Title

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A New Harmonized Approach to Estimate Busulfan Exposure Predicts Survival and Toxicity after Hematopoietic Cell Transplantation in Children and Young Adults: a Multicenter Retrospective Cohort Analysis

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Abstract

Background—Intravenous-busulfan (IV-busulfan) combined with therapeutic drug monitoring to guide dosing improves outcomes after allogeneic hematopoietic cell transplantation (allo-HCT). The best method to estimate busulfan exposure and the optimal exposure in children/young adults remains unclear. We therefore evaluated three approaches to estimate IV-Bu exposure (expressed as cumulative-area-under-the-curve; AUC) and associated busulfan-AUC with clinical outcomes in children/young adults undergoing allo-HCT.

Methods—In this retrospective analysis, patients (0.1–30.4 years) receiving busulfan-based conditioning regimen from 15 centers were included. Cumulative AUC was calculated by numerical integration using non-linear mixed effect modeling (AUC_{NONMEM}), non-compartmental analysis ($AUC_{0\text{-infinity}}$ and AUC to the end of the dose interval $AUC_{0\text{-tau}}$) and by individual centers using a variety of approaches (AUC_{center}). Main outcome of interest was event-free survival (EFS). Other outcomes of interest were overall survival, graft-failure, relapse, transplantation related mortality (TRM), acute toxicity (veno-occlusive disease (VOD) and/or acute graft versus-host disease (aGvHD), chronic GvHD (cGvHD) and cGVHD-free event-free survival (GEFS). Propensity score adjusted cox proportional hazard models, Weibull models, and Fine-Gray competing risk regressions were used.

Results—674 patients were included (41% malignant, 59% non-malignant) Estimated 2-year EFS was 69.7%. The median busulfan AUC_{NONMEM} was 74.4 mg*h/L (CI95% 31.1–104.6 mg*h/L). The median AUC_{NONMEM} correlated poorly with AUC_{center} (R^2 = 0.254). Patients with optimal IV-busulfan AUC of 78–101 mg*h/L showed 81% EFS at 2 years compared to 66.1% and 49.5% in the low (<78 mg*h/L) and high (>101 mg*h/L) busulfan AUC group respectively (P=0.011). Graft-failure/relapse occurred more frequently in the low AUC group (HR=1.75 P<0.001). Acute toxicity, cGvHD and TRM was significantly higher in the high AUC group (HR 1.69, 2.99 and 1.30), independent of indication.

Interpretation—These results demonstrate that improved clinical outcomes may be achieved by targeting the busulfan-AUC to 78–101 mg*h/L using a new validated pharmacokinetic-model for all indications.

Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is standard of care treatment for a variety of malignant and nonmalignant disorders (e.g. immunodeficiencies, inherited metabolic diseases and hemoglobinopathies).[1] Busulfan (Bu; Busulfex® for injection) is an alkylating agent routinely used in conditioning regimens prior to allo-HCT[2]. Intravenous (IV) busulfan shows large pharmacokinetic (PK) variability between children[3–7] and the optimal exposure range in children has not been precisely defined. Higher exposure (expressed as area-under-the-curve; AUC) is associated with an increased risk of toxicity: e.g. mucositis, graft-versus-host disease (GvHD), veno-occlusive disease/sinusoidal obstructive syndrome (VOD/SOS) and transplant-related mortality (TRM).[8–11] Low busulfan-AUC has been associated with a higher probability of graft-rejection or disease relapse.[12–14] Therapeutic drug monitoring (TDM) to optimally individualize the dose of IV-Bu is therefore often performed in children undergoing allo-HCT. However various targets (e.g. cumulative-AUC of 58–86 mg*h/L, or an $AUC_{0–6}$ per dose of 900–1350μM*min or the concentration at steady state from 0–6 hours (Css) of 600–900 ng*m/L[3,12,14,15]) and

methods to estimate the AUC are used (e.g. numeric integration or trapezoidal rule, AUC from 0 to infinity; ($AUC_{0\text{-infinity}}$), to the next dose ($AUC_{0\text{-tau}}$), Css. In addition, only a few small, retrospective studies have been performed to determine the optimal AUC of busulfan in children/young adults.[14,16–18] Recent studies in adults and children suggest that a busulfan-AUC of $AUC_{0\text{-inf}}$ 6000 μM*min/day × 4 (equivalent to a cumulative AUC of 100 mg*h/L) reaches optimal efficacy.[10,11,14] The optimal target may however vary with age, diagnosis, concomitant agents included in the preparative regimen and donor source.[15,19] Hence, there is an urgent need to comprehensively study busulfan exposure-response relationships to ensure optimal efficacy and prevent severe toxicity.

We therefore aimed to assess the relation between Busulfan exposure and clinical outcomes. To achieve this, we recalculated all AUCs by numerical integration using nonlinear mixed-effects modeling methodologies NONMEM (AUC_{NONMEM}) and non-compartmental analysis ($AUC_{0\text{-infinity}}$ and $AUC_{0\text{-tau}}$), based on raw time-concentration data and AUC values estimated by site-specific preference for routine TDM. We subsequently conducted a retrospective analysis to relate exposure measures of busulfan to various allo-HCT outcomes, such as event free survival (EFS), aGvHD, VOD, graft-failure/disease relapse, and cGvHD.

Methods

Study Design and Patients

In this analysis, we included all patients who received their 1st allo-HCT with IV-busulfan as part of the conditioning regimen who were enrolled at fifteen pediatric transplant centers between 2000 and 2015, and from whom raw time-concentration data was available. The minimum follow-up for surviving patients was six months. Although analyzed in retrospect, clinical data were collected by the individual institutes prospectively and registered to clinical databases. Patients were included and data collected after written informed consent in accordance with the Declaration of Helsinki. Patients were transplanted according to site-specific HCT protocols.

