REGULAR ARTICLE

blood advances

# Refining busulfan exposure enhances pediatric ALL HSCT outcomes: insights from the International FORUM study

Khalil Ben Hassine,[1,*] Yvonne Gloor,[2,*] Isabelle Dupanloup,[2] Chakradhara Rao Satyanarayana Uppugunduri,[2,3] Vid Mlakar,[2] Fanny Gonzales,[1] Tayfun Güngör,[4] Marianne Ifversen,[5] Peter J. Shaw,[6] Jochen Buechner,[7] Tony H. Truong,[8] Henrique Bittencourt,[9] Lochie Teague,[10] Jacek Toporski,[11] Petr Sedlacek,[12] Ulrike Poetschger,[13] Maja Krajinovic,[14] Krzysztof Kałwak,[15] Adriana Balduzzi,[16,17] Mattia Algeri,[18] Peter Bader,[19] Christina Peters,[20] Jean-Hugues Dalle,[21] and Marc Ansari,[1,2] on behalf of the FORUM BUPK Consortium

[1]Division of Pediatric Oncology and Hematology, Department of Women, Child and Adolescent, Geneva University Hospitals, Geneva, Switzerland; [2]CANSEARCH Research Platform for Pediatric Oncology and Hematology, Department of Pediatrics, Gynecology and Obstetrics, Faculty of Medicine, University of Geneva, Geneva, Switzerland; [3]Department of Medical Oncology, Jawaharlal Institute of Postgraduate Medical Education & Research, Puducherry, India; [4]Division of Stem Cell Transplantation, University Children's Hospital of Zurich, Zurich, Switzerland; [5]Department of Pediatric and Adolescent Medicine, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark; [6]The Children`s Hospital at Westmead, Sydney, Australia; [7]Department of Pediatric Hematology and Oncology, Oslo University Hospital, Oslo, Norway; [8]Division of Pediatric Oncology and Cellular Therapy, Alberta Children's Hospital, Calgary, Canada; [9]Hematology-Oncology Division, Centre Hospitalier Universitaire Sainte-Justine, Montréal, Canada; [10]Starship Blood and Cancer Center, Starship Children's Hospital, Auckland, New Zealand; [11]Center of Allogeneic Stem Cell Transplantation and Cellular Therapy (CAST), Karolinska University Hospital, Stockholm, Sweden; [12]Department of Pediatric Hematology and Oncology, Motol University Hospital, Prague, Czech Republic; [14]Centre Hospitalier Universitaire Sainte Justine Research Center Azrieli, Departments of Pediatrics and Pharmacology/Physiology, University of Montreal, Montreal, Canada; [15]Department of Pediatric Hematology/Oncology and BMT, Wroclaw Medical University, Wroclaw, Poland; [16]Pediatric Department, Fondazione Istituto di Ricovero e Cura a Carattere Scientifico San Gerardo dei Tintori, Monza, Italy; [17]School of Medicine and Surgery, University of Milano-Bicocca, Milano, Italy; [18]Department of Pediatric Hematology and Oncology, Cell and Gene Therapy, Istituto di Ricovero e Cura a Carattere Scientifico, Ospedale Pediatrico Bambino Gesù, Rome, Italy; [19]Division for Stem Cell Transplantation and Immunology, Department of Pediatrics, Goethe University Frankfurt, Frankfurt, Germany; [20]Department of Pediatrics and Adolescent Medicine, St. Anna Children's Hospital, St. Anna Children's Cancer Research Institute, Vienna, Austria; and [21]Hemato-Immunology Department, Robert Debré Hospital, Groupe hospitalo-universitaire de l'Assistance publique - Hôpitaux de Paris Nord Université Paris Cité, Paris, France

**Key Points**

- Busulfan exposure within a cumulative AUC of 73.3 to 98.0 mg.h/L correlates with improved HSCT outcomes in pediatric ALL.
- Model-based dosing of busulfan significantly lowers the risk of relapse, toxicity, and could present an alternative to TBI.

The optimal busulfan exposure window in pediatric patients with acute lymphoblastic leukemia (ALL) undergoing allogeneic hematopoietic stem cell transplantation (HSCT) remains to be defined. We identify this window for patients receiving busulfan within the prospective international, randomized controlled phase 3 ALLSCTped 2012 FORUM trial. We included 145 participants receiving fludarabine-busulfan-thiotepa conditioning, with available busulfan plasma levels. Busulfan exposure was estimated using a validated pediatric population pharmacokinetic model. Primary outcomes were event-free survival (EFS) and graft-versus-host disease-free relapse-free survival (GRFS). Patients were aged between 0.5 and 19.5 years, with a median follow-up of 5.0 years. The optimal busulfan exposure was defined as area under busulfan concentration curve (cAUC) of 73.3 to 98.0 mg.h/L based on EFS and GRFS. Patients with nonoptimal exposure had lower EFS (hazard ratio [HR], 1.93; p = 0.008) and GRFS (HR, 2.01; p = 0.002). Underexposure (cAUC <73.3 mg.h/L) was associated with higher relapse rates (HR, 1.93; p = 0.019), and overexposure (cAUC >98.0 mg.h/L) with an increased risk of treatment-related toxicities and graft-versus-host disease. Patients with optimal busulfan exposure showed no differences in EFS, GRFS, overall survival, or relapse with post hoc matched total-body irradiation (TBI) recipients (n = 51 in each group). This study identifies a favorable busulfan exposure window for pediatric patients with ALL undergoing HSCT from an HLA-matched donor. Therapeutic drug monitoring for optimized personalized busulfan dosing

Submitted 19 November 2025; accepted 7 February 2026; prepublished online on *Blood Advances* First Edition 4 March 2026; final version published online 26 May 2026. https://doi.org/10.1182/bloodadvances.2025019142.

*K.B.H. and Y.G. contributed equally to this study.

Summary data are available from the corresponding author, Marc Ansari (marc.ansari@hcuge.ch), on request. Individual participant data that underlies the results reported in this article, will be available for meta-analysis upon agreement by the study's international coordinator. Requests should be submitted to Marc Ansari. The study protocol is available from Peters et al.[1]

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

improves HSCT outcomes and might be validated for use as an alternative to irradiation. This trial was registered at EudraCT as 2012-003032-22 and on www.ClinicalTrials.gov as NCT01949129.

## Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) offers a potential cure for patients with high-risk acute lymphoblastic leukemia (ALL). Among different prognostic factors, the success of HSCT is influenced by the conditioning regimen administered before transplantation. The prospective international multicenter randomized controlled ALLSCTped 2012 FORUM study (NCT01949129) was designed to determine the most effective conditioning regimen for pediatric patients with ALL.[1,2] The randomized part of the study compared total-body irradiation (TBI) with chemotherapy-based conditioning for patients aged between 4 and 21 years receiving HSCT from a matched donor.[1] All patients younger than 4 years or receiving a transplantation from a mismatched donor were assigned to the chemotherapy conditioning (chemo-conditioning).[3] The interim analysis showed that TBI with etoposide was associated with significantly better outcomes than myeloablative chemo-conditioning, thus ending the randomization.[1] TBI/etoposide is now considered the standard-of-care conditioning regimen for pediatric patients with ALL aged >4 years.[4] Chemo-conditioning in the FORUM study included fludarabine and thiotepa with either busulfan (Bu) or treosulfan.[5] No significant differences in survival have been observed between the 2 chemo-conditioning regimens, neither in the randomized cohort nor in younger patients.[1,3,5] However, despite its demonstrated efficacy, TBI remains more often associated with long-term adverse effects than irradiation-free conditioning regimens.[6-8]

Busulfan is known for its exposure-outcome relationships, narrow therapeutic window, and high interpatient pharmacokinetic (PK) variability, making therapeutic drug monitoring (TDM)–guided dosing essential for optimizing HSCT outcomes.[7,9-12] The strongest evidence for optimal busulfan exposure to date comes from a 2016 retrospective study by Bartelink et al,[13] involving 674 pediatric and young adult patients with malignant and nonmalignant diseases, receiving busulfan mostly with cyclophosphamide. It established the widely accepted optimal therapeutic window for HSCT outcomes as a cumulative area under the busulfan concentration curve (cAUC) of 78.0 to 101.0 mg.h/L.[13] However, the applicability of this window to patients with ALL has to be confirmed due to the short follow-up time (median, 1.5 years; interquartile range, 0.5-3.6), outdated treatment period (2001-2015), and cohort heterogeneity with low ALL representation (5%, n = 31).[13] Indeed, a more recent study in patients with inborn errors of immunity reported a different optimal window of 70.0 to 90.0 mg·h/L,[14] highlighting the disease- and, most likely, protocol-specific nature of optimal busulfan exposure.[14]

These questions led us to hypothesize that the optimal busulfan target window may need to be fine-tuned for different diseases. Although previous studies report comparable clinical outcomes between conditioning regimens based on targeted busulfan and TBI,[15,16] the relationship between optimal busulfan exposure and HSCT outcomes has, to our knowledge, not been specifically assessed in a homogeneous cohort of pediatric patients with ALL. Using the prospectively enrolled FORUM study cohort, we aimed to define the optimal busulfan exposure window for myeloablative conditioning in thise patients by analyzing busulfan exposure-outcome relationships and then comparing outcomes in optimally exposed patients to matched patients receiving TBI-based conditioning.

