustine-containing regimens versus other systemic combinations. In terms of response to BT, patients were classified into two categories: *progressors* to systemic BT included those with progressive disease (PD) as best response to BT, whereas *non-progressors* to BT included patients in complete response (CR), partial response (PR) or stable disease (SD) after BT. Analyses were carried out for the full patient population and separately according to the CAR T-cell construct.

Efficacy endpoints after CAR T-cell infusion included overall response rate (ORR [CR/PR]), CR rate, progression-free survival (PFS, defined as the time from CAR T-cell infusion until relapse, progression, or death from any cause), and overall survival (OS, defined as the time from CAR T-cell infusion until death from any cause).

Absolute lymphocyte peak and CAR T-cell expansion analysis

To assess the lymphocyte peak after CAR T-cell therapy, each center provided the highest absolute lymphocyte count value and the date of this value reached by each patient in the first 28 days after infusion.

In terms of CAR T-cell kinetics, five centers contributed circulating CAR T-cell data, locally assessed by flow cytometry, using labeled CD19 protein methodology as previously described.[27,37–40] Peak expansion, days from CAR T-cell infusion to peak, and area under the curve for the first 28 days after infusion (AUC_{0-28}) were recorded.

Statistical analyses

We conducted a descriptive analysis of baseline variables in the bendamustine and non-bendamustine groups. Frequencies and percentages were reported for categorical variables and median with interquartile range (IQR) for numerical variables. We employed a logistic model to identify significant differences between the two cohorts.

Regarding response rates, we reported percentages and performed univariable and multivariable logistic models to identify significant differences. We reported odds ratios (OR) with 95% confidence intervals (CI) and *p*-values.

Both PFS and OS were analyzed using the Kaplan–Meier method. We used a univariable and multivariable Cox proportional hazards model to detect differences in survival endpoints. We reported hazard ratios (HR) with 95% confidence intervals and *p*-values.

[14]Haematology Department, Hospital Clínico Universitario, Valencia, Spain
[15]INCLIVA Research Institute, Valencia, Spain
[16]Hematology Department, Hospital Virgen del Rocío, Instituto de Biomedicina de Sevilla (IBIS)/CSIC, Universidad de Sevilla, Sevilla, Spain
[17]Cancer Research Centre (IBMCC, USAL-CSIC), Institute for Biomedical Research of Salamanca (IBSAL) and Department of Medicine and Cytometry Service (NUCLEUS Research Support Platform), University of Salamanca (USAL), Salamanca, Spain

To assess whether there were significant differences in CAR T-cell expansion between both cohorts, we utilized a test of proportions. We estimated the area under the curve (AUC) from 0 to 28 days using the trapezoidal rule.

Completely random missing values were imputed in the variables included in the multivariable model with the Multivariate Imputation via the Chained Equations method in order to avoid omitting cases.[41] All statistical analyses were conducted using R software version 4.2.2.[42]

RESULTS

Characteristics of the patient population

Of 439 patients with R/R LBCL who received commercial CAR T-cell therapy in the participating sites during the study period, we excluded patients who had been exposed to bendamustine before leukapheresis ($n = 80$), as well as those who did not receive any bridging after lymphocyte collection ($n = 64$) or only received steroids ($n = 26$) or radiotherapy ($n = 26$). The final study population included 243 patients who received systemic BT after leukapheresis for CAR T-cell manufacturing (Figure S1). In terms of the BT regimens, 62 (26%) patients received a benda-containing regimen (RBP in 53 patients and rituximab-bendamustine [RB] in nine patients) while 181 (74%) received a systemic approach based on different agents (gemcitabine [47%], cyclophosphamide [22%], etoposide [17%], lenalidomide [4%], or other [10%]) (Figure S2 and Table S2). Most patient- and lymphoma-related characteristics at the time of CAR T-cell therapy were balanced between the benda- and non-benda BT groups for the overall population (Table 1) and separately for axi-cel and tisa-cel recipients (Table S3). However, a higher proportion of patients from the benda-containing BT received axicabtagene ciloleucel (axi-cel), in comparison to the non-benda cohort (68% vs. 53%, $p = 0.04$). The median follow-up for the full cohort was 21.4 months (95% CI: 20–25).

Response to bridging therapy

Of the full study population, 234 (96%) patients had an available disease evaluation after BT, prior to LDC. Disease response included 13 (6%) patients achieving CR, 41 (17%) PR, 46 (20%) SD, and 134 (57%) with PD after BT. Patients in the benda-bridging group had a significantly lower rate of PD after BT (45% [benda] vs. 62% [non-benda], $p = 0.02$) and a numerically higher CR rate (10% vs. 4%, $p = 0.09$). Taking a closer

TABLE 1 Baseline characteristics of infused patients.

	All patients *N* = 243	Non-Benda *N* = 181	Benda *N* = 62	*p*
Median age, years (IQR)	60 (48–67)	60 (48–67)	61 (50–68)	0.64
Male gender, *n* (%)	147 (61)	110 (61)	37 (60)	0.88
ECOG > 1, *n* (%)*	22 (9)	17 (10)	5 (8)	0.74
Histology, *n* (%)				
DLBCL	190 (78)	138 (76)	52 (84)	Ref.
HGBL	32 (13)	27 (15)	5 (8)	0.17
PMBL	15 (6)	10 (6)	5 (8)	0.62
THRLBCL	6 (3)	6 (3)	0	0.99
Transformed lymphoma, *n* (%)*	43 (18)	34 (19)	9 (15)	0.44
>2 previous lines, *n* (%)	76 (31)	58 (32)	18 (29)	0.66
Primary refractory, *n* (%)*	116 (48)	86 (48)	30 (49)	0.85
IPI score 3–5, *n* (%)*	101 (47)	79 (48)	22 (44)	0.66
Previous HCT–*n* (%)				
Autologous	59 (92)	47 (92)	12 (92)	Ref.
Allogeneic	2 (3)	2 (4)	0	0.99
Construct				
Axi-cel	138 (57)	96 (53)	42 (68)	Ref.
Tisa-cel	105 (43)	85 (47)	20 (32)	**0.04**
Turnaround, median days (IQR)	42 (38–53)	42 (38–53)	41 (36–49)	0.16
LDH > ULN, *n* (%)[a,*]	157 (67)	123 (70)	34 (61)	0.22
CRP > ULN, *n* (%)[a,*]	145 (61)	110 (63)	35 (58)	0.57
Ferritin >ULN, *n* (%)[a,*]	172 (79)	124 (78)	48 (83)	0.40
CAR-HEMATOTOX ≥ 2, *n* (%)[a]	122 (58)	92 (59)	30 (54)	0.48

Note: % assessed with *N* of available data for each variable. Bold value indicates significant $p < 0.05$.