Busulfan Exposures and Evaluation of Methods to Calculate AUCs

All laboratories used validated methods to quantify busulfan in plasma, according to Good Laboratory Practices. In addition, cross validation of the methods between centers was previously performed.[20]

For patient care, busulfan exposures were calculated by individual centers using a variety of approaches (AUC_{center}, Appendix Table 1). To better understand differences in exposure when estimates for AUC are derived using these different methods, we first compared AUCs estimated by the individual centers (AUC_{center}) with the most commonly used approach: measuring $AUC_{0\text{-infinity}}$ by non-compartmental analysis using the individual raw time-concentration data. The optimal approach to estimate AUCs for this analysis was considered using validated population PK models. Therefore exposures were re-estimated using non-linear mixed effect modeling AUC_{NONMEM}. as described in the Supplement: Statistical

analysis.[4,5,21] The deviation and correlation and R^2 between the estimates by AUC_{NONMEM} with $AUC_{0\text{-infinity}}$ and $AUC_{0\text{-tau}}$ and Css were calculated using linear regression.

Outcomes and effect modifiers

Our main outcome of interest was event free survival (EFS) and was defined as survival from HCT to last contact whereby graft failure, relapse of disease, or death was regarded as events. All surviving patients were censored at day of last contact. Duration of follow-up was the time from allo-HCT to the last assessment for surviving patients or death.

We were also interested in graft-failure (defined as non-engraftment or rejection), disease relapse, transplant-related mortality (TRM), acute toxicity, chronic-GvHD (cGVHD), overall survival (OS) and cGVHD-free event-free survival (GEFS). TRM was defined as death unrelated to underlying disease. Acute toxicity was defined as moderate or severe VOD/SOS (graded according to Bearman),[22] or acute-GVHD grade II–IV (aGVHD, diagnosed and graded according to Glucksberg).[23] Chronic-GvHD (extensive or limited) was classified according to the Shulman criteria.[24]

Predictors of outcome considered were patient-specific variables (age at transplant, gender, cytomegalovirus (CMV) status), malignant/non-malignant disease First Complete Remission (CR1) or CR > 1 at baseline, donor-related factors (cell source, human leukocyte antigen (HLA)-disparity, match/mismatch), CMV status, conditioning regimen (one alkylating agent versus two or three alkylating agents), cumulative busulfan-AUC, use of serotherapy, aGvHD-prophylaxis/ ex vivo T cell depletion, calendar period (</>2006). Non-malignant was defined as having a diagnosis of primary immune deficiencies (PID), bone marrow failure, inherited metabolic diseases and hemoglobinopathies. Non-malignant disease were categorized by risk on graft failure: standard risk were classified; combined immunodeficiency (CID), severe combined immune deficiency (SCID), hemophagocytic lymphohistiocytosis (HLH), chronic granulomatous disease (CGD) or high-risk; inherited metabolic diseases and hemoglobinopathies). GvHD prevention was either *ex-vivo* T cell depletion of the graft of any immunosuppressive therapy given post-allo-HCT.

Statistical Considerations

The exposure-response models were built as described in Supplement: statistical analysis and Appendix Figure 1a. PK-PD analyses were performed using the regression analysis of survival data (PHREG) and procedures to estimate the parameters by maximum likelihood (LIFEREG) procedures from SAS software (version 9.3).

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Results

Patient Characteristics

In total 790 patients (41% malignant, 59% non-malignant) were initially included (Appendix Figure 1a). Eighty-nine patients were excluded as no raw concentration-time profile could be provided (Appendix Figure 1a). 27 patients were excluded as they received a re-transplant. From the remaining 674 patients the median age at allo-HCT was 4.5 years (range, 0.1–30). Graft-source was bone marrow (BM) in 311 (46%), umbilical cord blood (UCB) in 208 (31%) and peripheral blood stem cell (PBSC) in 144 (21%). The most frequently used conditioning regimen was busulfan/cyclophosphamide (n=363, 52%) followed by busulfan/fludarabine (n=265, 38%) and busulfan/cyclophosphamide/melphalan (n=73, 10%). Busulfan was given as once daily in 271 patients (39%) and in 430 patients (61%) in multiple administrations per day. At 13 of 15 centers, dose adjustments of busulfan were performed with routine TDM and using variety of approaches to calculate busulfan exposures (Appendix Table 1).

Cumulative AUCs provided by the individual centers estimated using various different methods are listed (Appendix Table 1 right). Nine institutes used trapezoid $AUC_{0\text{-infinity}}$, three used $AUC_{0\text{-tau}}$ and the other three were numeric integration by PK-models. All these centers used center-specific sampling schemes, used log-linear or linear trapezoidal rules during infusion and post-infusion, one institute used a test dose to estimate the cumulative exposures, in some institutes samples were repeated on one of the following dosing days and each institute varied in how to account for variability in exposure over time. The median $AUC_{0\text{-infinity}}$ estimated using the raw data in the current analysis was 3.6% higher than the AUC estimated by the individual centers (CI 95% −25% and +127%, Appendix Figure 2A). Due to large variability in estimation methods and sampling practices, cumulative AUCs estimated by the individual institutes showed a poor correlation compared to a standardized $AUC_{0\text{-infinity}}$ calculation method (Appendix Figure 2A, $R^2 = 0.254$).

Final estimates of the NONMEM-model used to estimate individual AUCs of all raw PK-data (except the data of UCSF as for this dataset was these specific raw concentration-time data were modeled previously)[4] are shown (Appendix Table 2). Calculated median busulfan-AUC by numerical integration using NONMEM was 74.4 mg*h/L (CI 95% 31.1–104.6 mg*h/L). NONMEM Plots of individual predicted concentrations and observed concentrations *versus* time shows that the predictions by NONMEM decreased variability due to sampling errors and measurement errors. In addition, trapezoidal AUC under-predicts the actual AUC, which is better captured using AUC_{NONMEM} (visualized in Figure 1). In addition, the models capture the increased exposure at day 2 to 4 in all patients. $AUC_{0\text{-infinity}}$ calculated using the raw data correlated well with AUC derived using NONMEM in respect of AUC prediction R^2 of 0.741, but under-predicted the AUC by 8.3% (CI 95% −35 to 17%, Appendix Figure 2B). $AUC_{0\text{-tau}}$ lead to more pronounced under prediction of −25% (CI 95% − 40 to −6%) compared to AUC_{NONMEM}. Css and $AUC_{0\text{-tau}}$ showed the poorest correlation (R^2=0.53, Appendix Figure 2C–2D). AUCs and Css values estimated by non-compartmental analysis were relatively low if measured on one occasion only *versus* multiple occasions, after prolonged infusion times, longer period between infusion and the

first sample, and when limited sampling schemes were used. For these reasons AUC_{NONMEM} was used to associate busulfan-exposure with outcomes.