## Methods

### Study design and patient population

All participants included in the current exposure-outcome analysis were busulfan-treated pediatric patients with high-risk or relapsed ALL enrolled in the international multicenter randomized open-label phase 3 ALLSCTped 2012 FORUM study and its pharmacogenetic add-on study (NCT02670564). The trial received approval from the ethics committee of the Medical University of Vienna, Austria, other local ethics committees and national competent authorities. It was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all patients and/or their parents/legal guardians. The study design is detailed in the previous reports from the FORUM study and the published protocol.[1,3,5] All patients were followed for a minimum of 2 years after transplantation. Data from patients who received TBI-based conditioning included in the analysis of the randomized cohort[1] were available for comparison. The study flowchart is available as supplemental Figure 1.

### Busulfan administration procedures and exposure estimation

Busulfan was administered intravenously once, twice, or 4 times daily, based on the center's preference, over 4 days. Infusions typically lasted 2 hours for 4 times daily dosing (q6h) and 3 hours for once (q24h) or twice daily (q12h) regimens. Initial doses followed the European Medicines Agency's actual body weight-based pediatric dosing nomogram.[17,18] Participating centers measured busulfan concentrations in their local TDM facilities and adjusted doses per local protocols, with a recommended per-dose target of $AUC_{0-\infty}$ of 3.7 to 5.3 mg.h/L or 16.4 to 22.6 mg.h/L for 4 times daily and once daily dosing, respectively. The analytical methods for busulfan quantification were cross validated in each local TDM laboratory.[19] Only data from cross-validated centers were included. Because centers used different methods to calculate busulfan exposure, we re-estimated exposure metrics from the individual measured concentrations, to harmonize our data set, as previously recommended.[12-14] We used a validated pediatric population pharmacokinetic model, taking into account patient age, an age-dependent allometric body weight model, and coadministration of fludarabine to perform Bayesian (a posteriori) estimations.[20,21] The model was implemented in Phoenix NLME software (version 8.2, Certara). Goodness-of-fit plots were used to validate the estimations of the model against the observed data from the current study. The cAUC in milligram-hours per liter was

selected as the main exposure metric of interest in relation to the outcomes as it was described as the most reliable and actionable metric for busulfan targeted dosing.[12,13,22,23]

### Clinical outcomes

The primary outcomes were event-free survival (EFS) and graft-versus-host disease (GVHD)–free relapse-free survival (GRFS). EFS included relapse or death, whichever occurred first; GRFS was defined as survival without relapse, grades III to IV acute GVHD, or extensive chronic GVHD. Secondary outcomes included overall survival (OS), cumulative incidence of relapse (CIR), postrelapse mortality (PRM), nonrelapse mortality (NRM), engraftment, and treatment-related toxicities (TRTs). TRTs included pneumonitis, pulmonary infiltrates, hematuria, bladder hemorrhage, liver failure, elevated transaminases, hyperbilirubinemia, and central or peripheral neurotoxicity. All TRTs were diagnosed and graded according to Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Sinusoidal obstructive syndrome, acute and chronic GVHD (aGVHD and cGVHD) were diagnosed and graded according to FORUM protocol recommendations.[1,2] A patient's TRT count score included all mentioned toxicities and cGVHD of respective grades. Since sinusoidal obstructive syndrome diagnosis overlaps with hepatotoxicity markers, only 1 count was added, if both were present. Neutrophil recovery time was defined as the first of 3 consecutive days with counts $>0.5 \times 10^9$/L. Platelet recovery time was defined as the first of 3 consecutive days with counts $>20 \times 10^9$/L at least 7 days after last transfusion.

Clinical variables other than busulfan exposure included age at transplantation, sex, first complete remission (CR1) vs second or greater (CR≥2) complete remission status at transplantation, stem cell source (bone marrow, cord blood, or peripheral blood), and number of busulfan daily doses (4 vs 2, or 1).

### Statistical analyses

Follow-up duration was defined as the time between HSCT and last visit, or death, and the median follow-up time was estimated using the inverse Kaplan-Meier method. Two approaches were used to determine the optimal cAUC window. The first approach identified the cAUC cutoff values associated with the maximum log-rank statistic obtained for EFS and GRFS across the entire range of observed values. The second method determined the optimal window's boundaries using a univariate Cox regression with a 2 degrees-of-freedom spline covariate relationship (equivalent to a quadratic relationship) between cAUC and either EFS or GRFS failure (1-EFS or 1-GRFS).[24] The resulting hazard ratio (HR) curve was plotted against cAUC, and the range where HR $\leq 1$ defined the optimal window. Patients were then classified as optimally exposed, overexposed, or underexposed.

Time-to-event outcomes (EFS, GRFS, OS, and PRM) were analyzed using the Kaplan-Meier approach. The log-rank test was used for comparisons across strata. Cox regressions were applied for univariate and multivariate analyses, with *P* values for each predictors level computed with the Wald test. HR estimates and their 95% confidence intervals (95% CI) from the multivariable regression model were assessed for stability and reliability using bootstrapping with 10 000 data sets randomly resampled from the original data. CIR was analyzed using competing risk cumulative incidence analysis, adjusting for death, and Gray's test was used for comparison. The competing risk analysis of NRM adjusted for PRM as a competing event, whereas the analysis of PRM adjusted for NRM. Fine-Gray regression models were used for all competing risk analyses with Wald tests to compute *P* values, and bootstrapping (10 000 resampled datasets) for model verification. Univariate and multivariate ordinal logistic and Poisson regression were used to compare TRT counts between busulfan exposure levels. All multivariate analyses used a full covariate model approach, following a thorough evaluation of collinearity. *P* values $<.05$ were considered statistically significant. Effect sizes were reported as HR for survival analyses, odds ratios (OR) for logistic regression, or incidence-rate ratios for Poisson regression with their 95% CI. EFS, GRFS, OS, and CIR were compared between patients optimally exposed to busulfan and matched patients receiving TBI, from the previously reported randomized part of the FORUM study.[1] The matching utilized propensity scores calculated through binomial logistic regression for stem cell source and immunophenotype, and Mahalanobis distance for age at HSCT and remission status. The matching considered a 1:1 TBI to busulfan ratio and a caliper of 0.2 standard deviation units. The statistical analyses were performed using R (version 4.1.3) with the packages gtsummary (version 1.7.2), survival (version 3.7-0), survminer (version 0.4.9), ggsurvfit (version 1.1.0), splines (version 4.1.3), tidycmprsk (version 1.0.0), ordinal (version 2023.12-4), boot (version 1.3-28), and MatchIt (version 4.5.3).

## Results

### Patient population and busulfan exposure

Of all the patients with available busulfan PK and clinical data, 10 patients who received mismatched donor grafts were excluded. The remaining 145 patients underwent transplantation between April 2014 and September 2022, from matched sibling or matched unrelated donors (with matching defined as 9 or 10 out of 10 HLA-compatible related or unrelated donors), at 26 centers across 9 countries (supplemental Table 1). Median follow-up time was 5.0 years (interquartile range, 4.8-5.4). Most patients were already described in previous reports of the FORUM study (n = 79 randomized patients,[1] and n = 54 below 4 years of age[4]), with longer follow-up in the present study. The demographic and clinical characteristics of the cohort are shown in Table 1. The patients' age at transplantation ranged from 0.5 to 19.5 years. Most patients were diagnosed with precursor B-cell ALL (84%) and received bone–marrow derived stem cells (74%). Patients in second or greater CR accounted for 46% of the cohort. The frequencies of clinical outcomes are reported in supplemental Table 2. In accordance with previous FORUM reports,[1,3] the proportion of patients experiencing NRM was low (4.1%), and relapse (41%) was the leading cause of treatment failure. Thus, the predominant event influencing EFS was relapse, making EFS more representative of treatment efficacy than toxicity. This motivated the consideration of GRFS, as a co-main outcome of interest to define the optimal therapeutic window.

