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Fludarabine exposure predicts outcome after CD19 CAR T-cell therapy in children and young adults with acute leukemia

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Key Points

- A cumulative fludarabine $AUC_{T0-\infty}$ ≥14 mg*h/L correlates with improved LFS after CD19 CAR T-cell infusion.
- Clinical outcome in patients receiving CD19 CAR T cells might be improved by optimizing fludarabine exposure in the lymphodepleting regimen.

The addition of fludarabine to cyclophosphamide as a lymphodepleting regimen prior to CD19 chimeric antigen receptor (CAR) T-cell therapy significantly improved outcomes in patients with relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (B-ALL). Fludarabine exposure, previously shown to be highly variable when dosing is based on body surface area (BSA), is a predictor for survival in allogeneic hematopoietic cell transplantation (allo-HCT). Hence, we hypothesized that an optimal exposure of fludarabine might be of clinical importance in CD19 CAR T-cell treatment. We examined the effect of cumulative fludarabine exposure during lymphodepletion, defined as concentration-time curve (AUC), on clinical outcome and lymphocyte kinetics. A retrospective analysis was conducted with data from 26 patients receiving tisagenlecleucel for r/r B-ALL. Exposure of fludarabine was shown to be a predictor for leukemia-free survival (LFS), B-cell aplasia, and CD19-positive relapse following CAR T-cell infusion. Minimal event probability was observed at a cumulative fludarabine $AUC_{T0-\infty}$ ≥14 mg*h/L, and underexposure was defined as an $AUC_{T0-\infty}$ <14 mg*h/L. In the underexposed group, the median LFS was 1.8 months, and the occurrence of CD19-positive relapse within 1 year was 100%, which was higher compared with the group with an $AUC_{T0-\infty}$ ≥14 mg*h/L (12.9 months; $P < .001$; and 27.4%; $P = .0001$, respectively). Furthermore, the duration of B-cell aplasia within 6 months was shorter in the underexposed group (77.3% vs 37.3%; $P = .009$). These results suggest that optimizing fludarabine exposure may have a relevant impact on LFS following CAR T-cell therapy, which needs to be validated in a prospective clinical trial.

Introduction

CD19 chimeric antigen receptor (CAR) T cells have demonstrated impressive early response rates in children and young adults with relapsed/refractory (r/r) B-cell acute lymphoblastic leukemia (B-ALL).[1-5] However, around 50% of patients relapse within 1 year after treatment with either CD19-positive or CD19-negative disease. Robust pretreatment predictors of a complete response to CAR T-cell therapy are lacking, which limits individualized management of patients eligible for treatment with CD19 CAR T cells.[4,6,7]

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Requests for data sharing may be submitted to Stefan Nierkens (s.nierkens-2@prinsesmaximacentrum.nl).

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

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While CD19-negative disease recurrence after initial complete response is associated with *TP53* mutation,[8] high tumor burden at infusion,[6] and blinatumomab pretreatment,[6,9] CD19-positive relapse is thought to occur due to lack of CAR T-cell expansion and/or persistence.[6,10] However, CD19-positive relapses can also arise after prolonged B-cell aplasia, the golden standard biomarker for CAR T-cell persistence.[11] As such, the role of product-intrinsic factors, such as cellular composition of the apheresis material and the actual product, and in vivo expansion for long-term functionality have not yet been fully elucidated.

The addition of fludarabine to cyclophosphamide as a lymphodepleting regimen significantly improved clinical CAR T-cell expansion, persistence, and event-free survival (EFS) in pediatric and adult B-ALL/B-cell non-Hodgkin lymphoma.[4,5,12,13] These data are in line with early clinical trials with CD19 CAR T-cell therapy demonstrating the superior effect of lymphodepletion on clinical outcome,[14] and the use of fludarabine to prolong the persistence of adoptive T cells in melanoma.[15] Lymphopenia-induced expansion of lymphocytes has been attributed to increased levels of proinflammatory cytokines IL7 and IL15.[16] In addition, Turtle et al suggested that intensified lymphodepletion dampens anti-CAR immune responses against the murine single-chain variable fragment.[12,13]

Standard dosing of fludarabine is currently based on body surface area (BSA). Most pediatric protocols consist of a daily dosage of 30 mg/m^2 on 4 consecutive days in combination with cyclophosphamide at a daily dosage of 500 mg/m^2 on 2 consecutive days. Protocols in the past used either lower fludarabine doses in combination with cyclophosphamide or other lymphodepleting regimens (supplemental Table 1); all studies use BSA for fludarabine dosing. We previously showed that BSA-based dosing results in a highly variable plasma exposure in children and adults receiving fludarabine in the conditioning for allogeneic hematopoietic cell transplantation (allo-HCT).[17,18] There, fludarabine exposure was significantly associated with EFS and transplant-related mortality,[18] and the validated assay was introduced in the clinical care of hematopoietic cell transplantation (HCT) patients.

