

Impact of fludarabine exposure on CAR T-cell outcomes in patients with large B-cell lymphoma

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Key Points

- Prospective serial fludarabine sampling was feasible, and PK-guided modeling identified optimal dosing ranges in the CAR T-cell setting.
- Optimal fludarabine exposure during lymphodepletion correlated with improved PFS in patients with R/R LBCL receiving CAR T-cell therapy.

The impact of fludarabine pharmacokinetics (PK) on chimeric antigen receptor (CAR) T-cell outcomes is not fully elucidated. We hypothesized that in patients with relapsed/refractory (R/R) large B-cell lymphoma (LBCL), measured fludarabine exposure during lymphodepletion would correlate with CAR T-cell results. We conducted a prospective, single-center study including patients with R/R LBCL treated with commercial, CD19-targeted CAR T-cell products. Up to 7 plasma samples per patient were collected at predefined intervals during lymphodepletion. Fludarabine exposure (area under the curve [AUC]) was estimated using a population PK model. An iterative cubic spline approach identified optimal AUC exposure ranges specific to each CAR T-cell product. The primary end point was progression-free survival (PFS). Among 54 patients with a median follow-up of 24.5 months, the median fludarabine AUC was 18.12 mg·h/L (interquartile range, 14.56-20.55). Optimal exposure windows were 17.5 to 21.5 mg·h/L for axicabtagene ciloleucel (axi-cel) and 14.0 to 18.0 mg·h/L for tisagenlecleucel, with 24 patients (44%) inside these ranges. Median PFS after CAR T-cell infusion was not reached vs 13.3 months in the optimal vs other cohort ($P = .03$). In the multivariable analysis, optimal exposure remained independently associated with improved PFS ($P = .03$), together with axi-cel treatment ($P = .03$). CAR T-cell expansion and rates of any grade cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome, and hematotoxicity were similar between groups. In conclusion, the prospective measurement of fludarabine exposure identified an optimal AUC window associated with prolonged PFS in patients with R/R LBCL receiving CAR T-cell therapy. These findings support the integration of PK-guided fludarabine dosing to enhance therapeutic outcomes.

Introduction

Chimeric antigen receptor (CAR) T-cell therapy targeting CD19 has become the standard of care for patients with relapsed/refractory (R/R) large B-cell lymphoma (LBCL). Despite the promising efficacy

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Data are available from the corresponding author, Marta Miarons (mmiarons@chv.cat), on request.

The full-text version of this article contains a data supplement.

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results,^{1,2} a substantial proportion of patients do not achieve a durable remission.³⁻⁵ Significant efforts have been made to elucidate variables that can predict efficacy and toxicity after infusion. Among them, patients' performance status,⁵ tumor burden,⁵⁻⁷ and inflammation biomarkers such as ferritin and C-reactive protein⁸ have been associated with long-term outcomes. CAR T-cell pharmacokinetics (PK) such as a higher expansion peak^{8,9} have also proven relevant. CAR T-cell PK are driven by a wide variety of factors including cell dose,¹⁰ density of target antigen,⁸ and the lymphodepleting chemotherapy (LDC) regimen.¹¹⁻¹³ Although intensity and response to LDC has been associated with better outcomes,¹⁴ there are scarce data regarding the specific impact of fludarabine PK parameters on CAR T-cell efficacy.

The predictive value of estimated fludarabine exposure was initially explored in the context of allogeneic stem cell transplantation (allo-HCT), suggesting that optimizing fludarabine dosage could significantly affect survival.¹⁵ In the CAR T-cell setting, adding fludarabine to cyclophosphamide enhances CAR T-cell expansion and persistence and is associated with prolonged event-free survival.^{11,16} Specifically, optimal fludarabine PK parameters in patients with B-cell acute lymphoblastic leukemia (B-ALL) have shown a relevant role in CAR T-cell outcomes.^{17,18} In multiple myeloma, Sweiss et al reported that estimated fludarabine overexposure was associated with a higher incidence of CAR T-cell-related toxicities (cytokine release syndrome [CRS], immune effector cell-associated neurotoxicity syndrome [ICANS], and hematotoxicity) in patients receiving idecabtagene vicleucel.¹⁹ In the LBCL setting, Scordo et al estimated a theoretical fludarabine exposure in patients treated with axicabtagene ciloleucel (axi-cel), identifying an optimal window linked to improved outcomes.²⁰ Although this estimation has inherent limitations,²¹ it highlights the potential for a PK-guided dosing approach that could potentially affect CAR T-cell results. Notably, previous studies reported an extremely high variability in measured and estimated fludarabine exposure,^{15,18,22} supporting the need for an optimized method of fludarabine exposure estimation in patients undergoing CAR T-cell therapy.