Dosing schedules, number of monitored doses, and sampling schedule varied across centers (supplemental Table 3). Most patients (55%) were only monitored after a single dose, typically the first. Sparse sampling was common for q6h dosing, whereas q24h, and q12h regimens involved more intensive sampling. The concordance between site-reported values and our PopPK model estimates was satisfactory[20,21] (supplemental Figure 2A). Despite TDM-guided dose adjustments, model-estimated exposures varied

**Table 1. Patients' demographic and clinical characteristics**

| Characteristic | N = 145* |
|---|---|
| **Sex** | |
| Female | 60 (41%) |
| Male | 85 (59%) |
| Age (years) | 6.9 (2.5, 11.3) |
| **Age group (y)** | |
| Above 4 | 88 (61%) |
| 2-4 | 28 (19%) |
| 1-2 | 13 (9.0%) |
| 0-1 | 16 (11%) |
| Height (cm) | 122 (89, 142) |
| Missing | 1 |
| Weight (kg) | 23 (13, 35) |
| **Remission status** | |
| CR 1 | 79 (54%) |
| CR ≥2 | 66 (46%) |
| **Donor** | |
| MD | 105 (72%) |
| MSD | 40 (28%) |
| **Stem cell source** | |
| Bone marrow | 108 (74%) |
| Cord blood | 22 (15%) |
| Peripheral blood | 15 (10%) |
| **Immunophenotype** | |
| Precursor B-cell ALL | 122 (84%) |
| T-cell ALL | 22 (15%) |
| Other | 1 (0.7%) |
| **Genetic aberration†** | |
| *BCR::ABL* | 10 (8.7%) |
| *ETV6::RUNX1* | 14 (12%) |
| *KMT2A::AFF1* | 17 (15%) |
| None of the above | 77 (67%) |
| Missing | 30 |
| Hyperdiploidy | 24 (18%) |
| Missing | 8 |
| Hypodiploidy | 7 (5.1%) |
| Missing | 8 |
| **Bu dosing schedule** | |
| 4 times daily | 104 (72%) |
| Once daily | 33 (23%) |
| Twice daily | 8 (5.5%) |
| **cAUC (mg.h/L)** | 86 (78, 93) |

cAUC, cumulative area under busulfan concentration curve; CR, complete remission; MD, matched donor; MSD, matched sibling donor.
*n (%); median (IQR).
†Three patients had >1 mutation.

widely (supplemental Figure 2B). The PopPK model predictions visually demonstrated a good correlation with the observed concentrations, thereby validating the precision of its estimations in this cohort (supplemental Figure 3). Busulfan doses were effectively adjusted in 79 (54%) of the patients (supplemental Table 3).

## Optimal exposure definition

Combining both methods for optimal exposure definition and considering both EFS and GRFS failures, the optimal exposure window was defined as cAUC 73.3 to 98.0 mg.h/L. The lower cutoff value (73.3 mg.h/L) corresponded to the maximal log-rank statistic value for the EFS outcome (Figure 1A). A cAUC of 98.0 mg.h/L, obtained from the curve of 1-GRFS HR against busulfan's cAUC, was defined as the upper cutoff (Figure 1B). In our cohort, 97 (67%) patients were within the optimal therapeutic exposure window, wheras a significant proportion (n = 48, 33%) were outside the optimal range; 26 (18%) underexposed and 22 (15%) overexposed. No differences in clinical variables were noted across the different exposure groups (supplemental Table 4).

## Exposure-outcome analysis

Patients outside the optimal busulfan exposure window (under- or overexposed) had significantly lower EFS compared to optimally exposed patients (HR, 1.93; 95% CI, 1.19-3.14; Figure 2A; supplemental Figure 4A). EFS did not differ significantly between optimal and overexposed groups (Figure 2A). In contrast, GRFS was significantly lower in both underexposed (HR, 1.93; 95% CI, 1.13-3.32) and overexposed patients (HR, 2.10; 95% CI, 1.20-3.68) (Figure 2B; supplemental Figure 4B). These findings remained significant in multivariate Cox regression analyses, where busulfan exposure impacted both EFS and GRFS in models accounting for other relevant clinical variables (Figure 3). The adjusted HR values of nonoptimally exposed groups were close to 2 for both EFS and GRFS underscoring the clinical significance of optimal busulfan exposure. Adjusted for busulfan exposure, patients in second or greater CR at transplantation had worse EFS and GRFS, whereas patients below 1 year-old had lower EFS, consistent with previously published results from the FORUM study.[1,3] Full univariate and multivariate Cox-regression results are reported in supplemental Table 5 and 6.

The lower EFS in underexposed patients is driven primarily by higher relapse rates (hazard ratio [HR], 1.93; 95% CI, 1.11-3.35; Figure 2C). This observation is confirmed by univariate and multivariate Fine-Gray analyses (supplemental Table 7), showing a significantly higher relapse risk in underexposed patients (adjusted HR, 2.00; 95% CI, 1.16-3.45) without difference in relapse risk between optimally exposed and overexposed patients. Validation using bootstraps confirmed the robustness of the multivariable estimates, showing strong concordance between those results and the original ones (supplemental Table 8). Subgroup analyses (Figure 4) stratified by remission status and age revealed significant benefits for patients in second or greater CR and younger patients (age <4 years) with busulfan exposure surpassing 73.3 mg.h/L compared to those below that threshold. No significant effect of busulfan exposure on OS, NRM, nor PRM (Figure 2D-F) were found. No neutrophil and platelet recovery differences were observed between busulfan exposure groups (supplemental Figure 5).

## Safety of the optimal therapeutic window

When considered individually, grade III or IV TRTs appeared slightly but nonsignificantly more frequently in busulfan-overexposed

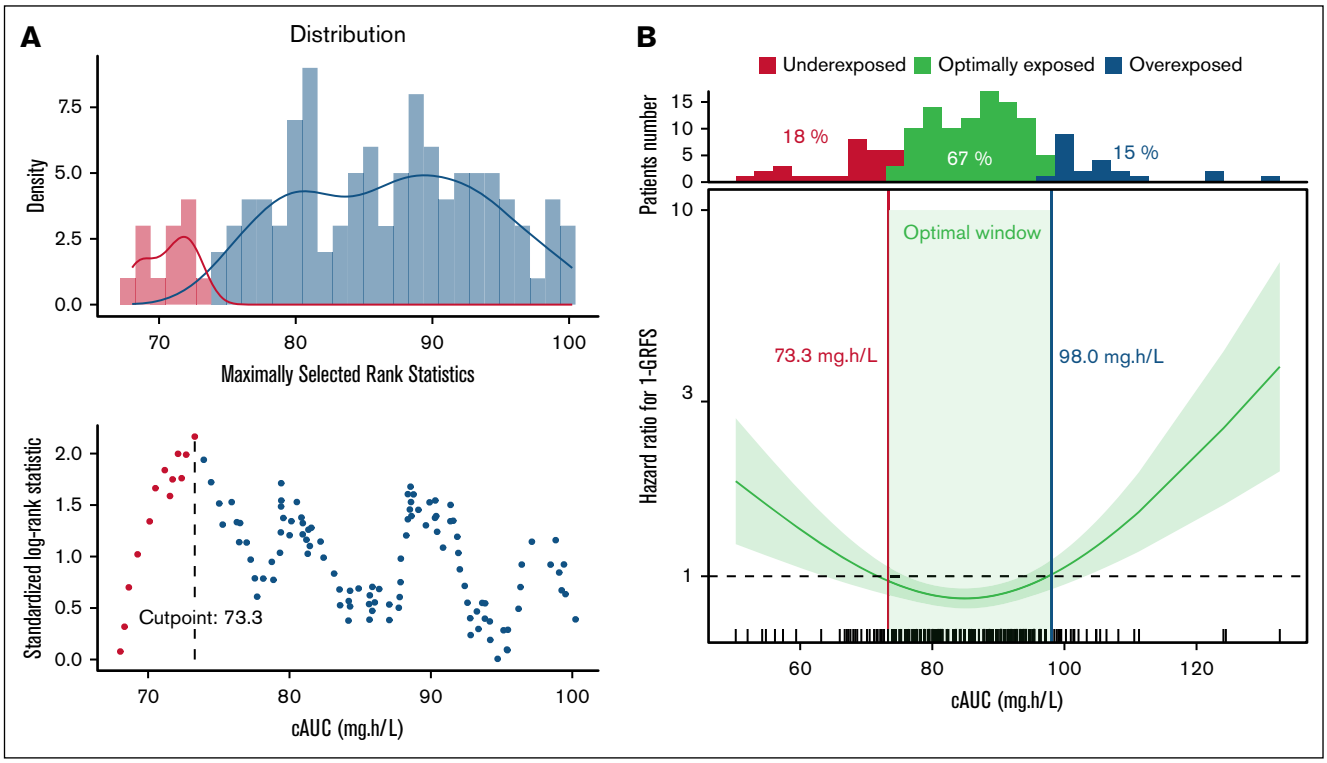



**Figure 1. Definition of busulfan optimal exposure windows.** (A) Definition of busulfan cAUC cutoff for 1-EFS using the maximal g-rank statistic. (B) Definition of optimal window based on the 2 degrees-of-freedom spline relationship between GRFS failure (1-GRFS) and cAUC in univariate Cox regression with shaded area showing +/- 1 standard error. The percentages displayed on the histogram correspond to the proportions of patients underexposed, optimally exposed, and overexposed.

patients compared to the others with 9.1% vs 4.9% for sinusoidal obstructive syndrome, 18.2% vs 7.3% for lung toxicities and 4.5% vs 0.8% for hematuria (supplemental Table 9). However, increasing busulfan exposure correlated with an increase in the sum of different TRTs affecting each patient both for grades III-IV and II-IV toxicities (Figure 5A,C, respectively). Moreover, when comparing TRT counts between busulfan exposure groups using regression models, cumulative TRTs were significantly higher in patients with busulfan exposures exceeding 98.0 mg.h/L (Figure 5B,D). Patients with busulfan overexposures had an incidence of death of 9.1% vs 3.3% in those exposed to less than 98 mg.h/L. In contrast, optimally exposed and underexposed patients showed similar incidences of toxicities. Thus, patients who were overexposed faced a greater risk of experiencing multiple severe toxicities. Due to the low number of events, only a nonsignificantly higher cumulative incidence of NRM ($P = .077$) was observed in patients outside the optimal exposure window (supplemental Figure 4). Extensive cGVHD was significantly higher in overexposed patients, whereas a nonsignificantly higher aGVHD garde III to IV incidence was observed in those patients (supplemental Table 10).