Based on these findings, and the fact that conditioning intensity is a potential determining factor in CAR T-cell persistence, we hypothesized that the clinical efficacy of CAR T treatment might be associated with an optimal exposure of fludarabine. We conducted a retrospective analysis to examine the effect of fludarabine exposure on outcome following CD19 CAR T-cell therapy, including leukemia-free survival (LFS), CAR T-cell persistence, and the occurrence of CD19-positive relapse, in patients with r/r B-ALL.

Patients and methods

Patients

Patients with r/r CD19+ B-ALL that received tisagenlecleucel between April 2019 and June 2021 in the Princess Máxima Center for Pediatric Oncology, The Netherlands, as a standard of care treatment with standard follow-up were included retrospectively. All patients gave broad consent for the use of clinical data and laboratory results. The BioBank protocols are in accordance with the ethical standards of our institution.

Determination of fludarabine exposure

Prior to CD19 CAR T-cell infusion, patients received fludarabine on 4 consecutive days at a daily dosage of 30 mg/m^2 and cyclophosphamide on 2 consecutive days at a daily dosage of 500 mg/m^2. IV hyperhydration (2.5 L/m^2) and sodium 2-mercaptoethanesulfonate (mesna) were administered according to standard protocols as a preventive method for hemorrhagic cystitis. Five blood samples (t = 0 and 1, 3, 7, and 11 hours after infusion) were longitudinally taken in clinical care on day 1 and day 2, 3, or 4 of fludarabine infusion. Concentrations of the circulating metabolite of fludarabine F-ara-A (hereafter referred to as fludarabine) were measured using a liquid chromatography-tandem mass spectrometry method validated according to European Medicines Agency guidelines with a lower limit of quantification of 0.001 mg/L.[19] The total exposure (area under the concentration-time curve [$AUC_{T0-\infty}$]) was determined by applying a fludarabine population pharmacokinetic model with maximum a posteriori Bayesian estimation using the software package NONMEM version 7.5.0.[17]

Clinical response to CAR T infusion

The primary outcome parameter was the effect of fludarabine exposure on LFS, defined as no response to CD19 CAR T-cell therapy or the time between CD19 CAR T-cell infusion and the moment of relapse defined as 1) >5% leukemic blasts in bone marrow (BM), 2) >0.01% to <5% leukemic blasts in BM at 2 different time points using flow cytometry or quantitative polymerase chain reaction (PCR), or 3) >0.01% leukemic blasts in the cerebral spinal fluid by 2 subsequent measurements using flow cytometry.

The overall response rate to CD19 CAR T infusion was defined as the minimal residual disease (MRD)-negative (≤0.01% leukemic blasts in BM at 2 different time points using flow cytometry and quantitative PCR) complete remission (CR) rate, including CR with incomplete hematologic recovery, at day 28.

Additional outcomes of interest were B-cell recovery (defined as >5 B cells/μL) and the occurrence of a CD19-positive or CD19-negative relapse. Infections were documented from CAR T infusion until the first event, either relapse, death, or consolidative HCT. Infections were defined as positive blood culture, PCR-proven viral infection, or defined clinical symptoms as for zoster reactivation or pulmonary fungal infection.

Cellular kinetics

Flow cytometry was used to detect the absolute CAR T-cell numbers in peripheral blood (PB) with the CD19 CAR detection reagent (CD19-CAR-Biotin and anti-Biotin-PE; Miltenyi Biotec). CD3+, CD4+, and CD8+ T- and B-cell numbers in PB were determined on days 1, 3, 7, 10, 14, and 28 after infusion (±1 day) and thereafter monthly. The peak CAR T expansion was defined as the highest number of CAR T cells in PB within 28 days after infusion. Antibodies used were CD3-FITC (BD Pharmingen), CD4-PE-Cy7 (BD Pharmingen), CD8-APC-Cy7 (BD Pharmingen), and CD19-APC (BD Pharmingen). B-cell recovery was considered the standard to determine CAR T-cell loss.

Statistical analysis

The association of fludarabine with LFS and the secondary outcome measures B-cell recovery and CD19-positive or CD19-negative relapse was explored using martingale residuals and further fitted by univariable Cox proportional hazards models. In addition, Kaplan-Meier and cumulative incidence curves were plotted and compared with log-rank tests. To compare CAR T-cell numbers in PB between exposure

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groups within the first 28 days after CAR T-cell infusion, the area under the curves (AUCs) were computed and compared between exposure groups with the Mann-Whitney *U* test. The peak CAR T-cell expansion was compared between the 2 defined exposure groups with the Mann-Whitney *U* test. Patient characteristics were compared between groups using the Mann-Whitney *U* test (continuous variables) or Fisher's exact test (categorical variables). LOESS regression curves were made to visualize CD4+ and CD8+ T-cell recovery in the 2 defined exposure groups within the first month after CAR T-cell infusion, which was subsequently compared by fitting linear mixed effect models. To study the correlation of real exposure with model-predicted fludarabine exposures, the total fludarabine dose was divided by the clearance. Clearance was calculated using total body weight (kg) and estimated globular filtration rate (eGFR; corrected to 70 kg in L/h), based on the previously published fludarabine population pharmacokinetic model.[17] Analyses were performed using R4.03 with packages *pknca*, *survival*, *survminer*, and *lme4*.