Given the key role of fludarabine in the LDC regimen, we hypothesized that achieving an optimal exposure to this agent could significantly influence CAR T-cell outcomes. To that extent, we conducted a prospective study focusing on measured fludarabine exposure in patients with R/R LBCL receiving commercial CAR T cells and assessing its impact on efficacy and safety results.

Methods

Study population

This study prospectively included all consecutive patients with R/R LBCL (de novo or transformed from an indolent lymphoma) who received a single infusion of commercially available CD19-targeted CAR T cells in the third or later line setting from March 2021 to September 2022 at Vall d'Hebron University Hospital.

Patient management

Patients received LDC according to the manufacturer recommendations of axi-cel, (fludarabine at 30 mg/m² per day for 3 days and cyclophosphamide at 500 mg/m² per day for 3 days) and tisagenlecleucel (tisa-cel; fludarabine at 25 mg/m² per day for

3 days and cyclophosphamide at 250 mg/m² per day for 3 days). Grading of CRS and ICANS followed the American Society for Transplantation and Cellular Therapy criteria²³ and management was in line with local institutional guidelines. Immune effector cell-associated hematotoxicity for neutropenia (N-ICAHT) was graded retrospectively using the European Hematology Association/European Society for Blood and Marrow Transplantation criteria.²⁴ CAR T-cells were assessed in peripheral blood using flow cytometry on days 7, 14, and 28 after CAR T-cell infusion, as previously described.²⁵

Determination of fludarabine exposure

In total, 7 blood samples were scheduled between the start of LDC and CAR T-cell infusion: on day 1 of LDC (90 minutes and 120 minutes after the start of fludarabine administration), on day 2 of LDC (before fludarabine administration), on day 3 of LDC (90 minutes, 120 minutes, and 24 hours after the start of fludarabine administration), and 30 minutes before CAR T-cell infusion. All patients received a 30-minute IV administration of fludarabine. Therefore, the time between the end of fludarabine and sample collection was 1 hour, 1.5 hours, and 23.5 hours. After 3 to 5 days of washout, patients received CAR T cells. Patients with <4 samples or who received CAR T cells after a washout longer than 5 days were excluded from the analysis.

Fludarabine is administered as the monophosphate (f-ara-AMP), the corresponding f-ara-A dose equivalents were obtained by multiplying the administered f-ara-AMP doses by 0.78, which is equivalent to the ratio of F-Ara-A molecular weight (285 g/mol) to fludarabine phosphate molecular weight (365.2 g/mol) as previously described.²⁶ Serum fludarabine concentrations were determined using a validated ultraperformance liquid chromatography coupled with tandem mass spectrometry assay based on liquid-liquid extraction and chromatographic separation on a C18 column with tandem mass detection in positive electrospray ionization mode. Calibration was linear over 1.5 to 1000 ng/mL, with accuracy and precision within $\pm 10\%$ and a lower limit of quantification of 1.5 ng/mL. Quality controls and stability tests met the European Medicines Agency/US Food and Drug Administration bioanalytical validation criteria. Samples were stored at -80°C until analysis. Chromatographic analyses were performed on a Waters Acquity UPLC system coupled to a Xevo TQ MS detector (Waters Corporation, Milford, MA).

Fludarabine exposure was defined as the cumulative area under the plasma concentration-time curve up to the last measured concentration ($\text{AUC}_{0-\text{last}}$), expressed in milligram-hour per liter. Both $\text{AUC}_{0-\infty}$ and $\text{AUC}_{0-\text{last}}$ were calculated. In this study, exposure was defined using $\text{AUC}_{0-\text{last}}$. This choice was driven by the sampling scheme, which included plasma concentrations collected at the end of the elimination phase. The availability of measured concentrations at late time points allowed $\text{AUC}_{0-\text{last}}$ to be defined based on observed data, without the need for extrapolation beyond the last concentration. In contrast, several previously published studies^{18,27,28} did not include late postinfusion samples, which may have limited the reliable estimation of $\text{AUC}_{0-\text{last}}$ and necessitated alternative definitions of exposure. Therefore, given the richness of the concentration-time data in our study, $\text{AUC}_{0-\text{last}}$ was considered the most appropriate and robust exposure metric.

Individual $\text{AUC}_{0-\text{last}}$ values were derived from a 3-compartment population PK (popPK) model developed through nonlinear

mixed-effects modeling in NONMEM.²⁹ Individual post hoc Bayesian estimates were then applied to compute cumulative fludarabine AUCs across the 3 lymphodepleting doses, which were subsequently used as exposure metrics in the pharmacodynamic and clinical outcome analyses.