### Optimal busulfan exposure vs TBI

In the current study, 52 patients aged 4 and older from the previously reported randomized cohort[1] were identified as being optimally exposed. Fifty-one of them were a posteriori matched to 51 out of 196 available TBI patients from the same cohort[1] based on their age, remission status, immunophenotype, and the stem cell source used for transplantation (matching assessment and population description in supplemental Figure 6 and supplemental Table 11, respectively). No significant differences in outcomes were found between patients optimally exposed to busulfan and those conditioned with TBI in terms of OS, EFS, GRFS, and CIR, and median differences were not clinically significant (Figure 6). NRM was observed only in 2 patients, both in the busulfan group. Interestingly for both EFS and GRFS, the differences between TBI and optimally exposed busulfan are the highest after 2 years and tend to decrease with time. Although Kaplan-Meier analyses stratified by remission status suggested a potential benefit of TBI in patients at CR1 in the first years after transplantation, this difference seems to decrease with time (supplemental Figure 7).

## Discussion

According to our data, pediatric patients with ALL receiving a transplant from HLA-matched donors with a busulfan exposure between 73.3 to 98.0 mg.h/L experienced improved EFS and GRFS and fewer severe toxicities compared to patients outside this exposure window.

In this contemporary and homogeneous cohort of pediatric patients with ALL, our results confirm that targeted dosing with TDM could significantly improve HSCT outcomes. Furthermore, our defined therapeutic window for children with ALL (73.3-98.0 mg.h/L) is in line with that defined by Bartelink et al[13] (78-101 mg.h/L). Another crucial finding was the significantly lower EFS only in underexposed patients, whereas GRFS was significantly lower in both underexposed and overexposed groups. This

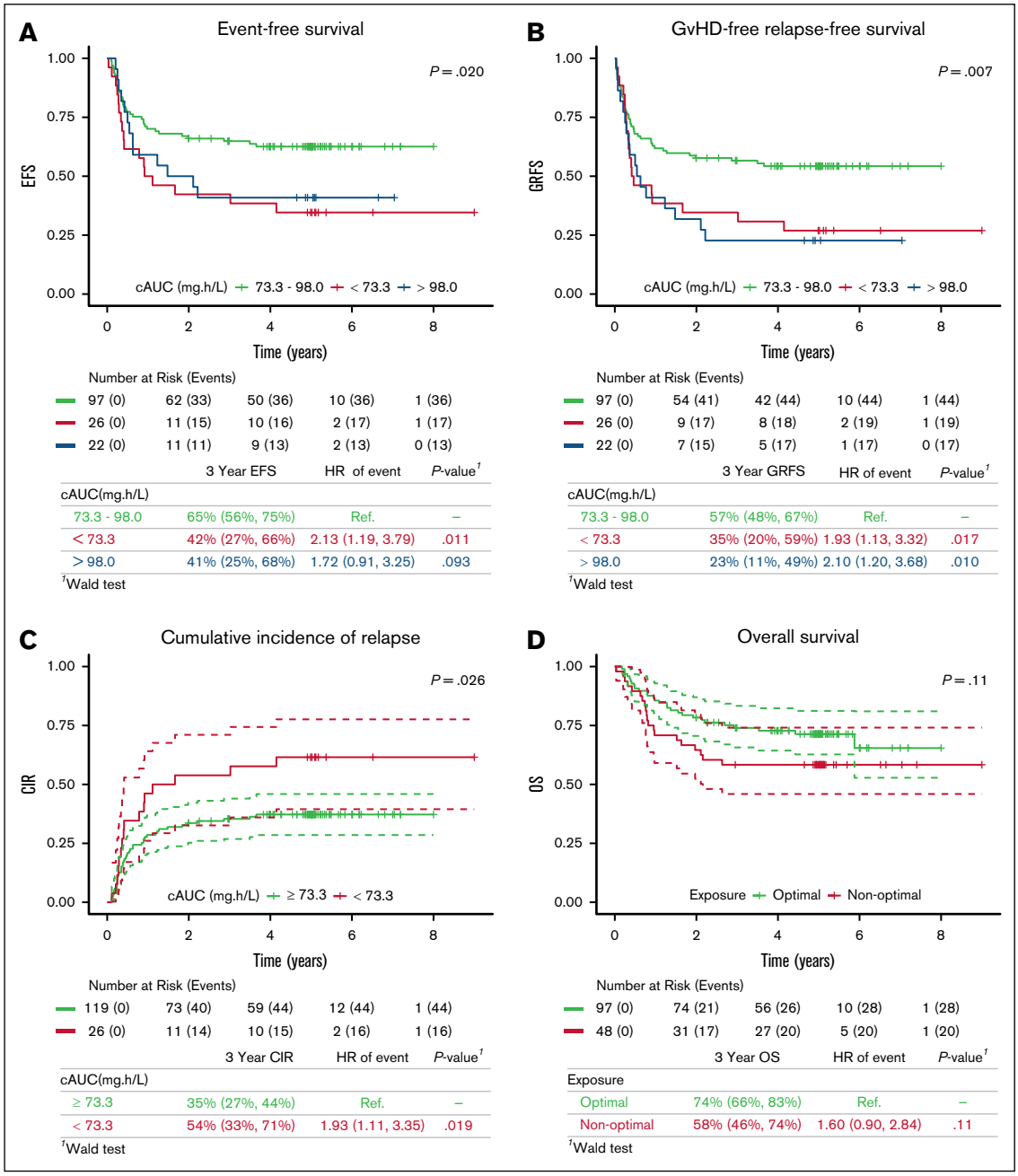



**Figure 2. Key outcomes according to busulfan exposure levels.** (A) Event-free survival (B) GvHD-free relapse-free survival (C) Cumulative incidence of relapse (D) Overall survival (E) Non-relapse mortality (F) Post-relapse mortality (A-B) Strata defined as: cAUC below 73.3 mg.h/L, within the optimal 73.3-98.0 mg.h/L window and above 98.0 mg.h/L busulfan (C) Strata defined as below or above 73.3 mg.h/L (D-F) Strata defined as: optimal exposure when cAUC were within 73.3 to 98.0 mg.h/L and non-optimal exposure when cAUC were below 73.3 mg.h/L or above 98.0 mg.h/L. *P* values annotated on the Kaplan-Meier plots were obtained from the log-rank test, or the Gray's test for relapse (C). HR, with 95% CI wee obtained from univariable Cox regression models (or the Fine-Gray model for relapse [C]) that compare the different exposure strata to the reference exposure stratum, with corresponding *P*-values calculated from the Wald test. Dashed lines on the Kaplan-Meier plots for panels C-F represent the 95% CIs. CIR, cumulative incidence of relapse; NRM, non-relapse mortality; PRM, post-relapse mortality.

highlights the importance of composite end points accounting for both treatment efficacy and toxicity when determining therapeutic windows.

We pondered whether elevating busulfan exposure levels beyond the toxicity threshold (98.0 mg.h/L) might further reduce relapse rates, especially in light of the low NRM and modest toxicity rates observed in this cohort (supplemental Table 1). However, there was no significant difference in relapse incidence between patients receiving optimal exposure and those overexposed (supplemental Table 2) indicating an efficacy plateau with the optimal exposure window we identified. As such, further increasing