Results

Patients and response

Twenty-eight consecutive children and young adults received tisagenlecleucel for r/r B-ALL in our center between April 2019 and May 2021. Twenty-six of these patients gave broad consent and were included (Table 1). The data cutoff point was 1 July 2021. The median age was 14.4 (range, 4.0-24.5) years at the time of CAR T-cell infusion. The median follow-up was 389 (range 53-800) days. Eleven patients had a history of an allo-HCT, and 8 patients had previously received blinatumomab (n = 7) or CD19 CAR T-cell therapy (n = 1). Nine patients had an M2/M3 marrow (≥5% marrow blasts) at the time of lymphodepletion. The 17 patients with an M1 marrow were further characterized by molecular PCR: 4 patients were MRD-negative (no detectable disease), 8 patients had an MRD of 0.01% to 1%, and 5 patients had 1% to 5% leukemic blasts. Twenty of 26 (77%) patients achieved an MRD-negative complete remission after CAR T therapy.

Table 1. Patient characteristics

	All patients, n (%)
Patients, n	26
Age at infusion, years (range)	14.4 (4.0-24.5)
Male	15 (58)
Follow-up, d (range)	389 (53-800)
MRD-negative CR on d 28	20 (77)
Indication for CAR T therapy	
Primary refractory	4 (15)
Relapse	22 (85)
Disease status at start of lymphodepletion	
M1 marrow	17 (65)
≥M2 marrow	9 (35)
Prior lines of systemic therapy, n	
1-2	21 (81)
3-5	5 (19)
Prior blinatumomab	
Yes	7 (27)
No	19 (73)
Prior allo-HCT	
Yes	11 (42)
No	15 (58)
TP53 mutation	
Yes	2 (8)
No	24 (92)

Fludarabine exposure and clinical outcome

The exposure of fludarabine was highly variable, resulting in a wide range of $AUC_{T0-\infty}$ 8.7-21.1 mg*h/L (Figure 1A). Exposure of fludarabine as a continuous variable was shown to be a predictor for LFS (P = .04), B-cell recovery (P = .02), and CD19-positive relapse (P < .0001) following CD19 CAR T-cell infusion. Fludarabine exposure as a continuous variable was not a predictor for CD19-negative relapse (P = .1), although it should be noted that only 5 CD19-negative relapses occurred. To further explore the effect of fludarabine exposure, martingale residuals of the null Cox proportional hazard model for LFS were plotted against the cumulative fludarabine $AUC_{T0-\infty}$ (Figure 1B). Martingale residuals can be used to investigate the functional form of a covariate with respect to the outcome.[20] The pattern of the martingale residuals showed a nonlinear relationship between fludarabine exposure and LFS and suggested a target window between 13.5 mg*h/L and 16 mg*h/L for $AUC_{T0-\infty}$. The Kaplan-Meier estimator was used to investigate differences in outcome between groups stratified by low and high exposure. A minimal event probability was observed at a cumulative fludarabine exposure ≥14 mg*h/L, and underexposure was defined as an $AUC_{T0-\infty}$ <14 mg*h/L.

To investigate potential confounding effects of clinical parameters, baseline characteristics (age, sex, disease status at start of lymphodepletion, number of prior lines of systemic therapy, indication for CD19 CAR T therapy, prior allo-HCT, and prior blinatumomab use) were compared between the 2 exposure groups stratified by cumulative fludarabine $AUC_{T0-\infty}$ of 14 mg*h/L, and no significant differences were found (Table 2). Infection after CAR T infusion was documented in 54% (14/26) of the patients, of which 4 out of 11 were in the low fludarabine exposure group and 10 out of 15 were in the high fludarabine exposure group (P = .23). Most of the infections were nonsevere gram-positive central line-associated bacterial systemic infections (2 vs 6 in the low vs high fludarabine exposure group) within the first 28 days after CAR T infusion. No life-threatening infections occurred in the cohort. Details on infections are given in supplemental Table 2 in the supplemental Appendix.

The median LFS after CAR T-cell infusion was 1.8 months in the group with an $AUC_{T0-\infty}$ <14 mg*h/L compared with 12.9 months in the group with an $AUC_{T0-\infty}$ ≥14 mg*h/L (P < .001) (Figure 2A). In addition, the cumulative incidence of CD19-positive relapse within 1 year following infusion was 100% in the underexposed group, which was significantly higher compared with 27.4% in the group with an AUC ≥14 mg*h/L (P = .0001) (Figure 2B). Furthermore, there was a trend toward a lower rate of CR in the group with an AUC <14 mg*h/L (6/11 vs 14/15; P = .05).