Definitions and end points

The primary end point was the impact of fludarabine exposure on progression-free survival (PFS) defined as the time from CAR T-cell infusion until relapse, progression of disease according to Lugano criteria,³⁰ or death from any cause, whichever happened first. Secondary end points included overall survival (OS) defined as the time from CAR T-cell infusion until death from any cause or last follow-up, the cumulative incidence of relapse, overall response rate (ORR; complete and partial responders), complete response rate (CRR), and occurrence of toxicity-related events, including CRS, ICANS, and ICAHT. Additional outcomes of interest included CAR T-cell expansion data, including CAR T-cell median peak and median AUC₀₋₂₈.

Statistical analysis

A descriptive analysis was performed for baseline variables across the overall population and the optimal and nonoptimal cohort. Categorical variables were summarized as frequencies and percentages, whereas numerical variables were reported as medians with interquartile ranges (IQR). Differences between cohorts were assessed using the Wilcoxon tests for numerical variables and the χ^2 tests for categorical variables.

Response rates were expressed as percentages, and differences between cohorts were evaluated using the χ^2 tests. A multivariable logistic regression model was used to compare response rates while adjusting for relevant covariates. Both PFS and OS were analyzed using the Kaplan-Meier method. Differences in survival outcomes were further evaluated using a Cox proportional hazards model, with hazard ratios (HRs), 95% confidence interval (CI), and *P* values reported. For the analysis of relapse incidence, a competing risk model was used, considering death as the competing event and relapse as the event of interest. The Fine and Gray subdistribution hazard model was applied to estimated HRs with their corresponding 95% CI and *P* values.

To visualize the impact of fludarabine exposure (AUC) on PFS at different time points along the AUC range, we applied cubic splines to a Cox proportional hazards model, treating AUC as a continuous variable and PFS as the end point (Figure 1). Optimal AUC intervals were identified using an iterative procedure that tested intervals of varying width and position, selecting the range that maximized the log-rank statistic. To limit computational cost, a minimum interval width of 0.5 units was imposed. The identified interval was then used as the optimal exposure range for all outcomes.

All statistical analyses were performed using R software version 4.2.2. Results were considered statistically significant if *P* < .05.

The Institutional Review Board of the Vall d'Hebron Hospital Board approved the study (PI21/00197). All patients provided written informed consent for the use of clinical data and laboratory results. All procedures in this study were performed in accordance with the Declaration of Helsinki.

Results

Characteristics of the patient population

A total of 60 patients were included in the study; 6 were excluded from the analysis due to insufficient sample collection, resulting in a final cohort of 54 patients. Baseline characteristics stratified by fludarabine exposure are summarized in Table 1. The median age at the time of infusion was 59 years (IQR, 51-68), with male predominance (56%). The median number of previous lines was 2 (IQR, 2-4). Fifteen (28%) patients had an International Prognostic Index of 3 to 5, and most (59%) patients had an advanced stage. After leukapheresis, 43 (80%) patients received bridging therapy. Disease assessment before LDC confirmed an ORR to bridging of 23% (complete response [CR] in 7%). The median body surface area and creatinine levels before the LDC were 1.8 m² (IQR, 1.6-1.9) and 0.77 mg/dL (IQR, 0.65-0.93), respectively. Regarding the CAR T-cell construct, 37 (69%) and 17 (31%) patients received axi-cel and tisa-cel, respectively. The median follow-up of the full study cohort was 24.5 months (95% CI, 24.1-28.7).

Determination of fludarabine exposure

The median number of samples per patient for fludarabine assessment was 7 (IQR, 6-7). A total of 342 (90% of those initially planned) were collected: 30 patients with 7 samples, 16 patients with 6 samples, 4 patients with 5 samples, and 4 patients with 4 samples (supplemental Figure 1). The median measured cumulative fludarabine exposure AUC for the entire cohort was 18.12 mg·h/L (IQR, 14.56-20.55; range, 10.17-33.83). When analyzing each CAR T-cell product separately, the median AUC was 18.43 mg·h/L (IQR, 17.03-21; range, 10.81-33.83) for axi-cel and 14.34 mg·h/L (IQR, 13.72-18.17; range, 10.17-23.97) for tisa-cel. The optimal AUC range was identified to be 17.5 to 21.5 mg·h/L (Figure 1A) for axi-cel recipients and 14.0 to 18.0 mg·h/L for tisa-cel (Figure 1B). Taking this into consideration, 24 (44%) patients were inside the optimal cohort and 30 (56%) were included in the nonoptimal cohort.