Abbreviations: Benda, Bendamustine; CRP, C-reactive protein; DLBCL, diffuse large B-cell lymphoma; ECOG, Eastern Cooperative Oncology Group; HCT, hematopoietic cell transplant; HGBL, high-grade B-cell lymphoma; IPI, International Prognostic Index; IQR, interquartile range; LDH, lactate dehydrogenase; PMBL, primary mediastinal B-cell lymphoma; THRLBCL, T-cell/histiocyte-rich large B-cell lymphoma; ULN, Upper Limit of Normal.

[a]Laboratory values at the time of lymphodepletion.

*Missing data in the following variables (*N* from the full infused data set): ECOG (2), transformed lymphoma (1), primary refractory (2), IPI (27), LDH (10), CRP (7), ferritin (25), HEMATOTOX score (31).

look at the non-benda group, PD following BT was high across all treatment regimens (53% in cyclophosphamide, 75% in etoposide, 63% in gemcitabine, and 71% in lenalidomide).

Impact of bridging therapy on CAR T-cell outcomes

Efficacy

Response rates. Of the full study population, 226/243 (93%) patients were evaluable for best response assessment after CAR T-cell infusion. Overall response rates were similar for the benda and non-benda BT cohorts (73% vs. 68%), with a significantly higher CR rate in the former (62% [benda] vs. 45% [non-benda], $p = 0.04$). Taking into account that the benda BT group was enriched with axi-cel recipients, we conducted separate analyses for each construct. In the axi-cel subgroup ($n = 125$), the CR rate was numerically increased in the benda BT cohort (70% [benda] vs. 53% [non-benda], $p = 0.12$). However, similar outcomes were noted when dividing axi-cel patients according to BT response (75% [benda] vs. 67% [non-benda] [$p = 0.70$] for *non-progressors* and 62% [benda] vs. 47% [non-benda] for BT *progressors* [$p = 0.54$]). For tisa-cel ($N = 101$), the CR rate after infusion was comparable between both BT groups (44% [benda] vs. 36% [non-benda], $p = 0.70$); the subanalysis according to BT response showed similar findings (71% [benda] vs. 53% [non-benda] [$p = 0.66$] for *non-progressors* and 27% [benda] vs. 26% [non-benda] for *progressors* [$p = 1$]) (Figure 1 and Table 2).

Finally, we conducted a multivariate analysis (MVA) for CR; type of CAR-T product (axi-cel vs. tisa-cel), LDH (normal vs. increased), and CRP values (normal vs. increased) at the time of CAR T-cell therapy maintained a significant impact on CR rate. The odds ratio for CR analyzed by type of BT (non-benda vs. benda) was 0.53 for the latter group, with a 95% confidence interval of 0.25–1.08 ($p = 0.084$).

Survival. Median PFS (mPFS) for the full study population was 4.90 months (95% CI: 3.06–7.16), with comparable results for the benda and non-benda groups (HR: 0.84 [95% CI: 0.58–1.21], $p = 0.35$). Restricting the analysis to axi-cel, 12-month PFS was similar for both BT cohorts (56% [benda] vs. 43% [non-benda], $p = 0.32$); no differences were observed when this analysis was further restricted to BT *non-progressors* (67% vs. 63%, $p = 0.57$) and *progressors* (37% vs. 32%, $p = 0.85$). In terms of tisa-cel recipients, 12-month PFS was also similar among BT groups (25% [benda] vs. 26% [non-benda], $p = 0.72$); again, no differences were noted across cohorts for BT *non-progressors* (38% vs. 39%, $p = 0.95$) and *progressors* (17% vs. 18%, $p = 0.59$) (Figure 2). In the MVA, the type of CAR-T product (axi-cel vs. tisa-cel), response to bridging (CR/PR/SD vs. PD), and ECOG (0-1 vs. >1) at the time of CAR T-cell therapy maintained their prognostic impact for PFS; type of bridging (non-benda vs. benda) was not a significant prognostic factor for PFS (HR: 1.03 [CI: 95% 0.70–1.51], $p = 0.87$).

Median OS (mOS) for all study patients was 16.46 months (95% CI: 13.04–26.55), without differences for the benda and non-benda BT groups (HR: 0.79 [95% CI: 0.51–1.22], $p = 0.29$). Focusing on axi-cel, there was a similar 12-month OS between BT cohorts (71% [benda] vs. 63% [non-benda], $p = 0.41$); no difference was observed between both groups when this analysis was restricted to *non-progressors* (70% vs. 82%, $p = 0.76$) and *progressors* (74% vs. 53%,