Cellular kinetics and fludarabine exposure

Cumulative incidence of B-cell recovery within 6 months after CD19 CAR T infusion, as an indicator for CAR T cell loss, was significantly higher in the underexposed group compared with the group with an $AUC_{T0-\infty}$ ≥14 mg*h/L (77.3% vs 37.3%; P = .009) (Figure 2C).

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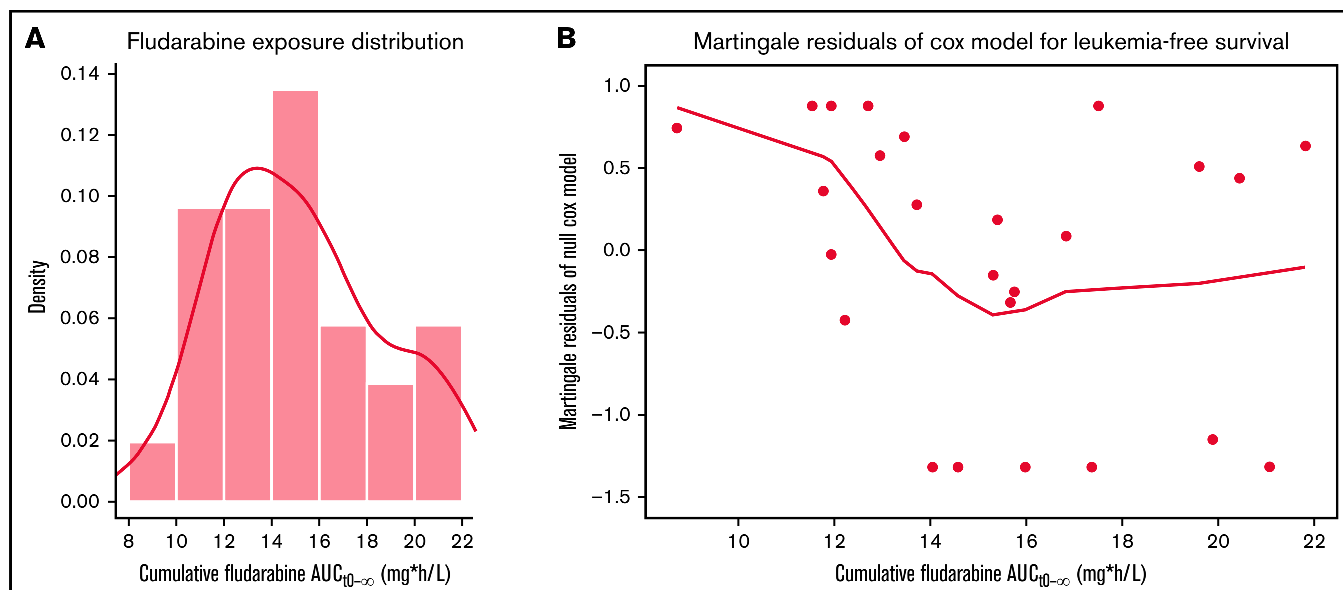

Figure 1. Fludarabine exposure and functional form. (A) Histogram (gray area) and density plot (black solid line) of the determined cumulative fludarabine $AUC_{T0-\infty}$. Density is the proportion of patients with fludarabine exposure within the specified limits. (B) Scatterplot of cumulative fludarabine $AUC_{T0-\infty}$ vs martingale residuals of null Cox proportional hazard model for LFS.

Table 2. Patient characteristics stratified on fludarabine exposure

	Fludarabine <4mg*h/L, n (%)	Fludarabine ≥14 mg*h/L, n (%)	*P* value
AUC mg*h/L, median (range)	11.9 (8.7-13.7)	16.8 (14.0-21.8)	
Patients, n	11	15	
Age at infusion, years (range)	11.6 (4.0-23.3)	16.8 (5.1-24.5)	.8
Male	7 (64)	8 (53)	.7
Follow up, d (range)	205 (107-695)	408 (53-800)	.26
MRD-negative CR on d 28	6 (55)	14 (93)	.05
Indication for CAR T therapy			1
Primary refractory	2 (18)	2 (13)	
Relapse	9 (82)	13 (87)	
Disease status at start of lymphodepletion			.68
M1 marrow	8 (73)	9 (60)	
≥M2 marrow	3 (27)	6 (40)	
Prior therapies, n			1
1-2	9 (82)	12 (80)	
3-5	2 (18)	3 (20)	
Prior blinatumomab			.66
Yes	2 (18)	5 (33)	
No	9 (82)	10 (67)	
Prior allo-HCT			.7
Yes	4 (36)	7 (47)	
No	7 (64)	8 (53)	
TP53 mutation			1
Yes	1 (9)	1 (7)	
No	10 (91)	14 (93)	