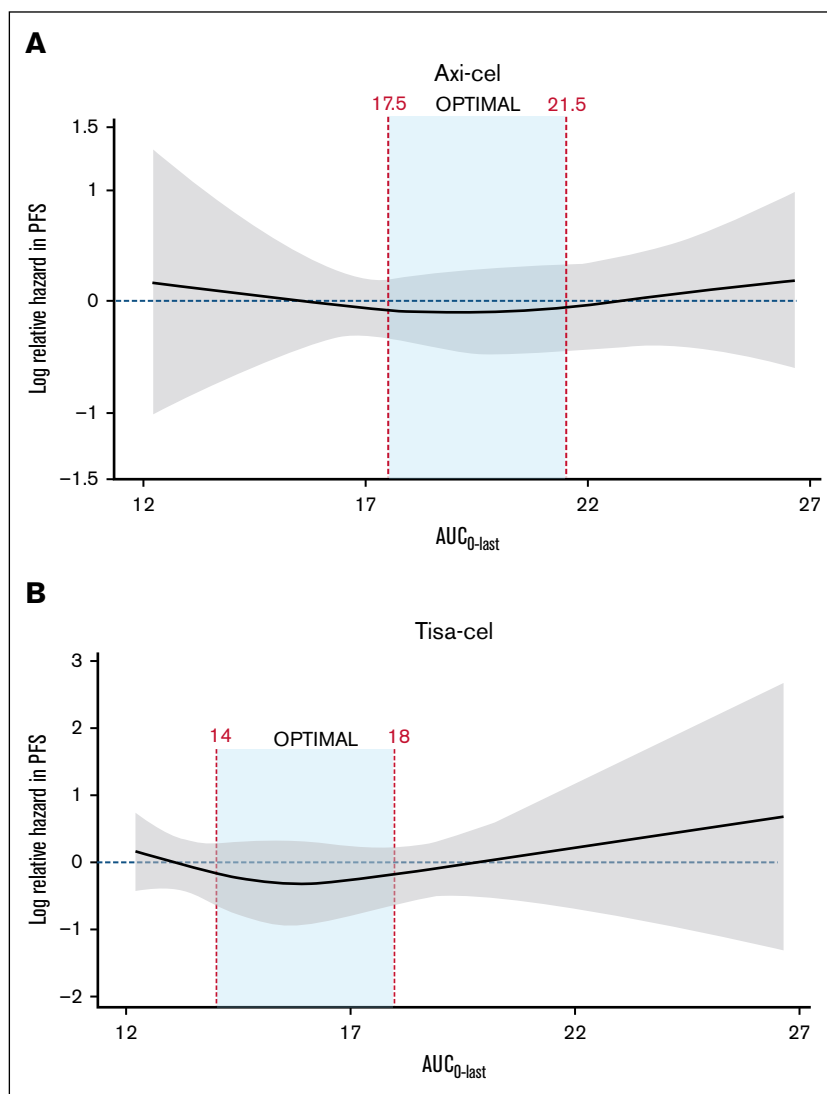
Fludarabine exposure and efficacy

Of the study cohort, 53 (98%) patients were evaluable for disease response. Complete response was observed in 34 (64%) patients, whereas 11 (21%) achieved a partial response. Progression disease (PD) was the best response in the remaining 8 (15%) patients. When evaluating the construct, axi-cel was associated with a significantly higher CRR compared with tisa-cel (69% vs 53%; *P* = .016). Patients in the optimal cohort had a numerically higher CRR than those in the nonoptimal cohort (supplemental Figure 2) (71% vs 59%; *P* = .3) (Table 2). Moreover, the multivariable analysis (supplemental Table 1) revealed that optimal fludarabine AUC was associated with a trend toward a higher ORR following CAR T-cell therapy (odds ratio, 0.06; 95% CI, 0.00-0.99; *P* = .08).

In terms of survival, median PFS after CAR T-cell infusion was not reached in the optimal cohort (95% CI, 26 months to not reached) compared with 13.3 months (95% CI, 3.5 months to not reached) in the nonoptimal group (Figure 2A; *P* = .03). In the multivariable analysis (supplemental Table 2), the optimal fludarabine AUC remained associated with improved PFS (HR, 0.31; 95% CI, 0.11-0.89; *P* = .03), as was axi-cel treatment compared with tisa-cel (HR, 0.40; 95% CI, 0.18-0.90; *P* = .03) (Table 2). The median

Figure 1. Relationship between AUC_{0-last} levels and PFS in patients receiving CAR T-cell therapy. (A) Axi-cel; (B) tisa-cel.

The x-axis shows AUC_{0-last} values, and the y-axis represents the log relative hazard for PFS. The black line depicts the estimated risk, with shaded areas indicating the 95% CI. The dashed blue line corresponds to a null effect (log HR = 0). The blue-shaded region highlights the AUC_{0-last} range associated with the lowest risk of progression, suggesting an optimal exposure window of 17.5 to 21.5 for axi-cel and 14 to 18 for tisa-cel.