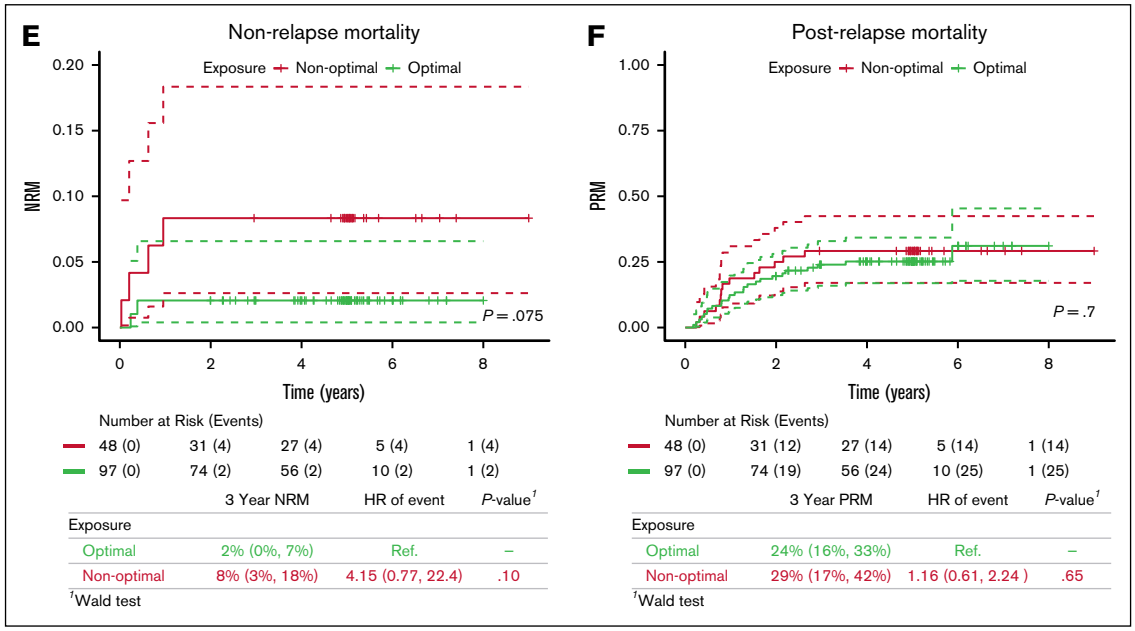



**Figure 2 (continued)**

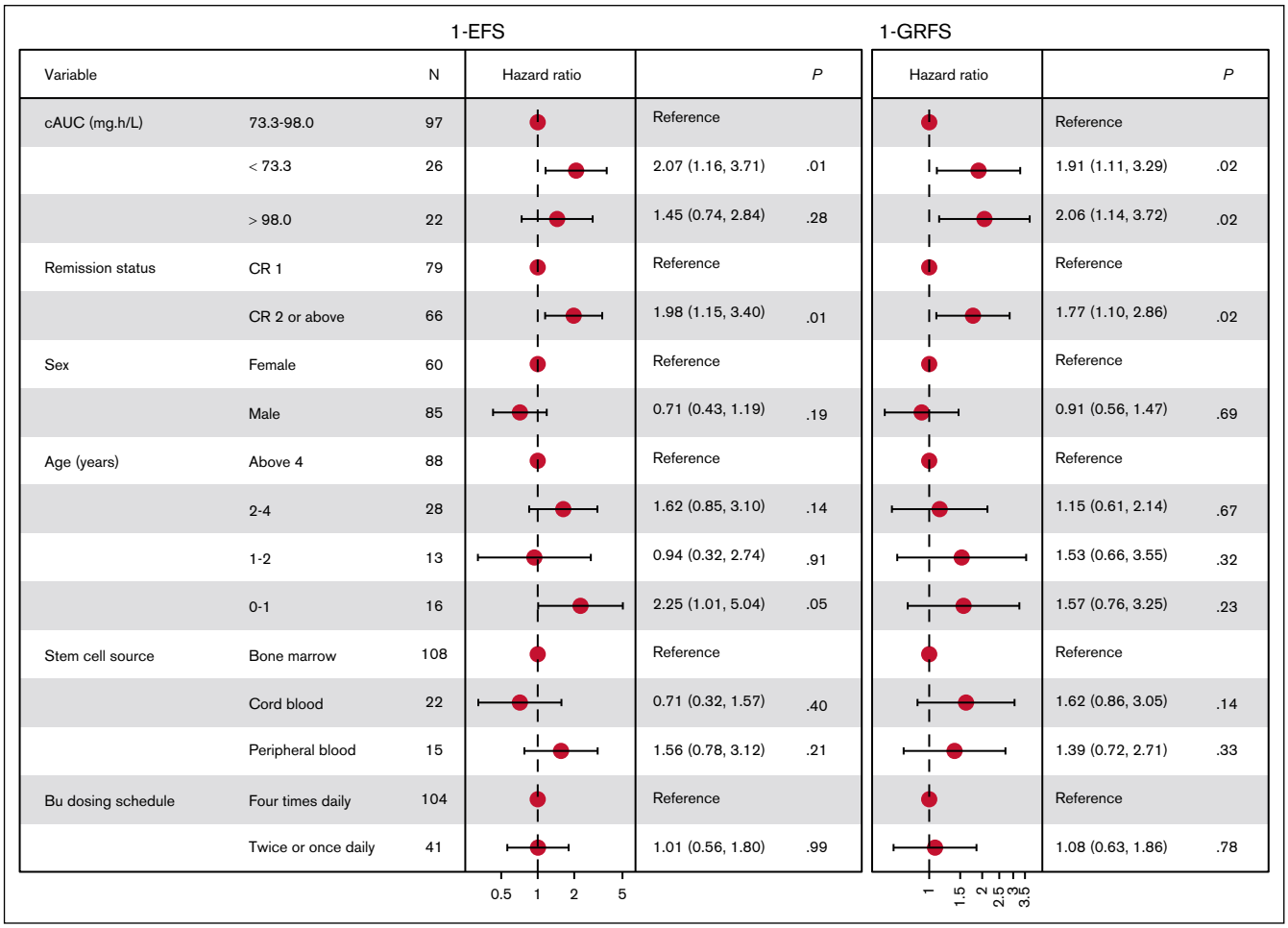
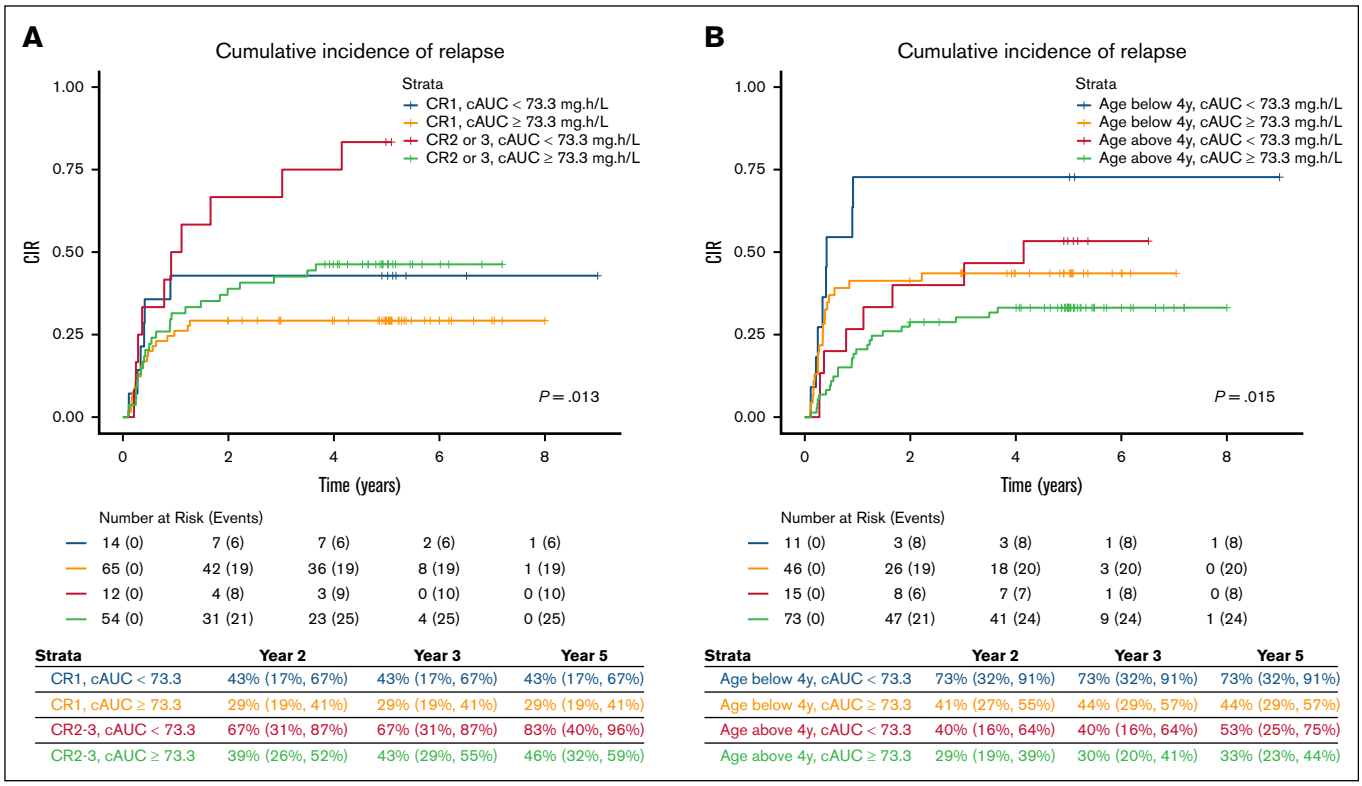



**Figure 3. Multivariable Cox-regression analysis results for event-free survival (EFS) and GVHD-free relapse-free survival (GRFS) failures.** *P* values were computed using the Wald test. Bu, busulfan.




| Strata | Year 2 | Year 3 | Year 5 |
|---|---|---|---|
| CR1, cAUC < 73.3 | 43% (17%, 67%) | 43% (17%, 67%) | 43% (17%, 67%) |
| CR1, cAUC ≥ 73.3 | 29% (19%, 41%) | 29% (19%, 41%) | 29% (19%, 41%) |
| CR2-3, cAUC < 73.3 | 67% (31%, 87%) | 67% (31%, 87%) | 83% (40%, 96%) |
| CR2-3, cAUC ≥ 73.3 | 39% (26%, 52%) | 43% (29%, 55%) | 46% (32%, 59%) |

| Strata | Year 2 | Year 3 | Year 5 |
|---|---|---|---|
| Age below 4y, cAUC < 73.3 | 73% (32%, 91%) | 73% (32%, 91%) | 73% (32%, 91%) |
| Age below 4y, cAUC ≥ 73.3 | 41% (27%, 55%) | 44% (29%, 57%) | 44% (29%, 57%) |
| Age above 4y, cAUC < 73.3 | 40% (16%, 64%) | 40% (16%, 64%) | 53% (25%, 75%) |
| Age above 4y, cAUC ≥ 73.3 | 29% (19%, 39%) | 30% (20%, 41%) | 33% (23%, 44%) |

**Figure 4. Stratified analysis of the cumulative incidence of relapse (CIR).** (A) Stratification by remission status and Bu exposure level. (B) Stratification by age and Bu exposure level. *P* values were obtained using Gray's test. The tables below the plot display CIR at 2, 3, and 5 years with the respective 95% CIs.

busulfan exposure beyond this threshold does not further reduce relapse rates. Instead, it increases toxicity risk, as evidenced by lower GRFS and higher TRT counts in overexposed patients (Figures 3 and 5).