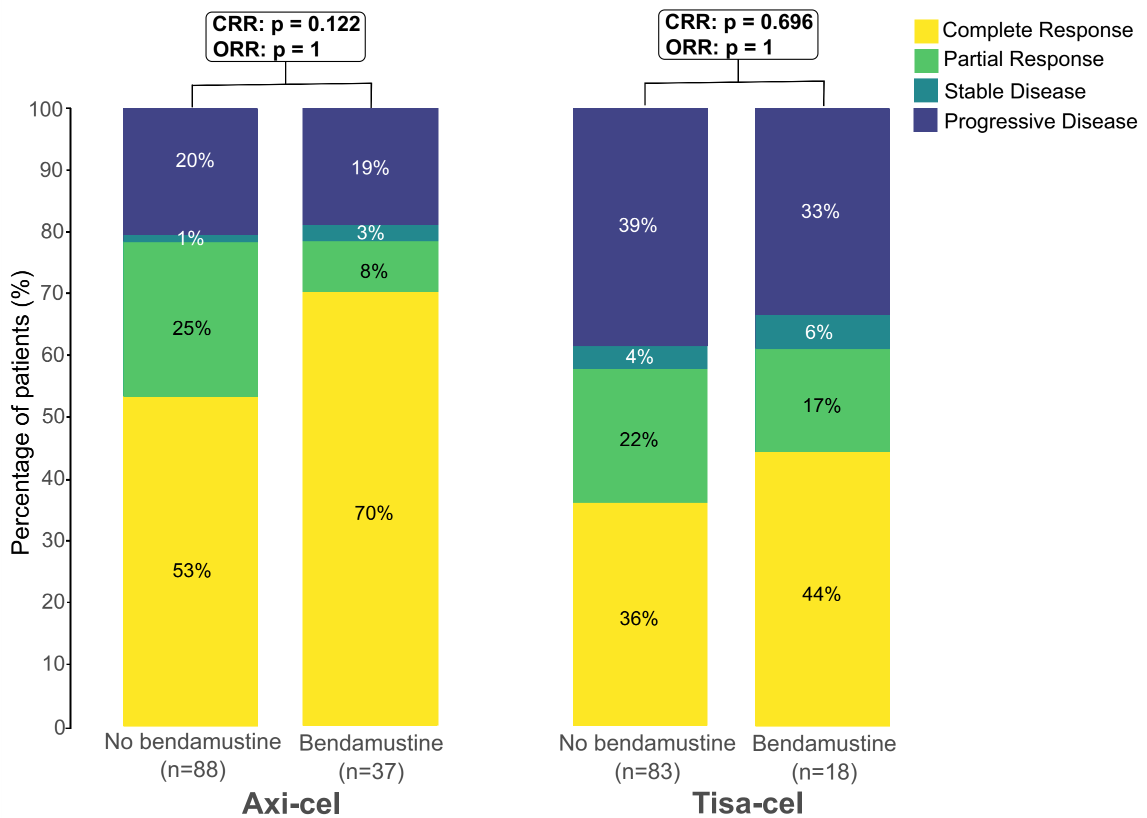

FIGURE 1 Response rates to axi-cel and tisa-cel according to the bridging treatment (including bendamustine and non-bendamustine regimens).

TABLE 2 Response rates and survival outcomes after CAR T-cell therapy according to the bridging regimen and response to bridging in the axi-cel and tisa-cel cohorts.

	All			Patients in CR/PR/SD after bridging			Patients in PD after bridging		
	Benda	No Benda	*p*	Benda	No Benda	*p*	Benda	No Benda	*p*
Axi-cel									
Response rate									
ORR, % (95% CI)	78 (61–90)	78 (68–86)	1	88 (67–97)	91 (75–98)	1	62 (32–85)	74 (59–84)	0.60
CRR, % (95% CI)	70 (53–84)	53 (43–64)	0.12	75 (53–89)	67 (48–81)	0.93	62 (32–85)	47 (34–61)	0.51
Survival									
PFS, median mo (95% CI)	23.5 (7.0–NR)	6.6 (3.7–26.6)	0.32	NR (NR–NR)	26.6 (3.5–NR)	0.09	5.5 (1.2–NR)	6.6 (3.2–23.6)	0.28
PFS HR (95% CI)	0.77 (0.46–1.29)			0.32 (0.08–1.20)			1.36 (0.78–2.37)		
OS, median mo (95% CI)	29.9 (23.5–NR)	23.6 (14.0–NR)	0.41	NR (NR–NR)	36.2 (18.1–NR)	0.50	23.5 (10.1–NR)	22.5 (13.0–NR)	0.88
OS HR (95% CI)	0.78 (0.43–1.41)			0.61 (0.14–2.62)			1.05 (0.54–2.03)		
Tisa-cel									
Response rate									
ORR, % (95% CI)	61 (36–82)	58 (46–68)	1	86 (42–99)	80 (61–92)	1	45 (18–75)	48 (33–63)	1
CRR, % (95% CI)	44 (22–69)	36 (26–47)	0.70	71 (30–95)	53 (35–71)	1	27 (7–61)	26 (15–41)	1
Survival									
PFS, median mo (95% CI)	3.1 (1.0–21.8)	2.8 (1.7–3.3)	0.72	7.2 (1.0–NR)	8.9 (2.7–NR)	0.61	2.9 (1.0–18.9)	2.8 (1.7–3.2)	0.29
PFS HR (95% CI)	1.10 (0.64–1.88)			0.71 (0.19–2.66)			1.38 (0.76–2.50)		
OS, median mo (95% CI)	14.5 (5.9–NR)	11.1 (7.8–15.1)	0.98	7.2 (5.9–NR)	13.3 (9.2–NR)	0.96	14.5 (3.4–NR)	11.9 (7.8–24.7)	0.83
OS HR (95% CI)	0.99 (0.52–1.91)			1.03 (0.27–3.92)			1.08 (0.51–2.32)		

Abbreviations: 95% CI, 95% confidence interval; Benda, bendamustine; CRR, complete response rate; HR, hazard ratio; mo, months; *n*, number of patients; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