OS for patients with optimal vs nonoptimal was 34.3 months (95% CI, 26.05 to not reached) vs not reached, respectively ($P = .5$) (Figure 2B). In the multivariable analysis (supplemental Table 3), the optimal fludarabine AUC showed a trend toward prolonged OS (HR, 0.29; 95% CI, 0.07-1.27; $P = .10$), whereas patients treated with axi-cel showed significantly longer OS (HR, 0.27; 95% CI, 0.09-0.77; $P = .01$). The 1-year cumulative incidence of relapse was 17% (95% CI, 6.8-41) vs 43% (95% CI, 29-65) in the optimal vs nonoptimal groups ($P = .05$) (Figure 2C). In the multivariable analysis, the optimal AUC group showed a trend toward a lower risk of relapse (HR, 0.36; 95% CI, 0.11-1.18; $P = .09$) compared with the nonoptimal group.

Fludarabine exposure and toxicity

Among the 54 patients, CRS of any grade was observed in 49 (91%) patients, grade ≥ 3 in 8 (15%) (supplemental Table 2). There were no significant differences between the incidence of any grade (or grade ≥ 3) CRS in the optimal vs nonoptimal cohorts (100% [4%] vs 83% [23%]). ICANS of any grade was observed in

28 (52%) patients, with 5 (9%) grade ≥ 3 events (supplemental Table 2); similarly, no significant differences in any grade (or grade ≥ 3) ICANS were observed between the optimal vs nonoptimal subgroups (54% [0%] vs 50% [17%]).

Regarding hematologic toxicity, N-ICAHT was reported in 45 (83%) patients, with grade ≥ 3 in 7 (13%). There were no significant differences between the incidence of any grade (or grade ≥ 3) N-ICAHT in the optimal vs nonoptimal cohorts (92% [17%] vs 77% [10%]).

In the optimal and nonoptimal cohorts, there were 5 (21%) and 10 (33%) deaths, respectively. The cause of death was relapse/PD ($n = 4$) and acute myeloblastic leukemia (AML) ($n = 1$) in the optimal cohort and relapse/PD ($n = 8$), COVID-19 infection ($n = 1$), and bacterial infection ($n = 1$) in the nonoptimal cohort.

Fludarabine exposure and CAR T-cell kinetics

Regarding the 46 (85%) patients with available CAR T-cell expansion data, median peak was 66 CAR T cells per μL (IQR, 33-128) and median AUC₀₋₂₈ was 637 CAR T cells per

Table 1. Baseline characteristics of infused patients according to fludarabine exposure

Characteristic	All patients (N = 54)	Optimal (n = 24)	Nonoptimal (n = 30)	P value
Median age (IQR), y	59 (51-68)	59 (53-65)	59 (49-69)	1
Male sex, n (%)	30 (56)	12 (50)	18 (60)	.646
ECOG >0, n (%)	30 (56)	17 (71)	13 (43)	.081
Disease type, n (%)				.360
DLBCL	29 (53)	14 (58.3)	15 (50)	
HGBL	3 (6)	2 (8.3)	1 (3)	
tFL	19 (35)	8 (33.3)	11 (37)	
Other	3 (6)	0	3 (10)	
Primary refractory, n (%)	27 (50)	13 (54)	14 (47)	.784
IPI score 3-5, n (%)	15 (28)	5 (21)	10 (33)	.476
Bulky (>7 cm), n (%)	16 (30)	6 (25)	10 (33)	.714
>2 previous lines, n (%)	11 (20)	5 (19)	6 (21)	1
Bridging therapy, n (%)				.745
Chemotherapy	33 (61)	16 (66.6)	17 (57)	
Radiotherapy	10 (18)	4 (16.6)	6 (20)	
None	11 (20)	4 (16.6)	7 (23)	
Creatinine, median (IQR), mg/dL	0.75 (0.64-0.98)	0.73 (0.66-0.97)	0.81 (0.64-1.01)	.614
Estimated GFR, median (IQR), mL/min	98.5 (83.3-122.5)	98 (87-110)	101.5 (81.3-140.8)	.645
LDH level >ULN, n (%)*	28 (53)	16 (67)	12 (40)	.094
CRP level >ULN, n (%)*	32 (59)	12 (50)	20 (67)	.337
Ferritin level >ULN, n (%)*,†	26 (53)	11 (52)	15 (54)	1
CAR-HEMATOTOX ≥2, n (%)†	21 (43)	8 (38)	13 (46)	.771
Construct, n (%)				.534
Axi-cel	37 (69)	18 (75)	19 (63)	
Tisa-cel	17 (31)	6 (25)	11 (37)	

CRP, C-reactive protein; DLBCL, diffuse LBCL; ECOG, Eastern Cooperative Oncology Group; GFR, glomerular filtration rate; HGBL, high-grade B-cell lymphoma; IPI, International Prognostic Index; LDH, lactate dehydrogenase; tFL, transformed follicular lymphoma; ULN, upper limit of normal.