Furthermore, higher fludarabine exposure was associated with increased CAR T-cell expansion within the first 28 days after CAR T-cell infusion. CAR T cells in PB were measured in 16 patients (7 with $AUC_{T0-\infty}$ <14 mg*h/L and 9 with $AUC_{T0-\infty}$ ≥14 mg*h/L), and the mean peak CAR T-cell expansion in PB was 102/µL in the underexposed group, which was significantly lower compared with 295/µL in the group with an $AUC_{T0-\infty}$ ≥14 mg*h/L (P = .03). The AUC of CAR T-cell numbers within the first 28 days after CAR T infusion did not differ significantly. However, the mean AUC of CAR T-cell numbers was 1609 (range, 58-3345) in the group with a fludarabine $AUC_{T0-\infty}$ >14, which showed a trend toward a higher AUC of CAR T cells compared with the underexposed group, where a mean AUC of 672 (range, 2-2570) was observed (P = .07) (Figure 3A). On the contrary, there was a trend toward higher recovery of CD4+ T cells (both non-CAR T and CAR T) within the first month in the underexposed group compared with the group with a fludarabine $AUC_{T0-\infty}$ >14 (Figure 3B). No difference in CD8+ T-cell recovery was observed between the 2 exposure groups (Figure 3C).

Fludarabine pharmacokinetic model in the CAR T setting

The observed effect of fludarabine exposure on outcome suggests that optimization of fludarabine exposure in the lymphodepleting regimen might be of clinical importance in patients receiving CD19 CAR T-cell therapy. To be able to up-front individualize fludarabine dosing in patients, a population pharmacokinetic model can be used to calculate the expected fludarabine exposure with a given dose. Therefore, we investigated whether our previously published model by Langenhorst et al[17] was applicable in the CAR T-cell setting. This model includes body weight (kg) and eGFR (corrected to 70 kg in L/h) to predict the fludarabine $AUC_{T0-\infty}$. We used these patient characteristics to predict the $AUC_{T0-\infty}$ and compared these to the $AUC_{T0-\infty}$ determined in this study. The predicted $AUC_{T0-\infty}$

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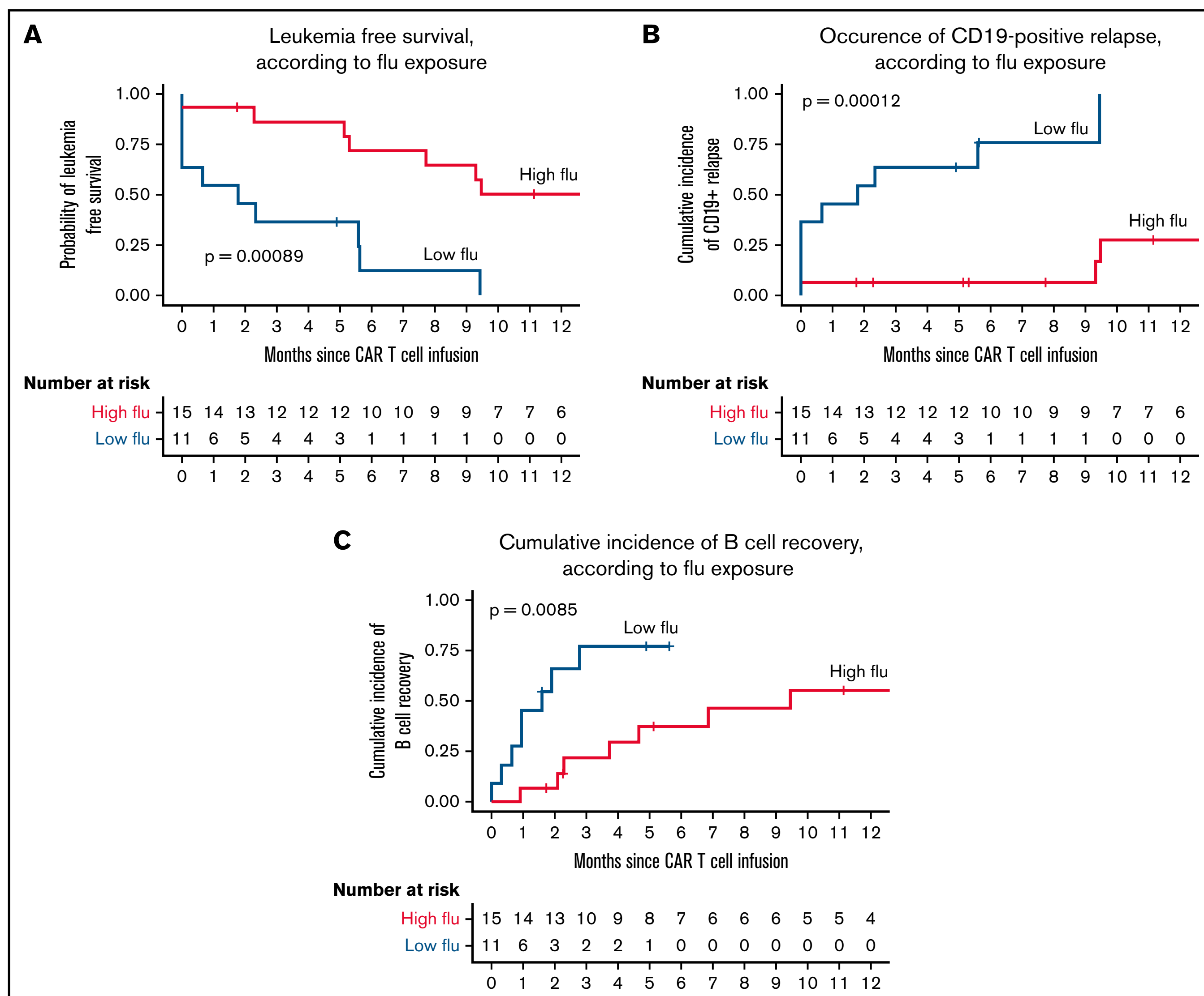