Outcomes

Estimated EFS at 1 and 2-years post-allo-HCT was 72.6% and 69.7%, respectively. Estimated probability of graft-failure, TRM, and relapse at 2-years was 6.2%, 11.8%, and 20.1, respectively. In the multivariate adjusted cox regression models busulfan-AUC (HR=0.64, P=0.04), malignant disease (HR=1.72, P=0.003), the addition of a third alkylating agent in the conditioning regimen (HR=1.6, P=0.049), and HLA-mismatch (HR=1.7, P=0.031) and year of transplantation (<2006, HR= 0.77 P=0.013) were independent predictors negatively influencing EFS (Appendix Table 3A).

To identify the optimal exposure, multivariate models correlating exposure with EFS were fitted. Given most events took place early after allo-HCT and decelerated with time, a Weibull model with decelerated hazard best described the baseline (Appendix Table 4). A fourth-order polynomial model was used to describe the association between cumulative AUC and EFS (Appendix Table 4, Figure 2A). Plots of model predictions *versus* observed events in the validation dataset shows that the model could well predict outcomes in new patients and the optimum determined using the validation set was within the 95% confidence interval of the originally defined optimum (Figure 2A, dotted line and Table 3). The Weibull model produced an optimal cumulative AUC of 90 mg*h/L (± 10% event probability optimum = 78–101 mg*h/L; Figure 2A). The EFS advantage of this ‘optimal exposure’ compared to the commonly used ‘historical’ busulfan target or an exposure above the ‘optimal exposure’ is demonstrated in Figure 3. A low cumulative AUC (< 78 mg*h/L) increased the probability of graft failure and disease relapse (HR =0.57, P =0.004), while a high AUC (>101 mg*h/L) increased risk of TRM (HR=2.99, P<0.001; Figure 4A, Appendix Table 3A). This observation was similar in malignant and non-malignant disorders (Appendix Figure 3A+B).

In addition, twelve models were designed to evaluate how other patient-specific variables could influence the exposure-EFS relationship (Table 2). None of the variables significantly interacted with busulfan cumulative exposure and outcome parameters, which was confirmed in the validation set. Specifically, no difference was noted in either the shape of curve or the optimum busulfan-AUC between indications (Figure 2B), or number of alkylating agents (Appendix Figure 4A). In a subset analysis, EFS differed significantly between CID, SCID / HLH, CGD, Common variable immunodeficiency disorders (CVID) *versus* other non-malignant diseases (HR = 0.44, P = 0.02), but the optimal busulfan-AUC did not differ (Appendix Figure 4B). Also when SCID was analyzed separately the optimum remains the same for all groups (Appendix Figure 4C).

The estimated probability of acute toxicity, VOD, or grade 2–4 aGVHD at day 100 was 22.9%, 9.1%, and 15.3%, respectively. Estimated probability of cGvHD (limited + extensive) at 2 years was 8.9%. A cumulative AUC above the ‘optimal exposure’ (> 101 mg*h/L) was associated with increased acute toxicities (HR 1.69, P=0.013) but not with cGvHD (HR = 1.3, P=0.374, Table 3, Figure 4B+C). Busulfan-AUC and the use of three alkylating agents (Appendix Fig 5A,B,C) were independent predictors for acute toxicity

(HR=1.69, P<0.013 and HR=2.12, P<0.013), and TRM (HR=2.99, P<0.001 and HR=2.33, P=0.048, Appendix Table 3B). In addition, a transplant after 2006 showed decreased risk of acute toxicity (HR=1.28, P=0.048). The lowest probability of aGvHD, VOD and cGvHD was noted in the single alkylating agent group (Appendix Figure 5B+C).

The estimated probability of GEFS at 1 year was 66.8% and 62.6% at 2-years post-allo-HCT. The shape of the curve and the optimal busulfan-AUC related to OS and GEFS was similar to the cumulative-AUC-EFS relationship with a HR of 0.71, P=0.016 and HR of 0.57, P<0.001 for optimal exposure (78–101 mg*h/L, Table 3). The validation dataset shows the same association between cumulative-AUC and all outcomes of interest (Table 3).

Discussion

To our knowledge this is the largest PK-PD analysis in children/young adults to investigate the relation between exposure and clinical outcome. This study was done to identify the optimal therapeutic window for busulfan in pediatric/young adult allo-HCT, aiming to improve survival chance and reduce toxicity, including TRM and chronic GvHD. With the limitations of a retrospective cohort study taken into account, our data suggests that optimizing the target for cumulative busulfan-exposure has a significant effect on survival chances.

Our data suggests that it is important to standardize the approach to AUC estimation among transplant centers. AUC estimations vary when derived using different calculation approaches (population PK model based or traditional non-compartmental analysis-based). Results of traditional non-compartmental analysis-based calculations vary when using different PK sampling schemes (limited or intensive), infusion time and the specific equations used to calculate AUC for first dose or at steady-state, $AUC_{0\text{-inf}}$, or $AUC_{0\text{-tau}}$). Using a population approach by NONMEM to calculate AUC_{NONMEM} limits the need to plan very specific sampling strategies and better approximates the actual cumulative AUC as it takes into account the exact time of infusion, accounts for errors in sampling and analysis and uses the individual clearance to calculate exposures. In addition, the models capture the increased exposure at day 2 to 4 in all patients. Using non-compartmental analysis, the latter effect can only be observed in patients when sampling occurs on multiple days. This suggests that for future studies it is important to harmonize the PK-estimation approach. This will also allow for better comparisons of busulfan-AUCs between institutions and help to facilitate prospective studies of individualized busulfan dosing strategies. Furthermore it reduces the number of blood samples required for AUC estimation, and will lead to better harmonization in clinical trial-design.[25] Population PK models (based the published models) are accessible for clinical use (http://www.insight-rx.com or http://doseme.com.au).