*Laboratory values at time of lymphodepletion.

†Missing data in the following variables: ferritin level (5) and CAR-HEMATOTOX score (5).

$\mu\text{L} \times \text{days}$ (IQR, 300-1500). Median CAR T-cell peak was similar in the optimal vs nonoptimal cohorts (59 [IQR, 36.25-123.75] vs 82 [33.55-143]; $P = .804$) (Figure 3A) as was median AUC_{0-28} (637 [IQR, 358.03-934.6] vs 650 [IQR, 261.54-1980.82];

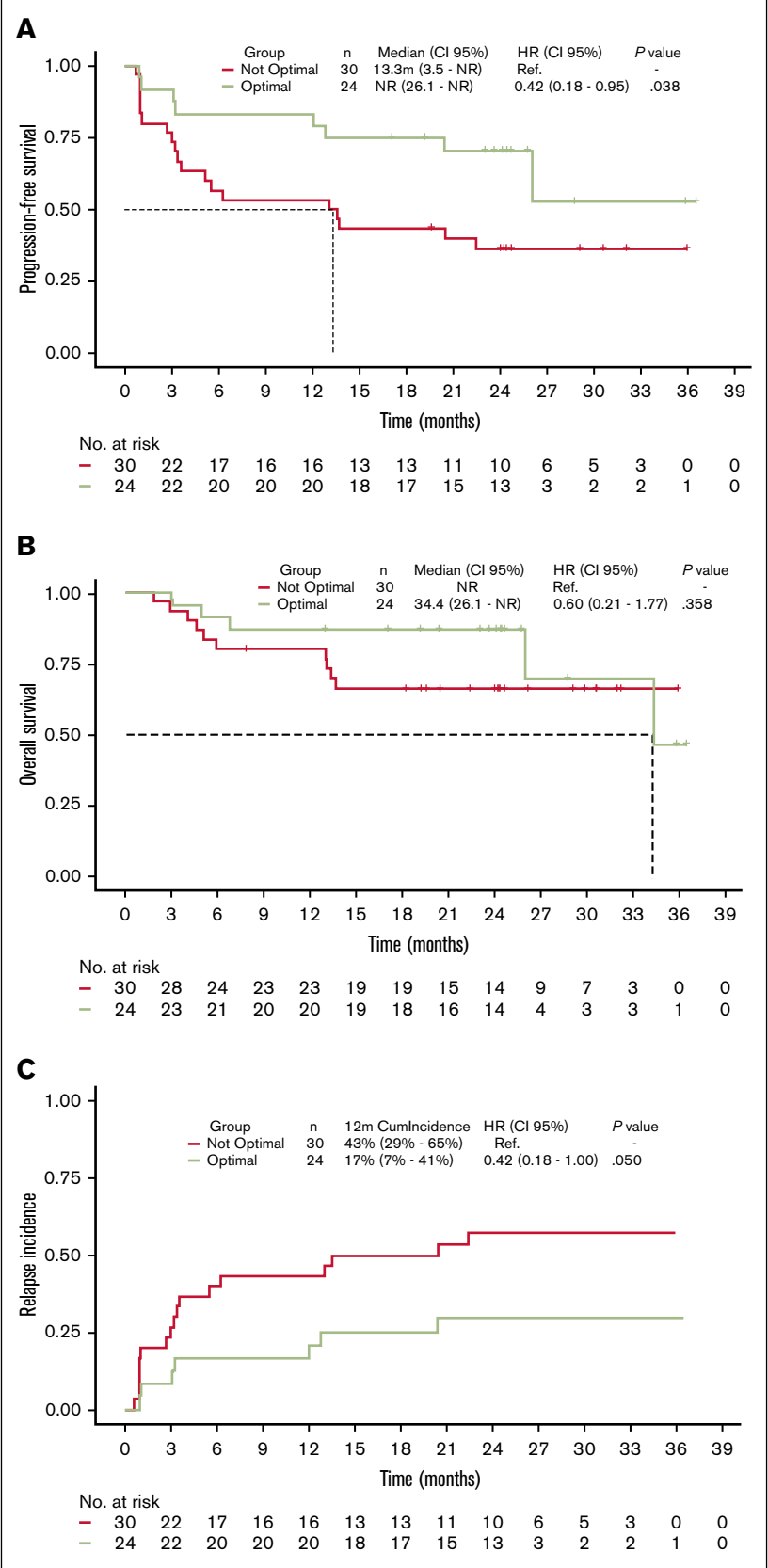
$P = .866$) (Figure 3B). The median time to CAR T-cell peak was 7 (IQR, 7-14) days for both cohorts. There were no significant differences in expansion results between axi-cel and tisa-cel (supplemental Figure 3).