Despite better EFS and GRFS, patients with optimal busulfan exposure did not have significantly better OS than patients outside that range. This could be due to the small sample size, low NRM, and the lack of comprehensive data on postrelapse management in the FORUM study.[5] Variations in practices among centers, including access to CAR T-cell therapy as a rescue treatment option following relapse, could play a key role among these potential confounders.[3,5]

In line with previous report, our analysis suggests that busulfan could be a valuable alternative to TBI, if the exposure is in the optimal window.[15,16] Although the original study was not designed for this purpose, we performed a matched analysis that does not show significant outcome differences between those 2 conditioning. Thus, even though these findings warrant cautious interpretation due to the limited sample size, they suggest that equivalent efficacy may be achievable through precise busulfan TDM. This is particularly important for patients who are ineligible for TBI due to high risk of long-term radiation toxicity, such as those with prior radiation exposure, young age, or comorbidities, or treated at centers who have limited access to TBI facilities. In addition, although a recent retrospective study even proposed lowering the TBI eligibility threshold from 4 to 2 years old,[25] the inherent risks associated with TBI, including secondary neoplasm or cognitive side effects, lessen the attractiveness of this conditioning regimen despite its efficacy.[6,7] Moreover, when considering longer follow-up periods, the late appearance of many severe TBI side effects could negatively influence long-term EFS in this group compared to the chemo-conditioning arm.[8] An adequately designed prospective trial comparing TBI vs busulfan with a protocol-mandated TDM procedure, using a single or cross-validated PK-model with a well-defined target, is warranted to confirm or refute TBI superiority. Ideally, this future study should also be powered to identify patient subgroups benefiting from each conditioning regimen. For instance, our results suggest that the outcomes of patients <2 years of age are considerably better when busulfan exposure is within the optimal window (supplemental Figure 8) with a 3-year CIR of 39% in patients exposed above 73.3 mg.h/L compared to 83% in patients below that threshold.

Our current study focused only on busulfan-PK, whereas fludarabine, thiotepa, and serotherapy (anti-thymocyte globulin [ATG], or anti-T-lymphocyte globulin) were also given to patients during conditioning. Several studies, including from the FORUM trial, suggested that model-based dosing of serotherapy might result in improved relapse and GVHD rates.[10,26-29] Thus, combined model-based dosing of serotherapy and busulfan may further improve HSCT outcomes in pediatric patients with ALL.[30]

Fludarabine PK exposure has also been associated with HSCT outcomes.[31] However, further studies are needed to determine whether targeted fludarabine dosing may impact HSCT outcomes in this context. Whether the combination of fludarabine, busulfan, and thiotepa is the optimal chemotherapy regimen for conditioning

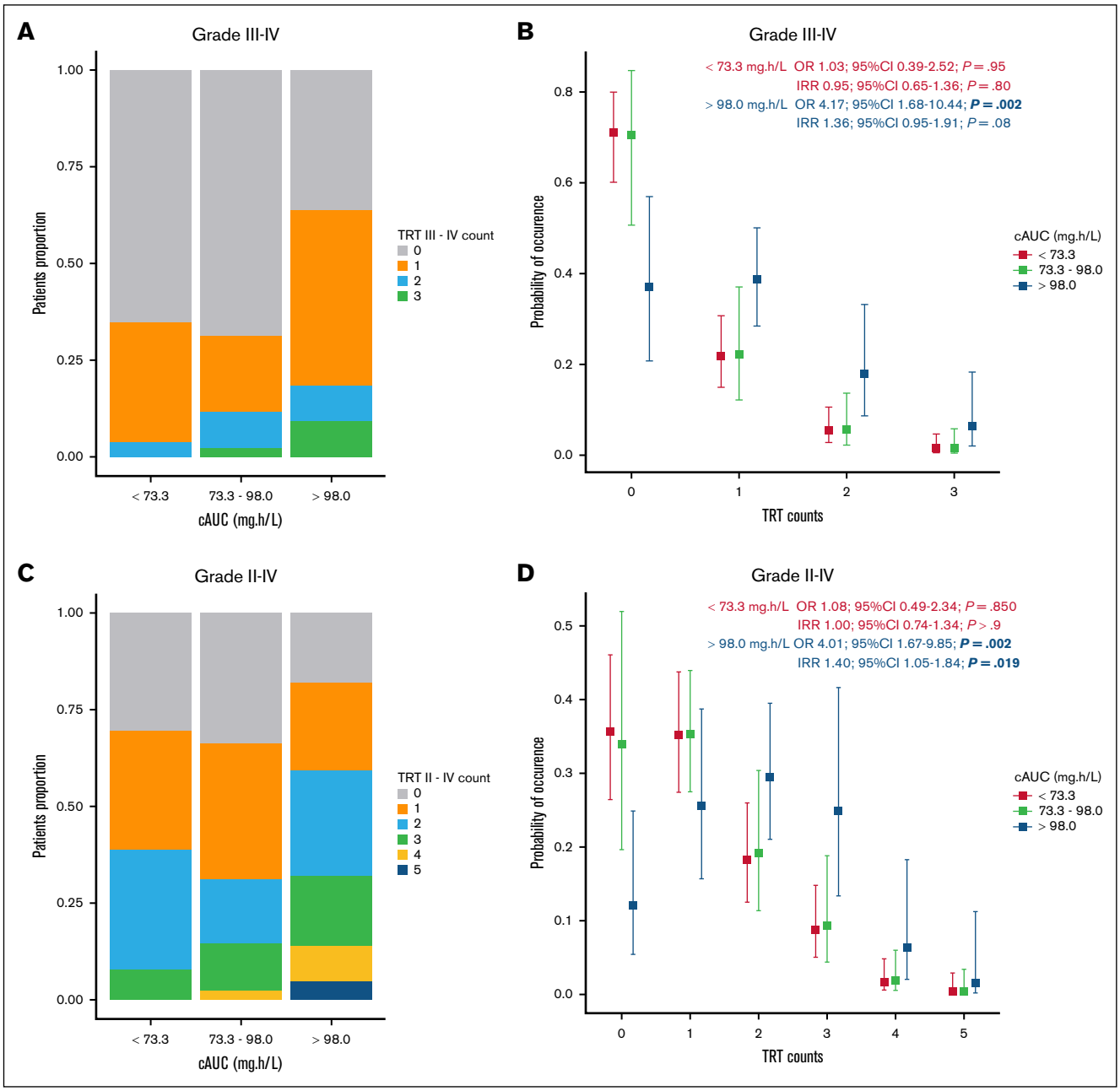



**Figure 5. Analysis of TRT counts according to busulfan exposure.** (A,C) Stacked density plots showing the cumulative count of TRT events of grades III-IV (A) and grades II-IV (C), experienced by each participant according to their busulfan exposure group. (B,D) Predicted probabilities for TRT grades III-IV (B) and II-IV (D) counts, respectively, according to busulfan exposure using an ordinal logistic regression model corrected for stem cell source. The odds ratios (OR) and incidence risk ratios (IRR) displayed on the plots are from the ordinal logistic regression and Poisson regression models, respectively, both corrected for stem cell source.

in pediatric ALL also remains uncertain.[7] Adding clofarabine to fludarabine and busulfan targeted to 85 to 95 mg.h/L yielded similar results to those achieved in the current study, with a 5-year EFS rate of around 64%,[16,32] vs 63% (95% CI, 54-73) in optimally exposed patients from our cohort.