This study demonstrates that the optimal busulfan-AUC_{NONMEM} of 78–101 mg*h/L predicts higher EFS in children/young adults, compared to lower and higher exposure groups. This is in line with previous publications showing that high busulfan-AUC predicts acute toxicity and TRM[8–10] and low busulfan-AUC leads to graft rejection or disease relapse.[12–14] Our data demonstrates the majority of children/young adults will experience suboptimal busulfan-AUC when using the lower, currently applied ‘historical target’ of 58–86 mg*h/

L[13,15,26,27]. Interestingly, studies conducted primarily in the US adult population target to higher cumulative busulfan-AUC (100 mg*h/L) either in combination with Cy or Flu, similar to the 'optimal exposure' identified in this study.[10,11] Given the optimal exposure range is small and higher than current practice and high inter-patient variability in busulfan-PK,[25] TDM of busulfan is essential to achieve this narrow 'optimal exposure'. The 95% confidence intervals of the models suggest that there is still some unexplained variability in outcomes. Therefore the optimized AUC should be considered with caution while applying the results to a single patient, such as in patients with high co-morbidity scores.

The exposure-EFS association was not influenced by any variable similar to previous studies in adults.[10,11] In line with higher EFS in this study is a recent retrospective study in adults showing that fludarabine added to high dose busulfan (12.8 mg/kg versus 6.4 mg/kg) improved EFS due to lower probability on relapse.[28] However, lower exposure is suggested to be sufficient in specific diseases: e.g. Gungur *et al.* reported in a prospective study that a cumulative busulfan-AUC of 45–65 mg/L*h combined with fludarabine resulted in a 2 year EFS of 89% in patients with CGD transplanted with BM/PBMC.[16] In this study it would be important to understand what the AUC would be when analyzed in a harmonized way. In our cohort 2-year EFS in non-malignant diseases with standard risk of graft failure (CID, SCID, HLH, CGD or CVID) and treated with BM/PBSCs at AUC of 45–65 mg*h/L was 71% while at 78–101 mg*h/L this was 81%, suggesting that further optimization in these patients may be possible, but this finding needs prospective validation. As our subset analyses were limited by the heterogeneity of the study population, a prospective comparison between exposures in specific cohorts of non-malignant and malignant patients is needed to address this further.

Given the retrospective nature of this study we acknowledge there may be other covariates not evaluated in our analysis, such as generalized improvements in post-allo-HCT care, GvHD prophylaxis, or the clinical status and risk of co-morbidities (Center for International Blood and Marrow Transplant Research (CIBMTR) risk) of the patient prior to transplant, as this may have influenced decision making. These factors may have contributed to clinical outcomes. Also a small number of patients receive defibrotide as VOD prophylaxes (most in context of the prophylaxis trial, mostly in BuCyMeL).[29] This may have influenced the endpoint VOD and potentially underestimated the risk of VOD. Other limitations are that for some variables like MRD status prior to allo-HCT, co-morbidity score, GvHD prophylaxis regimen, doses and exposures of each individual drug and ATG exposure before and after HCT[30] may have influence on the outcomes but could not be included in this retrospective analysis. Using a large sample size from fifteen different HCT centers and by applying propensity adjusted analyses we adjusted for possible group selection of low and high busulfan-AUC patients. However, a randomized controlled trial in a specific disease groups may be the best way to confirm this higher and narrow 'optimal exposure' to busulfan.

In conclusion, the use of a new, harmonized and validated approach to measuring the busulfan-exposure aims to target a new, optimal cumulative busulfan exposure in children/young adults undergoing allo-HCT. If this new approach is adopted, we expect higher survival chances with lower toxicity. Busulfan targeted to the 'optimal cumulative busulfan