Table 2. Response rate and survival outcomes according to fludarabine exposure

	Optimal (n = 24)	Nonoptimal (n = 30)	P value
Response rate			
ORR (95% CI), %	91.7 (71.5-98.5)	79.3 (59.7-91.3)	.39
CRR (95% CI), %	70.8 (48.8-86.6)	58.6 (39.1-75.9)	.53
Survival			
PFS, median (95% CI), mo	NR (26.05 to NR)	13.27 (3.52 to NR)	.038
PFS, HR (95% CI)		0.31 (0.11-0.89)	.03
OS, median (95% CI), mo	34.4 (26.05 to NR)	NR (NR to NR)	.3
OS, HR (95% CI)		0.55 (0.17-1.77)	.32

NR, not reached.

Figure 2. Impact of fludarabine exposure on efficacy outcomes after CAR T-cell therapy. Patients were stratified according to optimal and not optimal fludarabine exposure. (A) PFS; (B) OS; (C) relapse incidence. Median and HRs for PFS and OS are shown in panels A-B. Cumulative incidence and HRs are shown in panel C. CumIncidence, cumulative incidence; NR, not reached; Ref, reference.



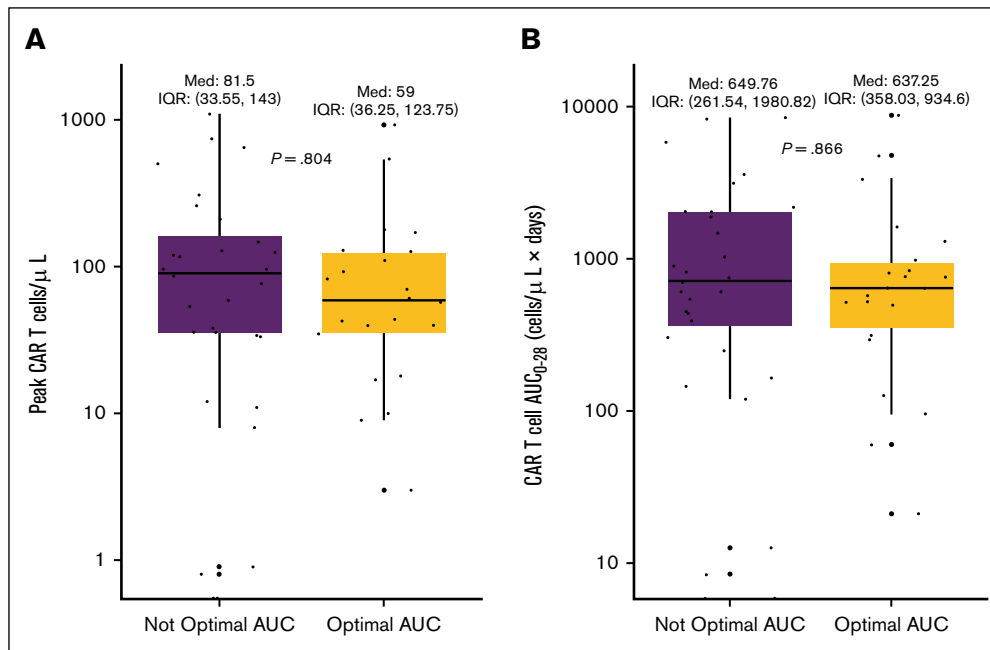


Figure 3. CAR T-cell expansion analysis. (A) Peak CAR T-cell expansion and (B) CAR T-cell AUC during the first 28 days after infusion, according to optimal and not optimal fludarabine exposure. The values stand for median peak (IQR). Med, median.

Discussion

In this study, we aimed to analyze the impact of measured fludarabine levels during LDC on the safety and efficacy of CAR T-cell therapy in patients with R/R LBCL. Our findings show that despite significant variability in fludarabine exposure, patients within an optimal exposure range had improved results compared with those outside the optimal range. To our knowledge, this is the largest analysis to date in patients with these characteristics using measured fludarabine levels. These data further support the growing body of evidence indicating that optimal fludarabine exposure is associated with improved clinical outcomes.

To conduct this study, we developed a popPK model to estimate the total fludarabine exposure. Previous reports relied on a popPK model developed for allo-HCT recipients,³¹ which can lead to inaccuracies when applied to CAR T-cell patients. To address this, we developed a new model incorporating body size, as represented by body weight with allometric scaling, estimated glomerular filtration rate, and type of CAR T-cell construct to estimate fludarabine exposure specifically in patients with lymphoma receiving CAR T cells.²⁹ As we have previously demonstrated, the use of theoretical values in popPK models tends to underestimate the total fludarabine exposure, expressed as AUC, compared with the use of direct serum levels,²¹ a finding also suggested in patients with B-ALL.¹⁸ To address this issue, we prospectively measured serum fludarabine values at 7 different time points during LDC to improve the accuracy of exposure estimation. With these measured levels and our refined model, we calculated individual fludarabine AUCs to identify optimal exposure ranges potentially associated with clinical outcomes.