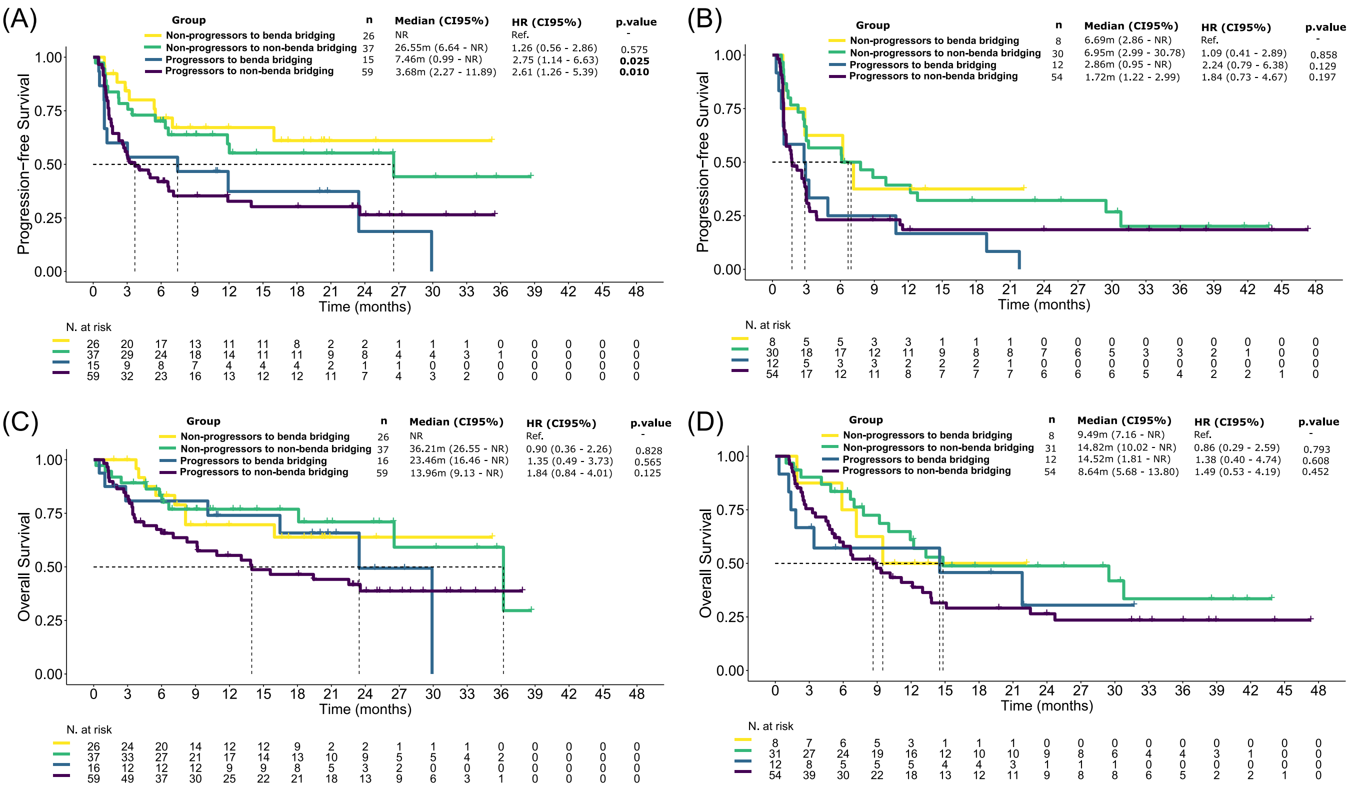

FIGURE 2 Survival outcomes according to the type of bridging (bendamustine vs. non-bendamustine) and the response to bridging (nonprogressors [CR/PR/SD] vs. progressors [PD]). (A) Progression-free survival for axi-cel patients. (B) Progression-free survival for tisa-cel patients. (C) Overall survival for axi-cel patients. (D) Overall survival for tisa-cel patients.

$p = 0.46$). For tisa-cel, 12-month OS was comparable for both BT groups (52% [benda] vs. 48% [non-benda], $p = 0.98$); the subanalysis for *non-progressors* (50% vs. 61%, $p = 0.74$) and *progressors* (57% vs. 41%, $p = 0.86$) showed no differences for the benda and non-benda BT cohorts (Figure 2). In the MVA, LDH (normal vs. high) and ECOG (0–1 vs. >1) at the time of CAR T-cell therapy maintained their impact; type of bridging (non-benda vs. benda) was not a significant prognostic factor for OS (HR: 0.92 [CI: 95% 0.59–1.44], $p = 0.72$).

Absolute lymphocyte count and CAR T-cell expansion analysis

In terms of the absolute lymphocyte count during the first 28 days after CAR T-cell infusion, there were no differences in the peak value between benda and non-benda BT patients (median 0.89 [IQR: 0.53–1.35] vs. 0.94 [IQR: 0.49–1.50], $p = 0.29$); median days to peak were also similar between cohorts (13 [benda] vs. 14 [non-benda], $p = 0.52$).

Focusing on the 141 (58%) patients of the full study population who had available CAR T-cell expansion data (Table S1), peak expansion was similar between the benda and non-benda BT groups, both for axi-cel (59 vs. 53 CAR T-cells/µL, $p = 0.23$) and tisa-cel (53 vs. 38 CAR T-cells/µL, $p = 0.25$). Comparable results were observed for AUC_{0-28} with axi-cel (725 [benda] vs. 588 [non-benda], $p = 0.19$) and tisa-cel (889 [benda] vs. 308 [non-benda], $p = 0.14$) (Figure 3). Median days to peak were not significantly different between the benda and non-benda BT cohorts ($p = 0.29$).

Safety

Of the full data set, 209 (86%) patients presented CRS and 97 (40%) developed ICANS, any grade; both the benda and non-benda BT cohorts had comparable rates of CRS (57 [92%]) vs. 152 [84%], $p = 0.15$) and ICANS (25 [40%] vs. 72 [40%], $p = 0.99$). Considering each construct separately, we observed similar rates of any grade CRS for the benda and non-benda BT cohorts with axi-cel (91% vs. 96%, $p = 0.41$) and tisa-cel (95% vs. 72%, $p = 0.06$). Furthermore, no difference between BT groups was observed for ICANS with axi-cel (52% vs. 57%, $p = 0.77$) and tisa-cel (15% vs. 21%, $p = 0.74$).

In terms of grade ≥3 CRS, there were no differences between the benda and non-benda BT cohorts for axi-cel (5% vs. 4%, p = n.s.) and tisa-cel (10% vs. 7%, $p = 1$). In terms of grade ≥3 ICANS, events were also comparable for axi-cel (19% benda vs. 28% non-benda, $p = 0.34$) and tisa-cel (5% benda vs. 4% non-benda, $p = 1$).

Focusing on other adverse events, the rate of grade ≥3 neutropenia was similar between the benda and non-benda BT cohorts in the first month (43% vs. 47%, $p = 0.64$) and third month (27% vs. 14%, $p = 0.09$) after CAR T-cell infusion (Figure S3). Also, there was a comparable rate of any grade and severe or worse infections in both cohorts (47% and 51% [$p = 0.58$] and 21% vs. 19% [$p = 0.78$])[43,44] (Figure 4). Non-relapse mortality (NRM) at 100 days post-CAR T-cell infusion for the benda and non-benda groups was 3% (2/62) and 5% (9/181), respectively ($p = 0.82$). Overall, the main cause for NRM was infections (8 of 11 patients, Table S4), followed by bowel perforation (2/11) and CRS (1/11).