exposure' combined with fludarabine further optimizes the balance between efficacy and toxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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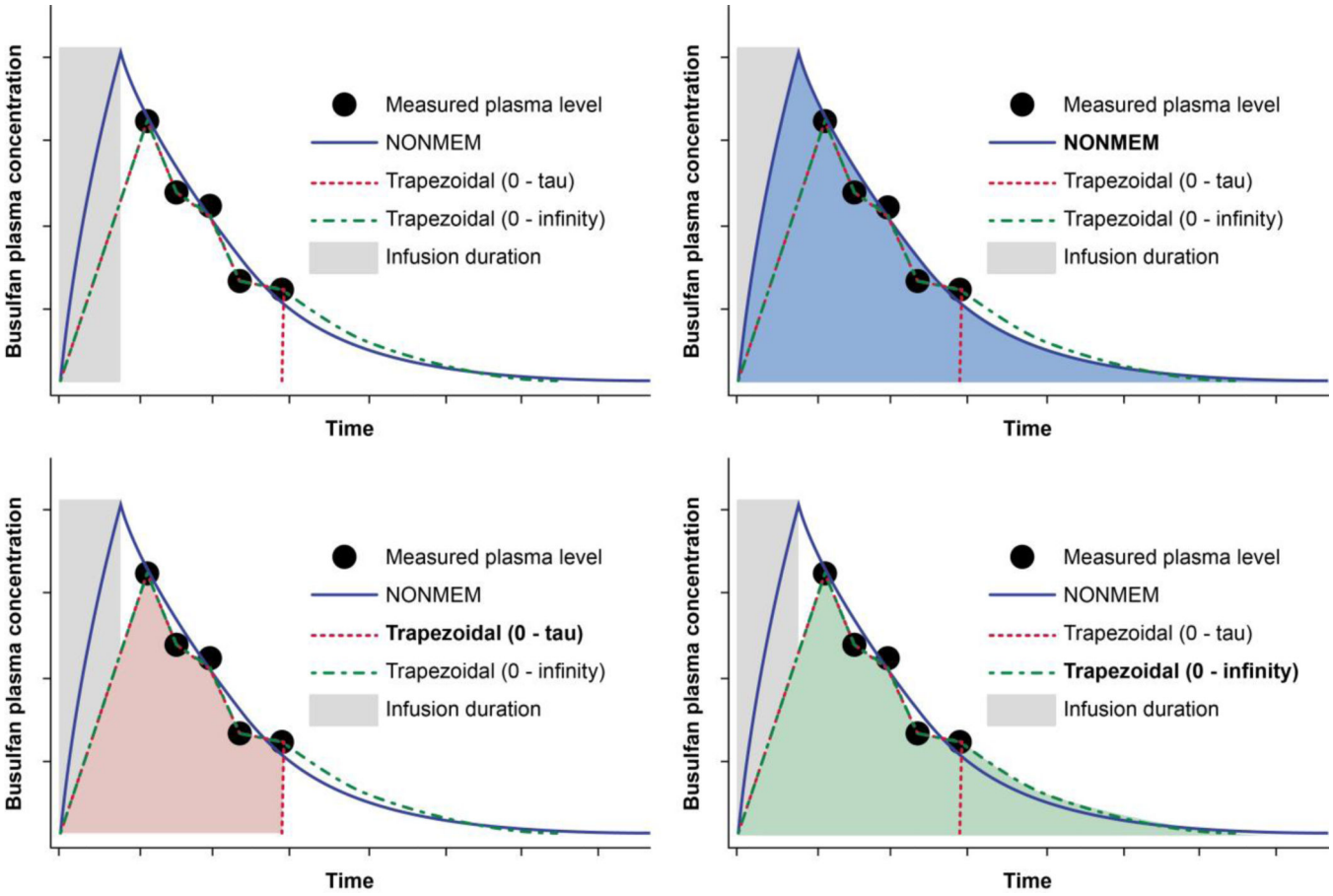

Figure 1.

Example plots showing individual concentration observations derived in individuals (black dots), the individual predicted concentrations (blue shaded area) and non-compartmental analysis* to calculate the exposure ($AUC_{-infinity}$ red shaded area and AUC_{-tau} green shaded area)

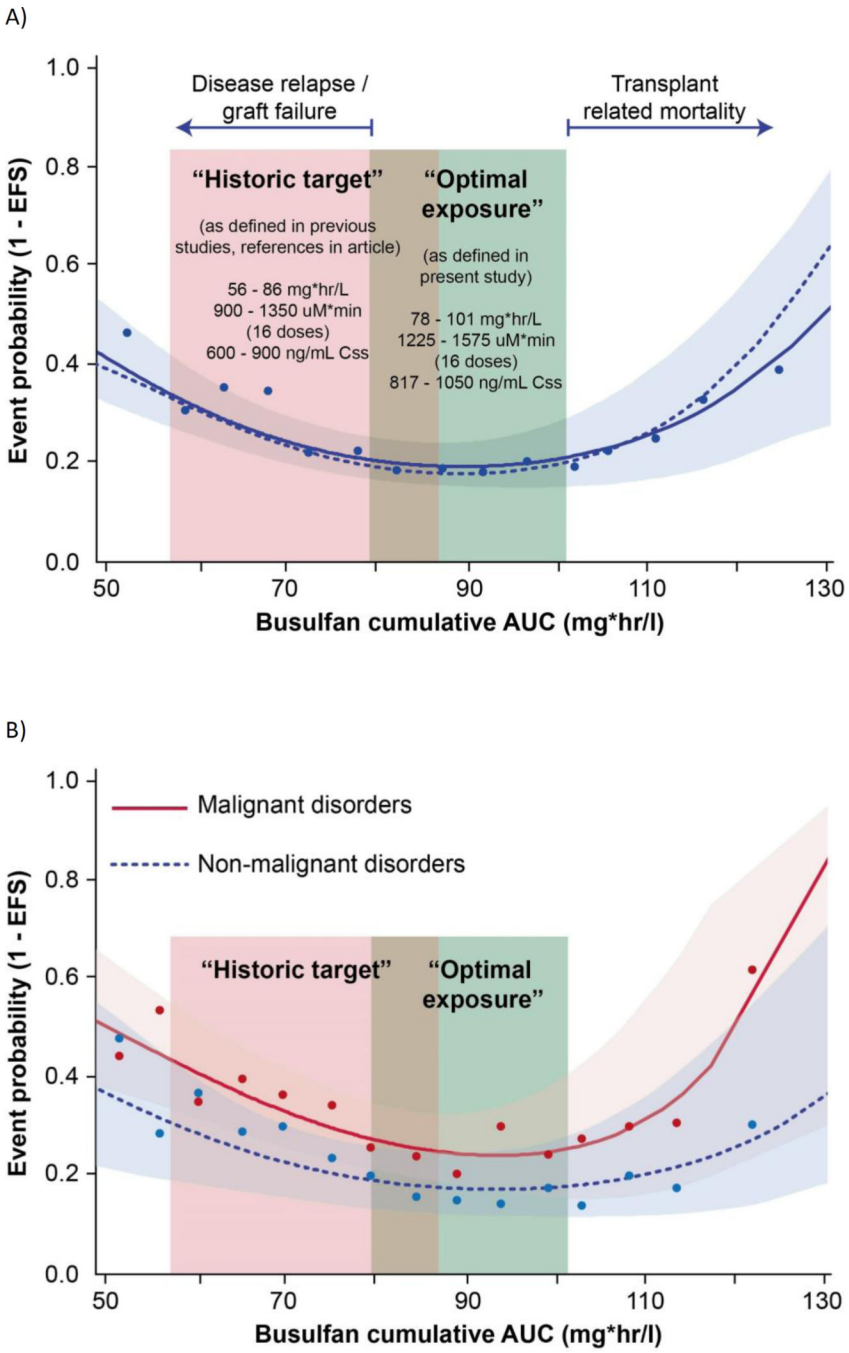

Figure 2.