Previous studies have established optimal fludarabine exposure thresholds in CAR T-cell recipients. Specifically, 2 retrospective

analyses performed in patients with B-ALL, using theoretical¹⁷ and measured¹⁸ fludarabine levels, suggested that patients above a specific fludarabine threshold had improved clinical outcomes, including leukemia-free survival, duration of B-cell aplasia, and lower incidence of CD19⁺ relapse. However, as suggested by Scordo et al, defining an optimal range of fludarabine exposure, rather than a single threshold, may better define the role of fludarabine in this setting and be more accurate to predict clinical outcomes.²⁰ Following this approach, we established optimal AUC ranges for each CAR T-cell construct (axi-cel and tisa-cel), accounting for differences in LDC fludarabine dosing. We then compared outcomes between patients in the optimal vs nonoptimal cohorts.

Our findings suggest a higher probability of response, prolonged PFS, and lower cumulative incidence of relapse in patients with an optimal exposure compared with those above or below it. Interestingly, although there were no significant differences in the incidence of CRS or ICANS, there were less cases of severe CRS (1 vs 7) and ICANS (0 vs 5) in the optimal cohort than in the nonoptimal cohort. In addition, we did not identify significant differences in CAR T-cell PK parameters between the optimal and nonoptimal exposure groups. Although previous studies have reported an association between optimal fludarabine exposure and enhanced CAR T-cell expansion,^{11,18} the absence of such differences in our study could be related to the moderate sample size, which reduces the statistical power to detect differences. A similar limitation may also explain the lack of differences in toxicity outcomes.

This study has additional limitations that need to be acknowledged. First, due to logistical challenges associated with multiple sample collections, our study was conducted at a single center with a relatively small cohort. Consequently, the nonoptimal

exposure group comprised both underexposed and overexposed patients who may experience different clinical outcomes. Owing to the moderate sample size, further stratification of this group was not feasible and would have substantially reduced statistical power; larger studies will be needed to assess the differential impact of underexposure and overexposure on efficacy and toxicity results. Second, despite our prospective study design and stringent sample collection plan, scheduling challenges prevented gathering all 7 samples from every participant, which may have decreased the accuracy of individual PK estimations and their effect on clinical outcomes. However, this study also has several strengths. First, we prospectively measured fludarabine levels at multiple time points, whereas previous studies relied on theoretical fludarabine levels. Second, to improve the accuracy of our analysis, we included only patients with 4 or more available samples. Finally, unlike previous studies that relied on models based on allo-HCT recipients who receive different fludarabine doses and have distinct clinical characteristics, our study determined fludarabine exposure using a recently developed model specifically designed to characterize fludarabine PK in the CAR T-cell setting (supplemental Figure 4). Future prospective studies should explore reduced sampling strategies combined with maximum a posteriori Bayesian estimation to enable practical, model-informed therapeutic drug monitoring of fludarabine in clinical settings.

In conclusion, this study confirms the association between optimal measured fludarabine exposure and improved clinical outcomes in patients with R/R LBCL receiving CAR T-cell therapy. These findings highlight the importance of developing PK models in CAR T-cell patients and the need for accurate fludarabine concentration measurements to enable personalized dosing and optimize treatment outcomes. However, several critical questions remain unanswered, such as considering the simplification of the sampling strategy if predictive accuracy can be maintained. In addition, it is unclear whether further model refinements could also improve accuracy in patients with severe renal impairment. Ongoing efforts are focused on addressing these questions, with the goal of developing a streamlined and personalized fludarabine dosing strategy that can enhance the efficacy and safety of CAR T-cell therapy.

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Authorship

Contribution: P.B. and M.M. conceived the study and were in charge of the overall direction and planning; J.V.-G.-A., M.M., and I.T. developed the population pharmacokinetics model to estimate the fludarabine exposure and analyzed the data; C.A.-M. and J.V.-G.-A. collected the samples; J.V.-G.-A. and M.A.S.-S. collected the data; V.N. provided statistical support for the study; M.A.S.-S., P.B., and G.I. wrote the original draft of the manuscript; J.V.-G.-A., V.N., I.T., C.C., C.A.-M., M.-J.C.-S., A.F.-D., M.G.-G., A.R.-D., L.R., S.F., F.B., C.V., and M.M. reviewed and edited the manuscript; and all authors approved the final version.

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