Figure 2. Cumulative fludarabine $AUC_{T0-\infty}$ <14 mg*h/L associates with worse outcome. (A-C) Kaplan Meier plots of LFS and the occurrence of CD19-positive relapse and B-cell recovery from the day of CAR T-cell infusion stratified by cumulative fludarabine $AUC_{T0-\infty}$ of 14 mg*h/L. Groups were compared using the log-rank test. *P* values $<$ .05 were considered statistically significant.

showed a range of 10.9 to 21.1 mg*h/L, which was comparable to the determined range of 8.7 to 21.1 mg*h/L (P = .26) (Figure 4). However, individual patients showed variation in $AUC_{T0-\infty}$, and for 13 patients, the predicted $AUC_{T0-\infty}$ value was 4 mg*h/L higher or lower than the determined exposure. In addition, 9/11 (82%) patients with a fludarabine $AUC_{T0-\infty}$ $<$14 mg*h/L were predicted to have a fludarabine $AUC_{T0-\infty}$ $>$14 mg*h/L. To be able to improve the prediction of the fludarabine $AUC_{T0-\infty}$, we investigated whether we could determine a common factor for patients showing these differences in predicted and determined fludarabine $AUC_{T0-\infty}$. No common factor, such as age, creatinine levels, height, gender, weight, dose, or BSA, was found.

Discussion

The high early response rate initially reported by the first pivotal study using CAR T cells[2] was confirmed in the real world.[21] Nevertheless, the 1-year EFS of 52% in children and young adults receiving tisagenlecleucel for r/r B-ALL, together with a dismal prognosis in patients relapsing after CAR T-cell therapy, highlights the need for improvement. In a cohort of children and young adults receiving CD19 CAR T-cell therapy as treatment for r/r B-ALL, we showed that a cumulative fludarabine $AUC_{T0-\infty}$ $\geq$14 mg*h/L was significantly correlated with a lower incidence of CD19-positive relapse and B-cell recovery and longer LFS. These results strongly suggest that optimizing fludarabine exposure may impact LFS following CAR T-cell therapy.

The observed clinical effect of high fludarabine exposure on the outcome is in line with the effect described by a recent publication of Fabrizio et al,[22] where the AUC was retrospectively estimated using the population pharmacokinetic model published by Langenhorst et al.[17] They show that patients with an estimated fludarabine AUC $<$13.8 mg*h/L had a significantly higher risk of relapse or B-cell