The polynomial Weibull model of the association between busulfan cumulative AUC and EFS (using uncensored data) is able to reproduce the central tendency in the observed EFS data, shown using Δ 5 mg*h/L AUC groups (dots) in the training (blue solid line) and internal validation dataset (blue dashed line) **(A).** The busulfan cumulative AUC and EFS model stratified by malignant (red solid line) and non-malignant (blue dashed line) underlying disease shows that the optimum AUC does not depend on indication **(B)**. Shaded areas represent 95% confidence intervals.

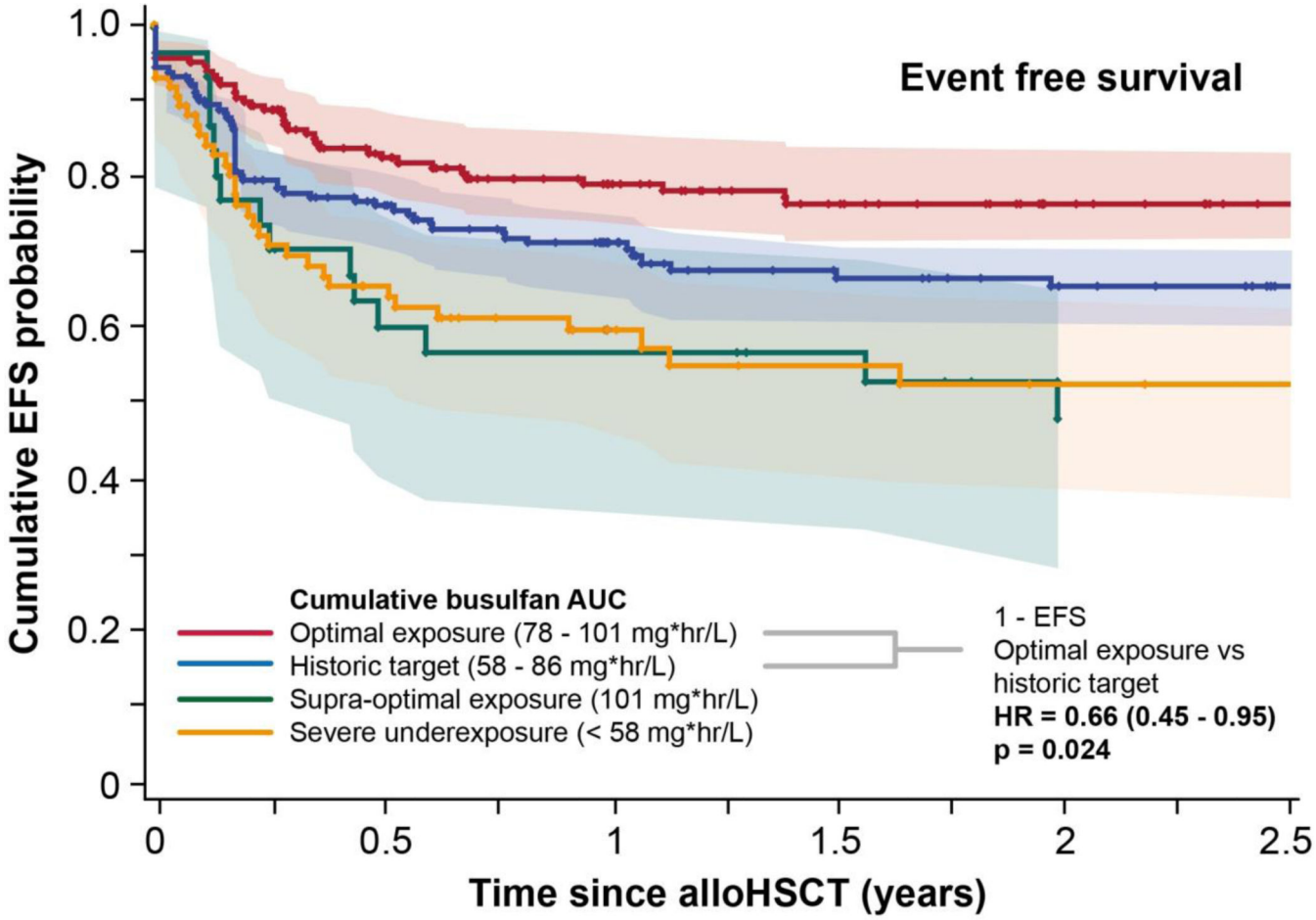

Figure 3.

Kaplan-Meier plots of event-free survival stratified by busulfan cumulative AUC historic, the new target and the AUC above the new target, defined in the current study. Observed EFS (straight lines) including 95%CI (shaded areas) (Fine & Gray) and modelled events (dotted line, using the final Weibull model) are shown. Two year EFS at AUC of < 58 mg*h/L was 52.3%, 'historic target' 58–86 mg*h/L was 66.1%, optimal IV-busulfan AUC of 78–101 mg*h/L was 81% and >101 mg*h/L was 49.5%.