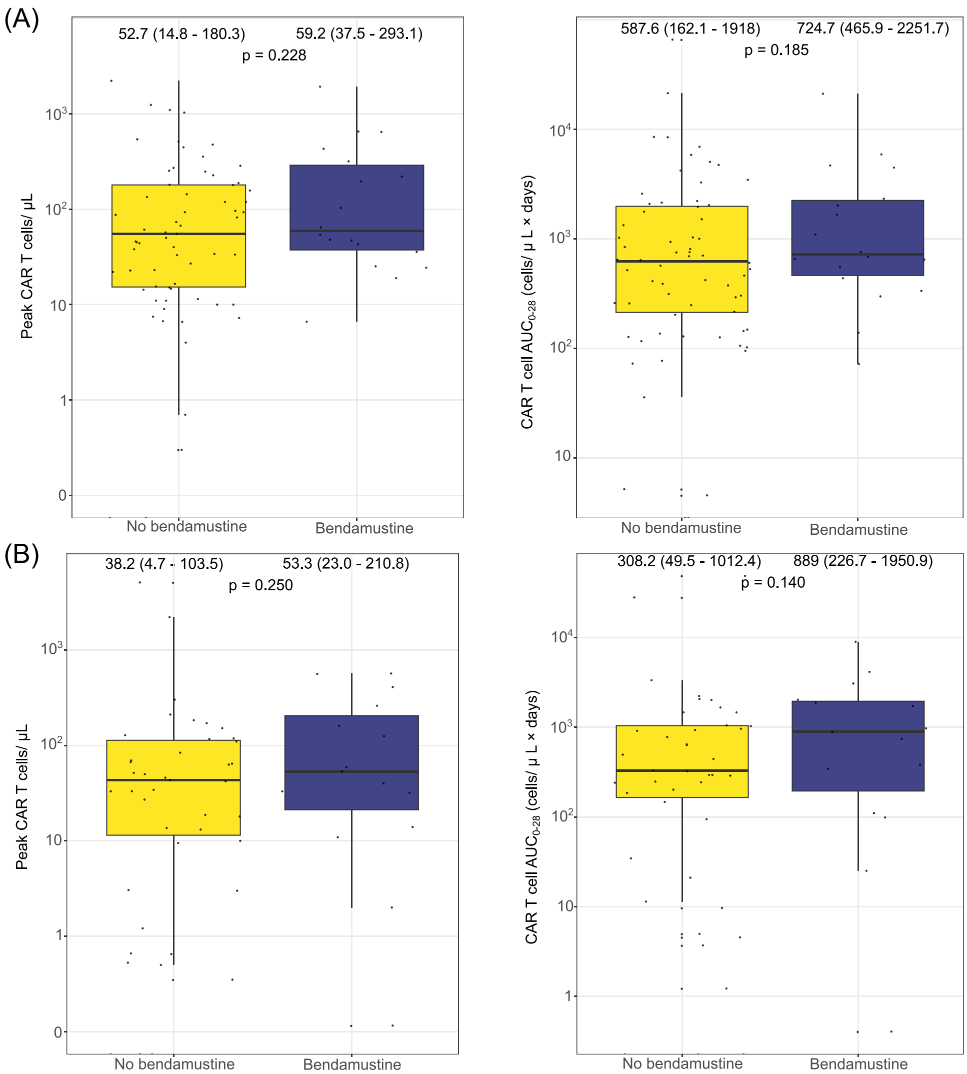

FIGURE 3 CAR T-cell expansion analysis according to the type of bridging. (A) Peak CAR T-cell expansion and area under the curve during the first 28 days after infusion (AUC_{0-28}) in axi-cel (A) and tisa-cel (B) patients receiving bendamustine or non-bendamustine bridging regimens. Values stand for median peak (interquartile range, IQR).

DISCUSSION

In light of the negative prognostic impact of pre-leukapheresis bendamustine exposure on CAR T-cell efficacy in the B-cell lymphoma setting,[26–28] and considering the scarce data focusing on the impact of this alkylating agent in the bridging period, we analyzed CAR T-cell outcomes in R/R LBCL patients receiving benda-containing BT and carried out a comparison with patients receiving other systemic bridging strategies. Both approaches showed similar safety and survival outcomes after CAR T-cell infusion, confirming that benda-containing BT is a feasible and effective approach after lymphocyte collection for CAR T-cell manufacturing.

In our patient population, both cohorts were balanced for most baseline characteristics. However, there was a higher rate of axi-cel in the benda cohort, possibly related to the more recent availability of RBP for R/R LBCL patients, in parallel to an increased use of axi-cel over the years in our European cohort (36% in 2018–2019, 61% in 2020–2021, and 74% in 2022). To overcome this limitation and the potential differences in the safety and efficacy profile between products,[5–8] we conducted separate analyses for each construct.

The first aim of this study was to analyze the response rate to bridging in both cohorts. Patients receiving bendamustine as part of BT were more likely to display a response than patients receiving other systemic approaches without bendamustine, suggesting improved disease control with this agent. This result is in line with the recently published study by Roddie et al. which highlighted that patients receiving RBP were twice as likely to respond to bridging than patients receiving other chemotherapy regimens.[21]

Second, we focused on CAR T-cell efficacy outcomes. Patients receiving bendamustine-based bridging had a higher chance of achieving a CR after infusion. However, these results were mitigated in the construct-specific subanalysis and when response to BT was considered, confirming that response to bridging is the key prognostic factor for long-term disease control after CAR T-cell infusion, as previously published.[6,7,21,22] Considering survival outcomes, we did not observe significant differences in terms of PFS nor OS after CAR T-cell infusion between the benda and non-benda BT cohorts.

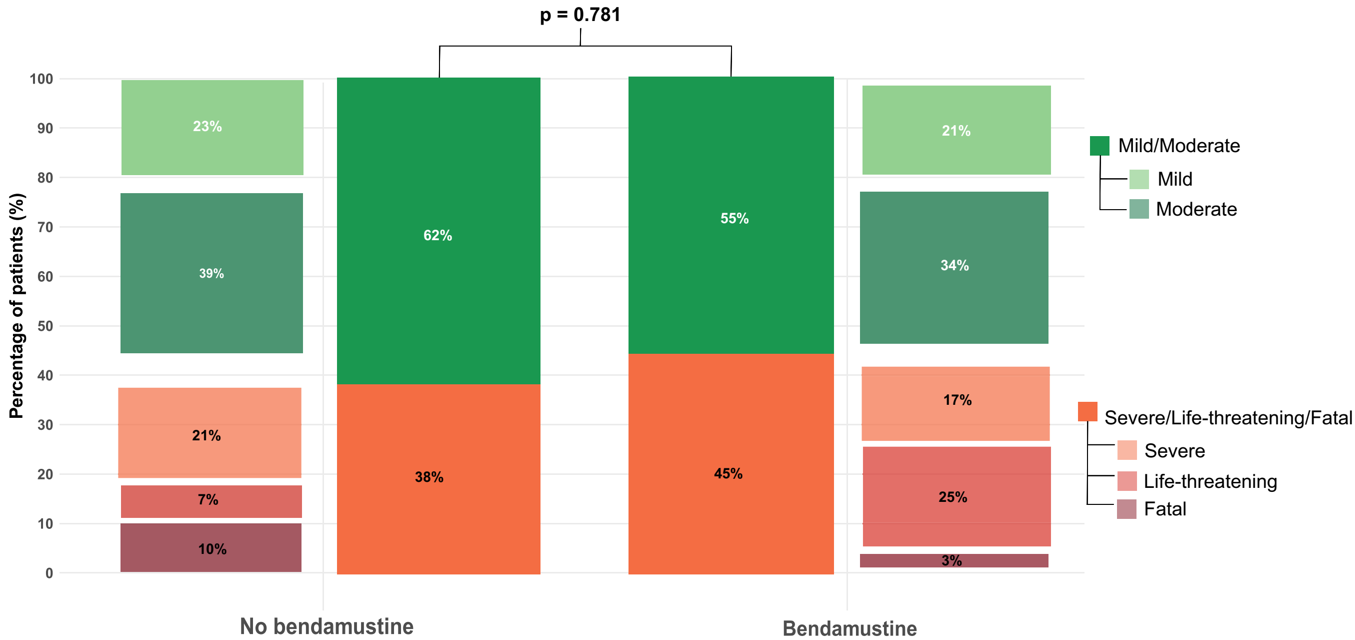

FIGURE 4 Comparison of the severity of infectious events after CAR T-cell infusion in patients receiving bendamustine and non-bendamustine bridging regimens. This analysis only included patients who experienced at least one infectious event.