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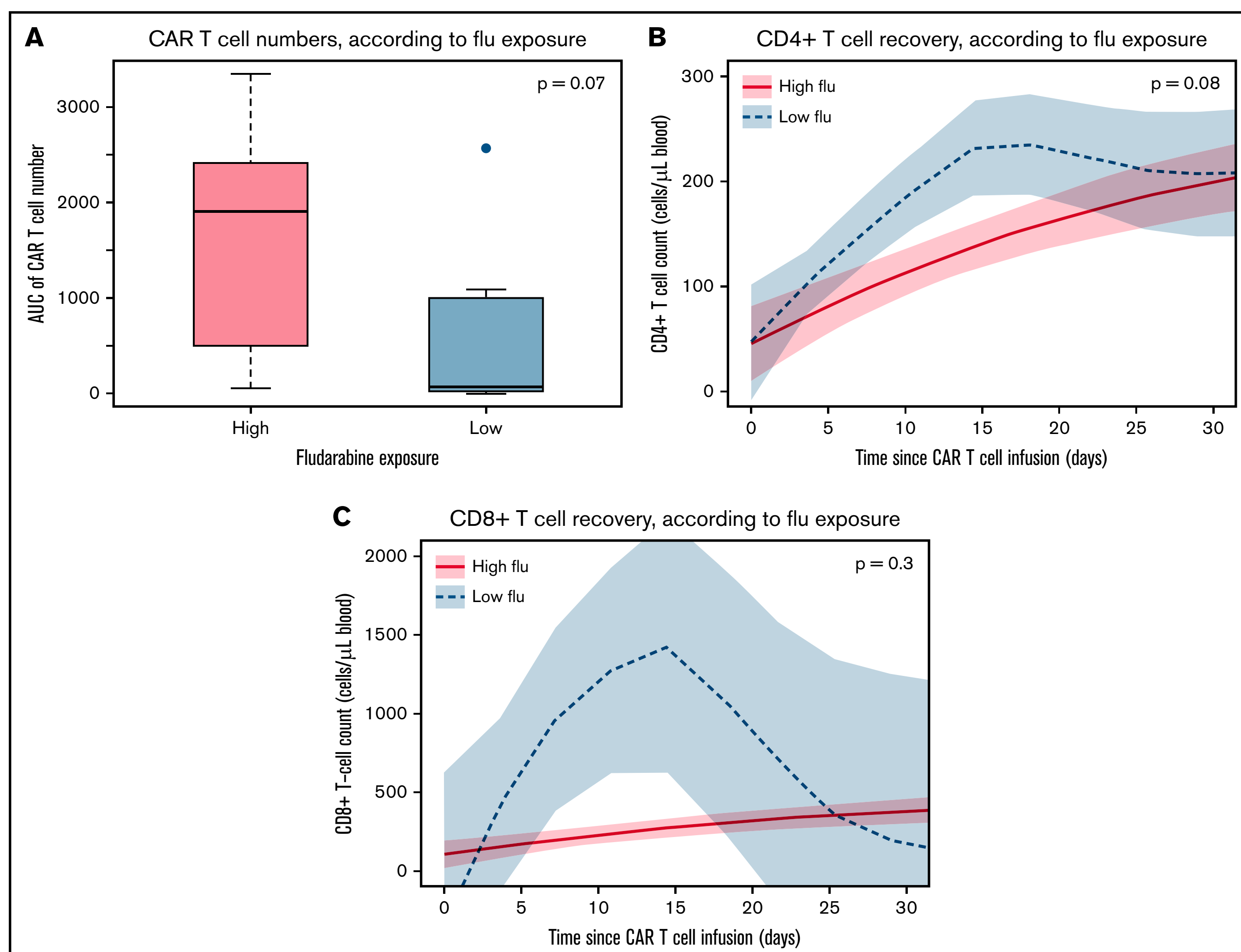

Figure 3. Cellular kinetics after CAR T-cell infusion in groups stratified by cumulative fludarabine $AUC_{T0-\infty}$ of 14 mg*h/L. (A) The AUCs of CAR T-cell numbers from the first 28 days after CAR T-cell infusion. The high and low fludarabine exposure groups consisted of 9 and 7 patients, respectively. Groups were compared using the Mann-Whitney *U* test. (B) LOESS regression curves of CD4+ T-cell recovery within the first month after CAR T-cell infusion in the whole cohort. (C) LOESS regression curves of CD8+ T-cell recovery within the first month after CAR T-cell infusion in the whole cohort. Groups were compared with linear mixed effect models. $P < .05$ considered statistically significant.

recovery compared with patients with an estimated fludarabine AUC ≥13.8 mg*h/L after CD19 CAR T-cell infusion. Our comparison between actual measured and estimated fludarabine exposures indicates that estimation of AUC through the model underestimates the true variation and that only actual measurement identifies all patients with too low exposure. This highlights the need for therapeutic drug monitoring of fludarabine in CD19 CAR T-cell therapy.

In addition, the clinical effect described here is comparable to the effect on LFS described by Turtle et al, comparing cyclophosphamide with and without fludarabine in the setting of a CD19 CAR T-cell product in adults with ALL.[12] In a comparable study by Gardner et al, a trend toward improved CAR T-cell persistence was observed when fludarabine was added to the lymphodepleting regimen.[5] Our data show that this effect is more profound at a cumulative fludarabine $AUC_{T0-\infty}$ ≥14 mg*h/L. In addition to CAR T-cell persistence, we observed a similar effect of fludarabine exposure on CAR T-cell peak expansion and total CAR T-cell numbers during the first 28 days after CAR T infusion. Multivariable regression models are needed to show the consistency of the relationship between fludarabine exposure and outcome, but the currently limited number of patients did not allow for the inclusion of potential covariates. Therefore, our results need to be prospectively confirmed in an independent cohort treated similarly. In such a cohort, MRD status should be considered as a covariate, as a leukemic blast count <5% is correlated with improved disease-free survival in both children[3,4,6] and (young) adults.[6,23,24] Furthermore, prior blinatumomab treatment,[6,9] the number of prior lines of therapy, and being primary refractory[4] should also be considered in the analysis. Nevertheless, all these characteristics were comparable between the 2 exposure groups, suggesting there might have been no confounding effects of clinical parameters on the observed association between fludarabine exposure and outcome in our small cohort.