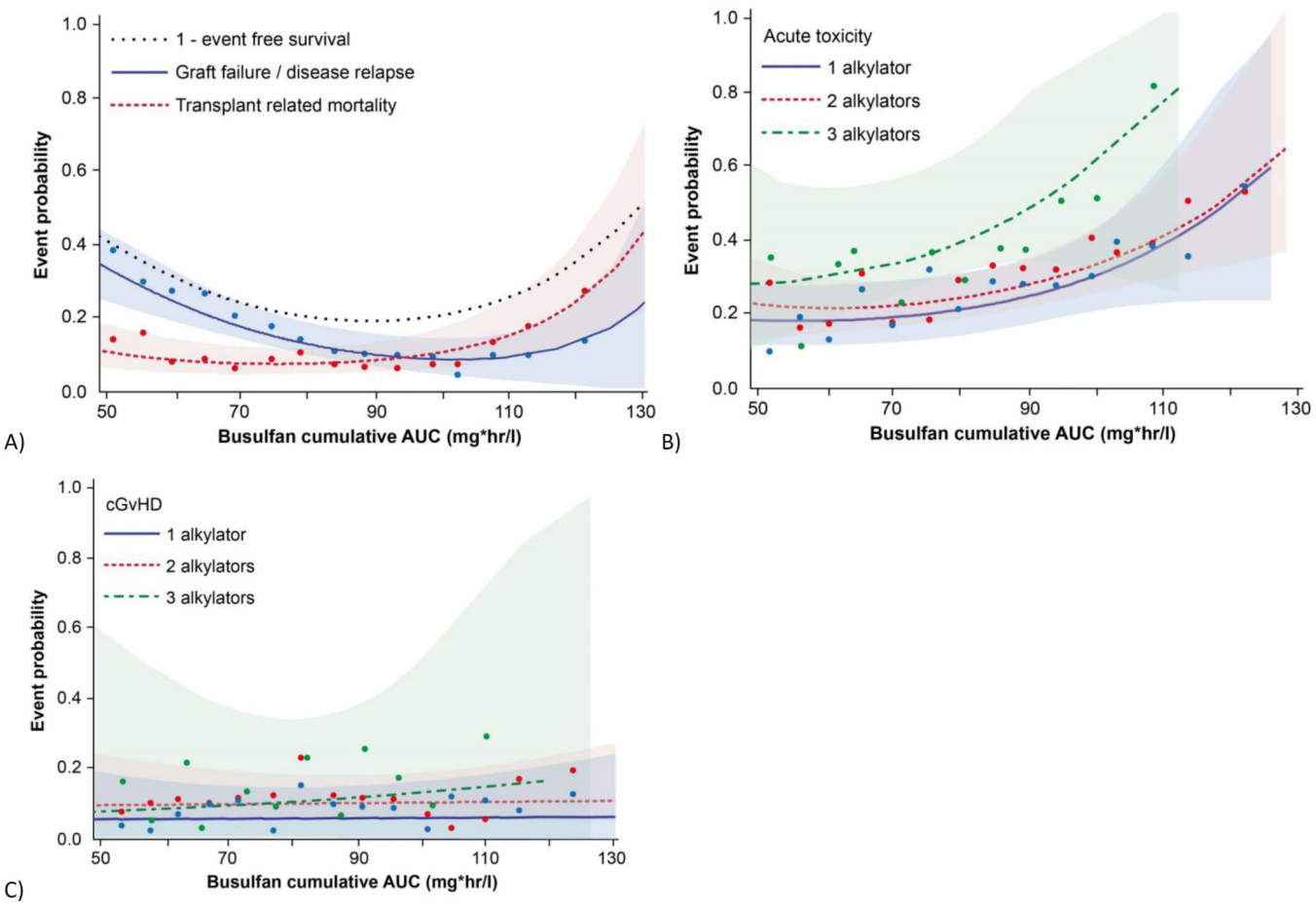

Figure 4.

The polynomial Weibull model of the association between busulfan cumulative AUC and graft failure/ disease relapse and TRM (using uncensored data) **(A)** and acute toxicity (at 6 months post-HCT) **(B)** and cGvHD **(C),** with toxicities stratified by number of alkylating agents showed that a low cumulative exposure (<78 mg*h/L) increased the probability of graft failure/disease relapse, but an decreased the risk of TRM. A high cum AUC (>101 mg*h/L) and the addition of a second or third alkylator increased the probability of VOD, aGvHD and cGvHD.

Table 1

Characteristics of the study population (n=674)

Characteristic		N	(%)
Patient demographics			
Age, years, median (range)		4.5	(0.1–30.4)
Year of transplant, year, median (range)		2008	(2000–2015)
Sex	Males	425	(63%)
missing, n = 0	Females	249	(37%)
CMV status recipient	Negative	332	(49%)
missing, n = 72	Positive	270	(40%)
Indication	**Malignant**	274	(41%)
missing, n = 0	AML	118	(18%)
	MDS	61	(9%)
	ALL	31	(5%)
	JMML	26	(4%)
	CML	17	(3%)
	Lymphoma, NHL	8	(1%)
	Infant ALL	5	(1%)
	Lymphoma, HD	4	(1%)
	Solid	3	(0%)
	Biphenotypical	1	(0%)
	Non-malignant	400	(59%)
	Metabolic	123	(18%)
	Hb-pathy	75	(11%)
	CID	61	(9%)
	SCID	43	(6%)
	HLH / XLP	36	(5%)
	CGD	29	(4%)
	Congenital BMF	20	(3%)
	SAA	7	(1%)
	CVID	3	(0%)
	Autoimmune	2	(0%)
	Bone marrow failure	1	(0%)
Remission status prior to transplantation	CR 1	69	(10%)
missing, n = 164 (malignancies only)	CR > 1	41	(6%)
Donor related factors			
HLA disparity *	Matched	373	(55%)
missing, n = 50	Mismatched	251	(37%)
Source	BM	311	(46%)
missing, n = 11	UCB	208	(31%)
	PBSC (+BM)	144	(21%)
CMV status donor	Negative	380	(56%)

Characteristic		N	(%)
missing, n = 57	Positive	219	(32%)
Conditioning regimen			
Number of alkylating agents in conditioning	1	252	(37%)
missing, n = 0	2	352	(52%)
	3	70	(10%)
GvHD prophylaxis / ex vivo T cell depletion	No	0	(0%)
missing, n = 15	Yes	659	(98%)
	GvHD prophylaxis	620	(92%)
	Ex vivo T cell depletion	39	(6%)
Serotherapy **	No	134	(20%)
missing, n = 57	Yes	483	(72%)
Busulfan dosing regimen	QD	267	(40%)
missing, n = 0	Q6H	324	(48%)
	Other	83	(12%)