Moreover, our results were consistent after dividing patients according to BT outcomes: *non-progressors* to benda versus non-benda BT had a similar PFS and OS after CAR T-cell infusion, as did *progressors* after both types of BT. These comparable results are further supported by the available benda LDC data, with non-inferior results to the standard fludarabine and cyclophosphamide conditioning regimen prior to tisa-cel and axi-cel infusion.[29,30]

Considering the significant role that CAR T-cell expansion plays in durable responses,[19] we also analyzed expansion kinetics. We did not observe significant differences in the peak expansion or AUC_{0-28} between both cohorts. Noteworthy, values of these parameters were numerically higher in the benda group for both axi-cel and tisa-cel. A deeper lymphodepletion with bendamustine followed by fludarabine and cyclophosphamide could potentially explain these CAR T-cell kinetics. The limited number of patients with available circulating CAR T-cell data in each subgroup could have underpowered this analysis and prevented the identification of significant differences.

It is a well-known fact that non-CAR lymphocytes and macrophages are the main source of IL-6, participating in the development of CRS and ICANS,[32] and T-cells play a key role in the prevention of opportunistic infections.[45–49] Therefore, we examined in detail the safety profile of both BT cohorts. Any grade and grade ≥3 CRS and ICANS rates were similar between the benda and non-benda BT groups and comparable to published reports.[5–11] We did not identify differences in severe infections after CAR T-cell therapy either. Overall, these results underline that benda-based BT does not negatively impact CAR T-cell expansion, toxicity outcomes, and long-term survival after CAR T-cell infusion.

The main limitations of this study are derived from its retrospective nature, preventing us from making a completely unbiased assignment of bridging regimens and CAR-T construct selection. The small number of patients in this study receiving RB bridging (without polatuzumab) or R-Polatuzumab (without bendamustine), precluded a specific analysis of these regimens. Additionally, we only included patients who received a CAR T-cell infusion, so we cannot confirm if the better disease control obtained with the benda-containing BT strategies reduced the drop-out rate from leukapheresis; this issue should be addressed in future studies carried out with an intention-to-treat analysis. However, the large, multicenter character of this study is also an advantage, including patients from focused European sites with extensive experience in CAR T-cell management. Also, unlike other studies evaluating BT in the CAR T-cell context,[20,21] we only included patients receiving systemic BT; radiotherapy and steroids as monotherapy were excluded. Hence, we were able to answer a clinically relevant question in a more homogeneous cohort who received similar BT intensity in both groups.

In conclusion, bendamustine-containing bridging regimens provide improved disease control prior to LDC compared to non-benda strategies, with similar safety and long-term survival outcomes after CAR T-cell infusion.

ACKNOWLEDGMENTS

The authors thank the patients and their families for their participation in this study.

AUTHOR CONTRIBUTIONS

Conception and design: Gloria Iacoboni, Mario Andrés Sánchez-Salinas and Pere Barba. Provision of study patients: Gloria Iacoboni, Mario Andrés Sánchez-Salinas, Kai Rejeski, Ana África Martín-López, Mi Kwon, Katarzyna Aleksandra Jalowiec, Rafael Hernani, Juan Luis Reguera-Ortega, Claire Roddie, Ana Benzaquén, Javier Delgado-Serrano, Rebeca Bailén, Cecilia Carpio, Lucia López-Corral, Mariana Bastos, Marion Subklewe, Maeve O'Reilly and Pere Barba. Data collection and analysis: Gloria Iacoboni, Mario Andrés Sánchez-Salinas, Kai Rejeski, Víctor Navarro, Laura Gallur, Viktoria Blumenberg, María Herrero-García, Paula Amat, Lourdes Martín-Martín and Pere Barba. Manuscript writing: All authors. Final approval of manuscript: All authors. Accountable for all aspects of the work: All authors.

CONFLICT OF INTEREST STATEMENT

Gloria Iacoboni: Consultancy and Honoraria: Novartis, Roche, Kite/Gilead, Bristol-Myers Squibb, Abbvie, Janssen, Sandoz, Miltenyi, AstraZeneca. Mario A. Sánchez-Salinas Honoraria for presentations: Kite. Support for attending meetings: Takeda. Kai Rejeski: Kite/Gilead: Research Funding, Consultancy, Honoraria and travel support; Novartis: Honoraria; BMS/Celgene: Consultancy, Honoraria; Pierre-Fabre: travel support. Mi Kwon Consulting and lectures: Gilead, Jazz, Pfizer. Katarzyna A. Jalowiec Honoraria: Kite/Gilead. Rafael Hernani: Research: Gilead. Travel support: Gilead. Honoraria: Gilead, Janssen, MSD, Celgene, Novartis. Viktoria Blumenberg: BMS/Celgene: Research Funding; Kite/Gilead: Consultancy, Honoraria, Research Funding; Janssen: Research Funding, Honoraria; Novartis: Research Funding, Honoraria; Roche: Consultancy, Research Funding; Takeda: Research Funding. Claire Roddie: Honoraria from Kite/Gilead, Novartis, BMS, Amgen. Javier Delgado-Serrano: Honoraria from Kite-Gilead, Novartis, Bristol Myers Squibb, Janssen. Rebeca Bailén: Speaker and travel: Kite. Cecilia Carpio: Regeneron: Consultancy/Advisory, BMS: Consultancy/Advisory, Takeda: Consultancy/Advisory/Honoraria, Novartis: Honoraria. Marion Subklewe: receives industry research support from Amgen, Bristol-Myers Squibb/Celgene, Gilead, Janssen, Miltenyi Biotec, Morphosys, Novartis, Roche, Seattle Genetics, and Takeda, and serves as a consultant/advisor to AvenCell, CDR-Life, Ichnos Sciences, Incyte Biosciences, Janssen, Molecular Partners, and Takeda. She serves on the speakers' bureau at Amgen, AstraZeneca, BMS/Celgene, Gilead, GSK, Janssen, Novartis, Pfizer, Roche, and Takeda. Maeve O'Reilly: Honoraria from Kite, Novartis, Janssen. Advisory boards Kite and Autolus. Travel grant Kite and Novartis. Pere Barba: Allogene: Honoraria; Amgen: Honoraria; BMS: Honoraria; Kite/Gilead: Honoraria; Janssen: Honoraria; Jazz Pharmaceuticals: Honoraria; Miltenyi: Honoraria; Novartis: Honoraria; Nektar: Honoraria.

DATA AVAILABILITY STATEMENT

Data are available upon request from the authors.

ETHICS STATEMENT

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

FUNDING

P. B. received research funding from the Carlos III Health Institute (PI21/0199, INT23/00072), Asociación Española contra el Cáncer (Ideas Semilla 2019), and a PERIS 2018-2020 grant from the Generalitat de Catalunya (BDNS357800).