Variable exposure of fludarabine with BSA-based dosing has been previously described in the allo-HCT setting.[18] Fludarabine overexposure was associated with lower EFS due to higher transplant-related mortality, while underexposure was associated with graft

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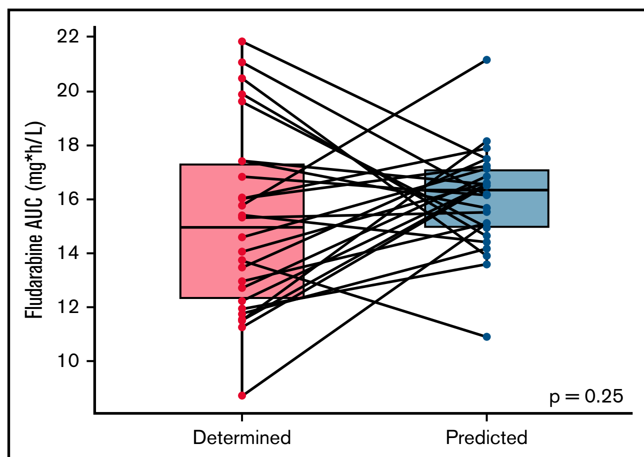

Figure 4. Distribution of determined and predicted fludarabine $AUC_{T0-\infty}$. Boxplots of the cumulative fludarabine $AUC_{T0-\infty}$ determined in our cohort (determined) and predicted using a previously published population pharmacokinetic model taking renal function and body weight as covariates (predicted).[17] Determined and predicted $AUC_{T0-\infty}$ of every patient ($n = 26$) is linked with a black solid line. Groups were compared with the Mann-Whitney *U* test. $P < .05$ considered statistically significant.

failure and also transplant-related mortality.[18] Interestingly, a high fludarabine exposure significantly delayed overall CD4+ T-cell reconstitution.[18] Also, in the CAR T setting, despite an effect on CAR T-cell persistence, high exposures of fludarabine showed a trend toward lower overall CD4+ T-cell reconstitution, especially around 2 weeks after CAR T-cell infusion. In addition, serum cytokine levels increase after lymphodepletion,[25,26] and the association between a higher intensity of lymphodepleting regimen with an increased probability of a favorable cytokine profile was reported in a recent study in lymphoma patients receiving CAR T-cell therapy.[25] Therefore, detailed immune monitoring is necessary to gain further insights into the effect of fludarabine on the functional dynamics of both CAR T cells and unmanipulated immune cells.

Interestingly, we observed that the previously published fludarabine population pharmacokinetic model[17] in the allo-HCT setting is not applicable without modification for the prediction of fludarabine exposure in the CD19 CAR T-cell setting. Perhaps, this can be explained by the differences in the pharmacological compounds in addition to fludarabine. In the study of Langenhorst et al, the allo-HCT conditioning regimen further consisted of either busulfan alone or busulfan with clofarabine in adults and children, respectively.[17] Furthermore, rabbit antithymocyte globulin was added in the unrelated donor setting. In our cohort, cyclophosphamide was part of the lymphodepleting regimen in all patients, accompanied by IV hyperhydration. Therefore, to achieve optimal fludarabine exposures in individual patients receiving CD19 CAR T-cell therapy, therapeutic drug monitoring is indicated.

After introducing the liquid chromatography-tandem mass spectrometry method[19] in clinical care to measure fludarabine concentrations in PB, the fludarabine dose can be adjusted based on the fludarabine measurement after the first fludarabine dose to target a cumulative fludarabine exposure ≥14 mg*h/L. This approach has previously been demonstrated to be feasible in a randomized clinical trial with patients receiving busulfan as a conditioning regimen for allo-HCT.[27] Notably, a multivariable analysis was not performed due to the limited number of patients included here. Larger studies validating the proposed target, including multivariable analysis and optimal dosing, using either adjusted predictive algorithms or therapeutic drug monitoring, are needed to implement therapeutic drug monitoring in clinical care.

In conclusion, clinical outcome in patients receiving CD19 CAR T-cell therapy is associated with fludarabine exposure in this retrospective cohort study. As one of the first identified controllable predictors for outcome, a fludarabine cumulative $AUC_{T0-\infty}$ ≥14 mg*h/L showed a strong correlation with improved LFS and a lower incidence of both CD19-positive relapse and B-cell recovery. Optimization of fludarabine exposure in each individual patient, using therapeutic drug monitoring in the lymphodepleting regimen, may improve CD19 CAR T-cell persistence and clinical outcome of currently available commercial CAR T therapies.

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Authorship

Contribution: F.G.C., C.A.L., S.N., and L.D. were involved in the study design; L.D. performed statistical analyses; Y.J. and H.V.T. advised on the statistical analysis and reviewed the manuscript; K.C.M.V.D.E. provided the fludarabine concentrations and reviewed the manuscript; A.L.N. and A.D.R.H. calculated the total fludarabine exposure and reviewed the manuscript; H.B. and S.R.V. were involved in the evaluation of immune cell kinetics and reviewed the manuscript; C.D.K., S.N., M.S., and R.A. reviewed the manuscript; and F.G.C., C.A.L., R.P., M.B., H.V., H.J.V., B.V., P.H., and M.V.D.V. treated patients and reviewed the manuscript.

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