The main limitations of the current study are related to the heterogeneity of supportive care and postrelapse therapeutic options among centers which have been previously discussed.[3,5] Our findings also reveal that busulfan administration and TDM practices vary among centers. Despite the cross-validation of the analytical methods to ensure the quantification accuracy of individual busulfan plasma levels,[19] dosing schedules, sampling schedules, number of monitored doses, PK exposure estimation, and subsequent dose calculations were not standardized. We attempted to mitigate this heterogeneity using our validated PopPK model for exposure estimation, which has previously demonstrated its predictive performance across varying sampling schedules.[21] Still, the calculated exposure of patients with sparse sampling might be less accurate than those with multiple samples

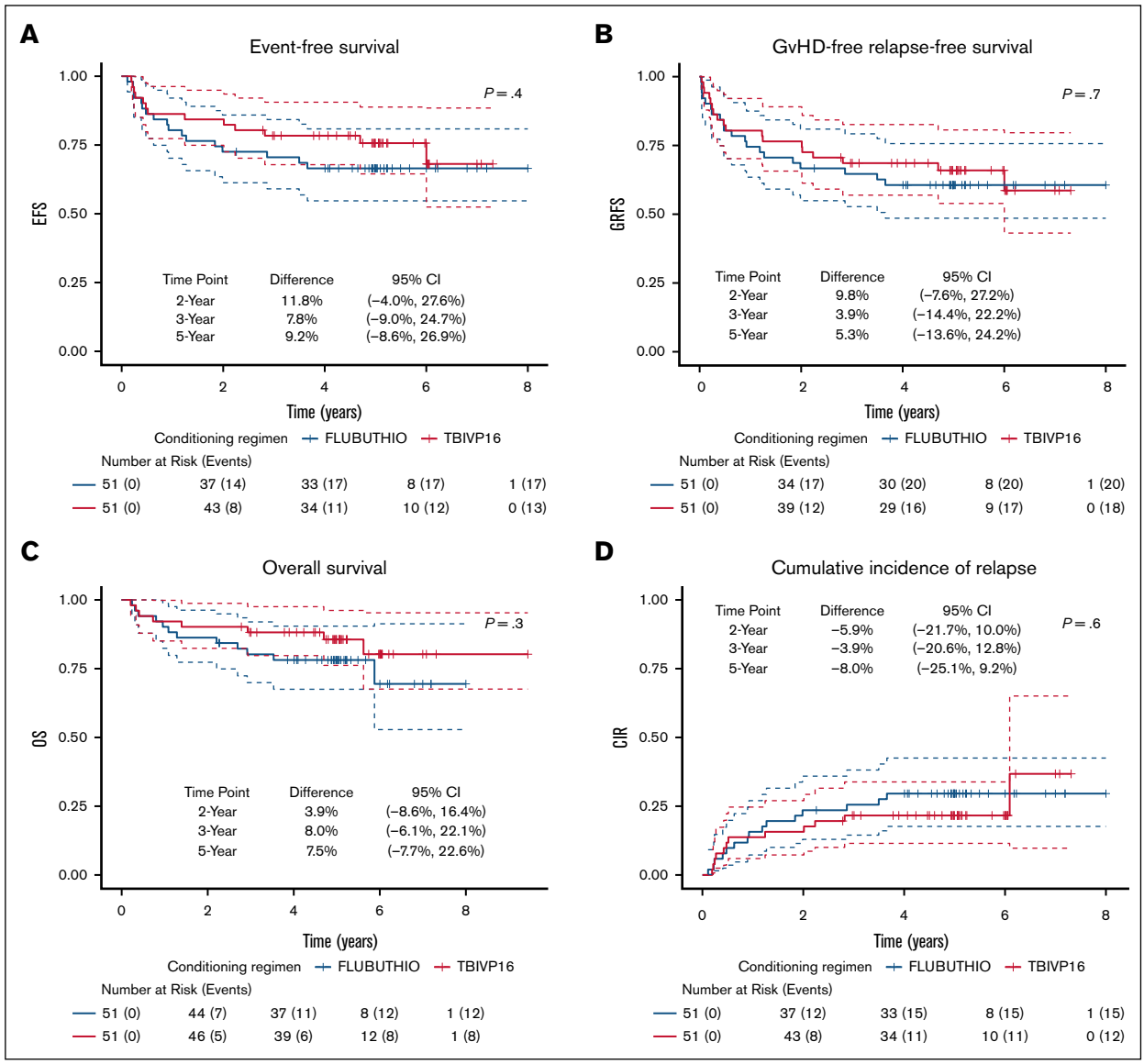



**Figure 6. Comparison between TBI with optimal busulfan in the matched patient cohort.** Survival differences at 2, 3, and 5 years are displayed on the plots. (A-C) A positive difference value indicates a higher survival with TBI, and a negative value indicates a higher survival with optimal busulfan. (D) Negative difference values indicate lower relapse incidence with TBI and vice-versa. FLU, fludarabine; THIO, thiotepa; VP16, etoposide.

across several doses. Importantly, our analysis revealed important interpatient differences in the dose adjustment following TDM suggesting that further studies would benefit from harmonized procedures (supplemental Figure 9). Another limitation was the lack of external validation of the optimal exposure window, due to a limited number of patients. Bootstrapping was applied to address this limitation by testing the consistency of HR estimates related to nonoptimal exposures. Finally, despite consistent reports of the predictive capacity of pre-HSCT minimal residual disease status on transplantation outcomes[15,16] and consistently with previous FORUM study reports,[33] we observed no association between minimal residual disease and CIR in the busulfan group of the FORUM cohort and did therefore not include this parameter as covariate in our analyses nor the matching with TBI patients. In conclusion, our findings demonstrate that maintaining a cumulative busulfan exposure within the defined optimal therapeutic cAUC window of 73.3 to 98.0 mg.h/L is efficacious and safe in pediatric patients with ALL receiving HSCT from a matched donor. Although overexposure increases the risk of TRTs, suboptimal exposure significantly elevates the risk of relapse. These results underscore the critical role of TDM busulfan dosing, to improve HSCT outcomes in pediatric ALL. Future prospective studies should adopt a unified PK-model for busulfan exposure to ensure reliable cross-study comparisons and further refine exposure targets.

## Acknowledgments

The authors thank the patients and their families for their invaluable contributions to the study. They thank physicians, pharmacists/pharmacologists, laboratory technicians, clinical research

associates, the statistical team of Vienna and the study manager of the Vienna central study office, and nurses from all participating centers for their commitment to the study. They also thank Rudolf Maibach and Stefanie Hayoz for their statistical expertise and advice.

This study was supported by grants from the CANSEARCH Foundation, Switzerland, the Swiss National Science Foundation, Switzerland (grant 153389), and the OAK Foundation, Switzerland (grant OCAY-17-642) to Marc Ansari. The FORUM study was funded by grants from the Deutsche Knochenmarkspenderdatei, Amgen, Neovii, Riemser, Medac and Jazz, and the study sponsor St. Anna Children's Cancer Research Institute, Vienna, Austria.

## Authorship

Contribution: M.A. conceptualized the study; K.B.H., Y.G., I.D., C.R.S.U., V.M., U.P., M.K., A.B., F.G., J.H.D., P.B., C.P. and M.A. designed research; K.B.H., Y.G., I.D., C.R.S.U. and M.A. performed the research; K.B.H., Y.G., I.D., M.A. performed statistical analysis; K.B.H., Y.G., I.D., C.R.S.U., V.M., M.A. wrote the manuscript on behalf of the FORUM BUPK Consortium; and K.B.H, Y.G., I.D., C.R.S.U., V.M., F.G., T.G., M.I., P.J.S., J.B., T.H.T., H.B., L.T., J.T., P.S., U.P., M.K., K.K., A.B., M.A., P.B., C.P., J.H.D., M. A. collected, analyzed, and interpreted data and revised and approved the manuscript.

Conflict-of-interest disclosure: K.B.H. is employed by AC Immune SA. F.G. serves on the advisory board for Merck Sharp & Dohme AG (MSD). T.G. serves on the advisory board for Novartis, Jazz, MSD; and received travel grants from Neovii. M.I. reports consulting or advisory role for Novartis; received research funding from Novartis; and travel grants from Novartis. P.J.S. reports consulting or advisory role for Novartis. T.H.T. received honoraria from Jazz; and reports consulting or advisory role for Jazz. J.B. reports advisory board participation for Janssen, Pfizer, and Novartis; and is a study steering committee member and speaker's bureau member of Novartis. K.K. reports speaker's bureau from Novartis, Medac, and Pierre Fabre; and travel grants from Pierre Fabre. A.B. reports speaker's bureau from Novartis, Amgen, Medac, and Neovii; meeting support from Medac and Neovii; and honoraria from Sanofi and Orchards. J.-H.D. reports consultancy and honoraria from Orchard, Pierre Fabre Medicaments, Novartis, Pfizer, Vertex, and Symbiopharm; travel grants from Pierre Fabre Medicaments; and is a current equity holder in a private company, Teva. P.B. reports travel grants from Medac, Novartis, and Vertex; speaker's bureau from Medac and Novartis; and research support from Neovii, Novartis, Medac. C.P. receives study support from Sanofi; and travel grants, study support, and speaker's bureau from Neovii, Jazz, Medac, Amgen, and Vertex. M.A. reports travel grants from Jazz and Novo Nordisk; and consulting or advisory role for Jazz Pharmaceuticals and Vertex. The remaining authors declare no competing financial interests.

A complete list of the members of the FORUM BUPK Consortium appears in supplemental Appendix.

ORCID profiles: K.B.H., 0000-0001-6760-8377; Y.G., 0000-0002-8790-0479; I.D., 0000-0002-9258-6963; C.R.S.U., 0000-0002-8928-7049; V.M., 0000-0003-4968-5592; F.G., 0009-0007-5883-391X; T.G., 0000-0002-3261-1186; M.I., 0000-0002-2558-1935; P.J.S., 0000-0003-0101-4482; J.B., 0000-0001-5848-4501; T.H.T., 0000-0002-0991-7961; J.T., 0000-0001-6012-0651; P.S., 0000-0001-7895-2886; U.P., 0000-0001-7024-7082; M.K., 0000-0003-4772-7031; K.K., 0000-0003-1174-5799; A.B., 0000-0002-5879-0610; M. Algeri, 0000-0002-6673-2664; P.B., 0000-0003-4554-0265; C.P., 0000-0003-0369-8515; J.-H.D., 0000-0002-8406-0793; M. Ansari, 0000-0002-9649-6498.