ORCID

Gloria Iacoboni http://orcid.org/0000-0003-0805-9288
Víctor Navarro http://orcid.org/0000-0001-6925-4605
Katarzyna A. Jalowiec http://orcid.org/0000-0003-3284-8308

SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

REFERENCES

1. Locke FL, Ghobadi A, Jacobson CA, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1-2 trial. *Lancet Oncol*. 2019;20(1):31-42. doi:10.1016/S1470-2045(18)30864-7
2. Schuster SJ, Tam CS, Borchmann P, et al. Long-term clinical outcomes of tisagenlecleucel in patients with relapsed or refractory aggressive B-cell lymphomas (JULIET): a multicentre, open-label, single-arm, phase 2 study. *Lancet Oncol*. 2021;22(10):1403-1415. doi:10.1016/S1470-2045(21)00375-2
3. Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet*. 2020;396(10254):839-852. doi:10.1016/S0140-6736(20)31366-0
4. Neelapu SS, Jacobson CA, Ghobadi A, et al. 5-year follow-up supports curative potential of axicabtagene ciloleucel in refractory large B-Cell Lymphoma (ZUMA-1). *Blood*. 2023;141:2307-2315. doi:10.1182/blood.2022018893
5. Bachy E, Le Gouill S, Di Blasi R, et al. A real-world comparison of tisagenlecleucel and axicabtagene ciloleucel CAR T cells in relapsed or refractory diffuse large B cell lymphoma. *Nat Med*. 2022;28(10):2145-2154. doi:10.1038/s41591-022-01969-y
6. Kwon M, Iacoboni G, Reguera JL, et al. Axicabtagene ciloleucel compared to tisagenlecleucel for the treatment of aggressive B-cell lymphoma. *Haematologica*. 2023;108(1):110-121. doi:10.3324/haematol.2022.280805
7. Bethge WA, Martus P, Schmitt M, et al. GLA/DRST real-world outcome analysis of CAR T-cell therapies for large B-cell lymphoma in Germany. *Blood*. 2022;140(4):349-358. doi:10.1182/blood.2021015209
8. Kuhnl A, Roddie C, Kirkwood AA, et al. A national service for delivering CD19 CAR-Tin large B-cell lymphoma—The UK real-world experience. *Br J Haematol*. 2022;198(3):492-502. doi:10.1111/bjh.18209
9. Jacobson CA, Locke FL, Ma L, et al. Real-world evidence of axicabtagene ciloleucel for the treatment of large B cell lymphoma in the United States. *Transpl Cell Therapy*. 2022;28(9):581.e1-581.e8. doi:10.1016/j.jtct.2022.05.026
10. Pasquini MC, Hu ZH, Curran K, et al. Real-world evidence of tisagenlecleucel for pediatric acute lymphoblastic leukemia and non-Hodgkin lymphoma. *Blood Adv*. 2020;4(21):5414-5424. doi:10.1182/bloodadvances.2020003092
11. Iacoboni G, Villacampa G, Martinez-Cibrian N, et al. Real-world evidence of tisagenlecleucel for the treatment of relapsed or refractory large B-cell lymphoma. *Cancer Med*. 2021;10(10):3214-3223. doi:10.1002/cam4.3881
12. Alarcon Tomas A, Fein JA, Fried S, et al. Outcomes of first therapy after CD19-CAR-T treatment failure in large B-cell lymphoma. *Leukemia*. 2023;37(1):154-163. doi:10.1038/s41375-022-01739-2
13. Zurko JC, Nizamuddin I, Epperla N, et al. Peri-CAR-T practice patterns and survival predictors for all CAR-T patients and post-CAR-T failure in aggressive B-NHL. *Blood Adv*. 2023;7(12):2657-2669. doi:10.1182/bloodadvances.2022008240
14. Di Blasi R, Le Gouill S, Bachy E, et al. Outcomes of patients with aggressive B-cell lymphoma after failure of anti-CD19 CAR T-cell therapy: a DESCAR-T analysis. *Blood*. 2022;140:2584-2593. doi:10.1182/blood.2022016945
15. Vercellino L, Di Blasi R, Kanoun S, et al. Predictive factors of early progression after CAR T-cell therapy in relapsed/refractory diffuse large B-cell lymphoma. *Blood Adv*. 2020;4(22):5607-5615. doi:10.1182/bloodadvances.2020003001
16. Iacoboni G, Simó M, Villacampa G, et al. Prognostic impact of total metabolic tumor volume in large B-cell lymphoma patients receiving CAR T-cell therapy. *Ann Hematol*. 2021;100(9):2303-2310. doi:10.1007/s00277-021-04560-6
17. Dean EA, Mhaskar RS, Lu H, et al. High metabolic tumor volume is associated with decreased efficacy of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv*. 2020;4(14):3268-3276. doi:10.1182/bloodadvances.2020001900

18. Rejeski K, Perez A, Iacoboni G, et al. The CAR-HEMATOTOX risk-stratifies patients for severe infections and disease progression after CD19 CAR-T in R/R LBCL. *J Immunother Cancer*. 2022;10(5):e004475. doi:10.1136/jitc-2021-004475
19. Locke FL, Rossi JM, Neelapu SS, et al. Tumor burden, inflammation, and product attributes determine outcomes of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv*. 2020;4(19):4898-4911. doi:10.1182/bloodadvances.2020002394
20. Pinnix CC, Gunther JR, Dabaja BS, et al. Bridging therapy prior to axicabtagene ciloleucel for relapsed/refractory large B-cell lymphoma. *Blood Adv*. 2020;4(13):2871-2883. doi:10.1182/bloodadvances.2020001837
21. Roddie C, Neill L, Osborne W, et al. Effective bridging therapy can improve CD19 CAR-T outcomes while maintaining safety in patients with large B-cell lymphoma. *Blood Adv*. 2023;7(12):2872-2883. doi:10.1182/bloodadvances.2022009019
22. Bücklein V, Perez A, Rejeski K, et al. Inferior outcomes of EU cersus US patients treated with CD19 CAR-T for relapsed/refractory large B-cell lymphoma: association with differences in tumor burden, systemic inflammation, bridging therapy utilization, and CAR-T product use. *HemaSphere*. 2023;7(8):e907. doi:10.1097/HS9.0000000000000907
23. Jain MD, Jacobs MT, Gao F, et al. Bridging therapy with axicabtagene ciloleucel for large B-cell lymphoma: results from the US lymphoma CAR-T consortium. *Blood Adv*. 2024;8(4):1042-1050. doi:10.1182/bloodadvances.2023011489
24. Sehn LH, Hertzberg M, Opat S, et al. Polatuzumab vedotin plus bendamustine and rituximab in relapsed/refractory DLBCL: survival update and new extension cohort data. *Blood Adv*. 2022;6(2):533-543. doi:10.1182/bloodadvances.2021005794
25. Jain T, Bar M, Kansagra AJ, et al. Use of chimeric antigen receptor T cell therapy in clinical practice for relapsed/refractory aggressive B cell non-hodgkin lymphoma: an expert panel opinion from the american society for transplantation and cellular therapy. *Biol Blood Marrow Transplant*. 2019;25(12):2305-2321. doi:10.1016/j.bbmt.2019.08.015
26. Wang M, Munoz J, Goy A, et al. Three-year follow-up of KTE-X19 in patients with relapsed/refractory mantle cell lymphoma, including high-risk subgroups, in the ZUMA-2 study. *J Clin Oncol*. 2023;41(3):555-567. doi:10.1200/JCO.21.02370
27. Iacoboni G, Navarro V, Martín-López A, et al. Recent bendamustine treatment before apheresis has a negative impact on outcomes in patients with large B-cell lymphoma receiving chimeric antigen receptor T-cell therapy. *J Clin Oncol*. 2024;42(2):205-217. doi:10.1200/JCO.23.01097
28. Neelapu SS, Chavez JC, Sehgal A, et al. Three-year follow-up analysis of axicabtagene ciloleucel in relapsed/refractory indolent non-hodgkin lymphoma (ZUMA-5). *Blood*. 2024;143(6):496-506. doi:10.1182/blood.2023021243
29. Ghilardi G, Chong EA, Svoboda J, et al. Bendamustine is safe and effective for lymphodepletion before tisagenlecleucel in patients with refractory or relapsed large B-cell lymphomas. *Ann Oncol*. 2022;33(9):916-928. doi:10.1016/j.annonc.2022.05.521
30. Ong SY, Pak S, Mei M, et al. Bendamustine lymphodepletion is a well-tolerated alternative to fludarabine and cyclophosphamide lymphodepletion for axicabtagene ciloleucel therapy for aggressive B-cell lymphoma. *Am J Hematol*. 2023;98(11):1751-1761. doi:10.1002/ajh.27069
31. Ghilardi G, Paruzzo L, Svoboda J, et al. Bendamustine lymphodepletion before axicabtagene ciloleucel is safe and associates with reduced inflammatory cytokines. *Blood Adv*. 2024;8(3):653-666. doi:10.1182/bloodadvances.2023011492
32. Chen PH, Lipschitz M, Weirather JL, et al. Activation of CAR and non-CAR T cells within the tumor microenvironment following CAR T cell therapy. *JCI Insight*. 2020;5(12):e134612. doi:10.1172/jci.insight.134612
33. Scholler N, Perbost R, Locke FL, et al. Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. *Nat Med*. 2022;28(9):1872-1882. doi:10.1038/s41591-022-01916-x
34. Locke FL, Filosto S, Chou J, et al. Impact of tumor microenvironment on efficacy of anti-CD19 CAR T cell therapy or chemotherapy and transplant in large B cell lymphoma. *Nat Med*. 2024;30:507-518. doi:10.1038/s41591-023-02754-1
35. Lee DW, Santomasso BD, Locke FL, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant*. 2019;25(4):625-638. doi:10.1016/j.bbmt.2018.12.758
36. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014;32(27):3059-3067. doi:10.1200/JCO.2013.54.8800
37. Demaret J, Varlet P, Trauet J, et al. Monitoring CAR T-cells using flow cytometry. *Cytometry Part B: Clin Cytom*. 2021;100(2):218-224. doi:10.1002/cyto.b.21941
38. Brando B, Barnett D, Janossy G, et al. Cytofluorometric methods for assessing absolute numbers of cell subsets in blood. *Cytometry*. 2000;42(6):327-346. doi:10.1002/1097-0320(20001215)42:6<327::AID-CYTO1000>3.0.CO;2-F
39. Peinelt A, Bremm M, Kreyenberg H, et al. Monitoring of circulating CAR T cells: validation of a flow cytometric assay, cellular kinetics, and phenotype analysis following tisagenlecleucel. *Front Immunol*. 2022;13:830773. doi:10.3389/fimmu.2022.830773
40. Blumenberg V, Busch G, Baumann S, et al. Early quantification of anti-CD19 CAR T cells by flow cytometry predicts response in R/R DLBCL. *Blood Adv*. 2023;7(22):6844-6849. doi:10.1182/bloodadvances.2023010364
41. van Buuren S, Groothuis-Oudshoorn K. mice: multivariate imputation by chained equations in R. *J Stat Softw*. 2011;45(3):1-67. doi:10.18637/jss.v045.i03
42. Team R. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing; 2008. http://www.r-project.org/
43. Hill JA, Li D, Hay KA, et al. Infectious complications of CD19-targeted chimeric antigen receptor-modified T-cell immunotherapy. *Blood*. 2018;131(1):121-130. doi:10.1182/blood-2017-07-793760
44. Young JAH, Logan BR, Wu J, et al. Infections after transplantation of bone marrow or peripheral blood stem cells from unrelated donors. *Biol Blood Marrow Transplant*. 2016;22(2):359-370. doi:10.1016/j.bbmt.2015.09.013
45. Martínez-Calle N, Hartley S, Ahearne M, et al. Kinetics of T-cell subset reconstitution following treatment with bendamustine and rituximab for low-grade lymphoproliferative disease: a population-based analysis. *Br J Haematol*. 2019;184(6):957-968. doi:10.1111/bjh.15722
46. Cona A, Tesoro D, Chiamenti M, et al. Disseminated cytomegalovirus disease after bendamustine: a case report and analysis of circulating B- and T-cell subsets. *BMC Infect Dis*. 2019;19(1):881. doi:10.1186/s12879-019-4545-7
47. Yutaka T, Ito S, Ohigashi H, et al. Sustained CD4 and CD8 lymphopenia after rituximab maintenance therapy following bendamustine and rituximab combination therapy for lymphoma. *Leuk Lymphoma*. 2015;56(11):3216-3218. doi:10.3109/10428194.2015.1026818
48. Saito H, Maruyama D, Maeshima AM, et al. Prolonged lymphocytopenia after bendamustine therapy in patients with relapsed or refractory indolent B-cell and mantle cell lymphoma. *Blood Cancer J*. 2015;5(10):e362. doi:10.1038/bcj.2015.86
49. Gafter-Gvili A, Polliack A. Bendamustine associated immune suppression and infections during therapy of hematological malignancies. *Leuk Lymphoma*. 2016;57(3):512-519. doi:10.3109/10428194.2015.1110748