Correspondence: Marc Ansari, Division of Pediatric Oncology and Hematology, Department of Women, Child, and Adolescent, Geneva University Hospitals, Rue Willy Donzé 6, 1205 Geneva, Switzerland; email: marc.ansari@hcuge.ch.

## References

1. Peters C, Dalle JH, Locatelli F, et al. Total body irradiation or chemotherapy conditioning in childhood ALL: a multinational, randomized, noninferiority phase III study. *J Clin Oncol*. 2021;39(4):295-307.
2. Willasch AM, Peters C, Sedlacek P, et al; EBMT Paediatric Diseases Working Party. Myeloablative conditioning for allo-HSCT in pediatric ALL: FTBI or chemotherapy? a multicenter EBMT-PDWP study. *Bone Marrow Transpl*. 2020;55(8):1540-1551.
3. Bader P, Potschger U, Dalle JH, et al. Low rate of nonrelapse mortality in under-4-year-olds with ALL given chemotherapeutic conditioning for HSCT: a phase 3 FORUM study. *Blood Adv*. 2024;8(2):416-428.
4. Pulsipher MA. Continued role for radiation in the conditioning regimen for children with ALL. *J Clin Oncol*. 2021;39(4):262-264.
5. Kalwak K, Moser LM, Potschger U, et al. Comparable outcomes after busulfan- or treosulfan-based conditioning for allo-HSCT in children with ALL: results of FORUM. *Blood Adv*. 2025;9(4):741-751.
6. Lawitschka A, Peters C. Long-term effects of myeloablative allogeneic hematopoietic stem cell transplantation in pediatric patients with acute lymphoblastic leukemia. *Curr Oncol Rep*. 2018;20(9):74.
7. Ben Hassine K, Powys M, Svec P, et al. Total body irradiation forever? Optimising chemotherapeutic options for irradiation-free conditioning for paediatric acute lymphoblastic leukaemia. *Front Pediatr*. 2021;9:775485.
8. Eichinger A, Poetschger U, Glogova E, et al. Incidence of subsequent malignancies after total body irradiation-based allogeneic HSCT in children with ALL - long-term follow-up from the prospective ALL-SCT 2003 trial. *Leukemia*. 2022;36(11):2567-2576.
9. Lawson R, Staatz CE, Fraser CJ, Hennig S. Review of the pharmacokinetics and pharmacodynamics of intravenous busulfan in paediatric patients. *Clin Pharmacokinet*. 2021;60(1):17-51.

10. van der Stoep M, Oostenbrink LVE, Bredius RGM, et al. Therapeutic drug monitoring of conditioning agents in pediatric allogeneic stem cell transplantation; where do we stand? *Front Pharmacol*. 2022;13:826004.
11. Combarel D, Tran J, Delahousse J, Vassal G, Paci A. Individualizing busulfan dose in specific populations and evaluating the risk of pharmacokinetic drug-drug interactions. *Expert Opin Drug Metab Toxicol*. 2023;19(2):75-90.
12. Domingos V, Nezvalova-Henriksen K, Dadkhah A, et al. A practical guide to therapeutic drug monitoring in busulfan: recommendations from the Pharmacist Committee of the European Society for Blood and Marrow Transplantation (EBMT). *Bone Marrow Transpl*. 2024;59(12):1641-1653.
13. Bartelink IH, Lalmohamed A, van Reij EM, et al. Association of busulfan exposure with survival and toxicity after haemopoietic cell transplantation in children and young adults: a multicentre, retrospective cohort analysis. *Lancet Haematol*. 2016;3(11):e526-e536.
14. Bognar T, Garcia-Rosa M, Lalmohamed A, et al. Association of busulfan exposure and outcomes after HCT for patients with an inborn error of immunity. *Blood Adv*. 2024;8(19):5137-5145.
15. Friend BD, Bailey-Olson M, Melton A, et al. The impact of total body irradiation-based regimens on outcomes in children and young adults with acute lymphoblastic leukemia undergoing allogeneic hematopoietic stem cell transplantation. *Pediatr Blood Cancer*. 2020;67(2).
16. Versluijs AB, de Koning CCH, Lankester AC, et al. Clofarabine-fludarabine-busulfan in HCT for pediatric leukemia: an effective, low toxicity, TBI-free conditioning regimen. *Blood Adv*. 2022;6(6):1719-1730.
17. Nguyen L, Fuller D, Lennon S, Leger F, Puozzo C. I.V. busulfan in pediatrics: a novel dosing to improve safety/efficacy for hematopoietic progenitor cell transplantation recipients. *Bone Marrow Transpl*. 2004;33(10):979-987.
18. European Medicines Agency. Busulfan Fresenius-Kabi product information. European Medecines Agency, Amsterdam, The Netherlands. 2021. https://www.ema.europa.eu/en/medicines/human/EPAR/busulfan-fresenius-kabiPublisher
19. Choong E, Uppugunduri CRS, Marino D, et al. Therapeutic drug monitoring of busulfan for the management of pediatric patients: cross-validation of methods and long-term performance. *Ther Drug Monit*. 2018;40(1):84-92.
20. Ben Hassine K, Nava T, Theoret Y, et al. Precision dosing of intravenous busulfan in pediatric hematopoietic stem cell transplantation: Results from a multicenter population pharmacokinetic study. *CPT Pharmacometrics Syst Pharmacol*. 2021;10(9):1043-1056.
21. Ben Hassine K, Daali Y, Gloor Y, et al. Simulation-based optimization of sampling schedules for model-informed precision dosing of once-daily and 4-times-daily busulfan in pediatric patients. *Ther Drug Monit*. 2024;46(6):786-796.
22. McCune JS, Quinones CM, Ritchie J, et al. Harmonization of Busulfan Plasma Exposure Unit (BPEU): a community-initiated consensus statement. *Biol Blood Marrow Transpl*. 2019;25(9):1890-1897.
23. Palmer J, McCune JS, Perales MA, et al. Personalizing busulfan-based conditioning: considerations from the American Society for Blood and Marrow Transplantation Practice Guidelines Committee. *Biol Blood Marrow Transpl*. 2016;22(11):1915-1925.
24. Chen Y, Huang J, He X, et al. A novel approach to determine two optimal cut-points of a continuous predictor with a U-shaped relationship to hazard ratio in survival data: simulation and application. *BMC Med Res Methodol*. 2019;19(1):96.
25. Eichinger A, Szmit Z, Kleinschmidt K, et al. Total body irradiation versus chemotherapy conditioning before allogeneic hematopoietic stem cell transplantation for pediatric acute lymphoblastic leukemia patients aged 2 to 4 years: a retrospective study of the EBMT Pediatric Diseases Working Party. *Blood*. 2024;144(suppl 1):3540.
26. Oostenbrink LV, von Asmuth EG, Jol-van der Zijde CM, et al. Anti-T-lymphocyte globulin exposure is associated with acute graft-versus-host disease and relapse in pediatric acute lymphoblastic leukemia patients undergoing hematopoietic stem cell transplantation: a multinational prospective study. *haematologica*. 2024;109(9):2854-2863.
27. Admiraal R, Nierkens S, Bierings MB, et al. Individualised dosing of anti-thymocyte globulin in paediatric unrelated allogeneic haematopoietic stem-cell transplantation (PARACHUTE): a single-arm, phase 2 clinical trial. *Lancet Haematol*. 2022;9(2):e111-e120.
28. Admiraal R, van Kesteren C, Jol-van der Zijde CM, et al. Association between anti-thymocyte globulin exposure and $CD4^+$ immune reconstitution in paediatric haemopoietic cell transplantation: a multicentre, retrospective pharmacodynamic cohort analysis. *Lancet Haematol*. 2015;2(5):e194-e203.
29. Admiraal R, van Kesteren C, Jol-van der Zijde CM, et al. Population pharmacokinetic modeling of Thymoglobulin((R)) in children receiving allogeneic-hematopoietic cell transplantation: towards improved survival through individualized dosing. *Clin Pharmacokinet*. 2015;54(4):435-446.
30. Meesters-Ensing JI, Admiraal R, Ebskamp L, et al. Therapeutic drug monitoring of anti-thymocyte globulin in allogeneic stem cell transplantation: proof of concept. *Front Pharmacol*. 2022;13:828094.
31. Langenhorst JB, van Kesteren C, van Maarseveen EM, et al. Fludarabine exposure in the conditioning prior to allogeneic hematopoietic cell transplantation predicts outcomes. *Blood Adv*. 2019;3(14):2179-2187.
32. Dekker L, de Koning CCH, Nijstad AL, et al. Effect of clofarabine and fludarabine exposure on outcome after pediatric allogeneic hematopoietic cell transplantation. *Blood Neoplasia*. 2024;1(3).
33. Balduzzi A, Glogova E, Peters C, et al. Impact of minimal residual disease on the outcome of hematopoietic stem cell transplantation for childhood acute lymphoblastic leukemia within the FORUM Trial. *Haematologica*. 2025;111(1):122-134.