Abbreviations: HLA, human leukocyte antigen; Bu, busulfan; Flu, fludarabine; Cy, cyclophosphamide; Mel, melphalan; QD, once daily; Q6H, four times daily; UBM, unrelated bone marrow; UCB, umbilical cord blood; PBSC, peripheral blood stem cell; AML, acute myeloid leukemia; ALL, acute lymphatic leukemia; CML, chronic myeloid leukemia; JMML, juvenile myelomonocytic leukemia; HD, Hodgkin's disease; NHL, non-Hodgkin lymphoma; MDS, myelodysplastic syndrome; CGD, chronic granulomatous disease; CID, combined immunodeficiency; BMF, bone marrow failure; CVID, common variable immune deficiency; HLH, hemophagocytic lymphohistiocytosis; XLP, X-linked lymphoproliferative disease; SAA, severe aplastic anemia; SCID, severe combined immunodeficiency; CR, Complete Remission; CMV, cytomegalovirus.

*HLA matching was based on high-resolution typing for class I and class II (10 alleles) for bone marrow or peripheral blood stem cell donors. For cord blood donors, intermediate resolution criteria were used on 6 loci (low resolution for loci HLA-A, -B, and -DRB1 by high resolution typing). One or more allele or antigen mismatches was considered a mismatch.

**Serotherapy was defined as the use of alemtuzumab (Campath®) or ATG (Thymoglobulin®).

Table 2

Multivariate Weibull models showing the optimal busulfan cumulative AUC target for EFS

	training dataset (n = 449)			validation set (n = 225)
	Optimal AUC target (±10%) (mg*hr/L)	**P value model**	**P value optimum vs other stratum**	**Median optimal AUC (mg*hr/L)**
All patients	90 (78 – 101)	0.011	-	86
Malignant underlying disease				
No	88 (75 – 101)	0.035	-	89
Yes	94 (82 – 103)	0.094	0.868	84
By baseline remission				
CR 1	97 (80 – 110)	0.487		81
CR 2+	91 (79 – 107)	0.612	0.910	89
HLA disparity				
Matched	87 (77 – 96)	0.351	-	84
Mismatched	94 (77 – 107)	0.095	0.891	87
By donor relationship				
MRD	87 (77 – 95)	0.032	-	90
MMRD	90 (86 – 100)	0.446	0.930	84
MUD	87 (71 – 103)	0.086	0.894	85
MMUD	98 (83 – 112)	0.184	0.726	86
Number of alkylating agents				
1	92 (76 – 102)	0.102	-	85
2	88 (80 – 100)	0.120	0.892	88
3	92 (84 – 96)	0.224	0.930	88
Age at HSCT				
< 2 years	94 (77 – 106)	0.032	-	82
2–5 years	84 (70 – 96)	0.112	0.801	89
5–12 years	93 (85 – 103)	0.134	0.882	83
> 12 years	92 (80 – 99)	0.198	0.891	89
HSCT source				
UCB	90 (80 – 100)	0.284	-	88
BM / PBSC	89 (79 – 98)	0.408	0.791	83
By year of transplantation				
< 2006	89 (81 – 98)	0.043	-	86
≥ 2006	93 (79 – 106)	0.054	0.326	86
Busulfan dosing regimen				
Once daily dosing	89 (79 – 99)	0.700	-	85
Four times daily dosing	93 (82 – 102)	0.530	0.811	87
By serotherapy				
No	88 (70 – 102)	0.326	-	90
Yes	92 (73 – 104)	0.153	0.882	82

Abbreviations: AUC, area under the curve; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; UCB, umbilical cord blood; BM, bone marrow; PBSC, peripheral blood stem cell; CR, Complete Remission

Table 3

Multivariate Weibull models showing the association between busulfan cumulative AUC and clinical outcomes

		training dataset (n = 469)					validation set (n = 235)
		N patients	**N events**	**HR**	**(95% CI)**	**P value**	**HR**
1 - EFS							
Cumulative AUC (mg*hr/L)	< 78	280	95		1		1
	78 – 101	141	32	**0.64**	**(0.47 – 0.87)**	**0.004**	0.61
	> 101	28	14	1.21	(0.73 – 2.00)	0.454	1.04
Graft failure / relapse							
Cumulative AUC (mg*hr/L)	< 78	280	62		1		1
	78 – 101	141	20	**0.57**	**(0.39 – 0.84)**	**0.004**	0.46
	> 101	28	5	0.41	(0.14 – 1.17)	0.094	0.41
TRM							
Cumulative AUC (mg*hr/L)	< 78	280	22		1		1
	78 – 101	141	7	1.07	(0.61 – 1.89)	0.816	1.05
	> 101	28	5	**2.99**	**(1.82 – 4.92)**	**<0.001**	2.43
Acute toxicity *							
Cumulative AUC (mg*hr/L)	< 78	280	88		1		1
	78 – 101	141	52	1.14	(0.88 – 1.47)	0.324	1.13
	> 101	28	17	**1.69**	**(1.12 – 2.57)**	**0.013**	1.57
cGvHD ** / ***							
Cumulative AUC (mg*hr/L)	< 78	280	12		1		1
	78 – 101	141	11	1.30	(0.73 – 2.33)	0.374	1.02
	> 101	28	1				
1 - OS							
Cumulative AUC (mg*hr/L)	< 78	280	79		1		1
	78 – 101	141	28	**0.71**	**(0.53 – 0.94)**	**0.016**	0.66
	> 101	28	10	1.03	(0.63 – 1.68)	0.915	1.21
1 - GEFS ****							
Cumulative AUC (mg*hr/L)	< 78	280	101	1	1		1

		training dataset (n = 469)					validation set (n = 235)
		N patients	**N events**	**HR**	**(95% CI)**	**P value**	**HR**
	78 – 101	141	36	**0.57**	**(0.44 – 0.73)**	**<0.001**	0.45
	> 101	28	15	1.38	(0.90 – 2.12)	0.139	1.40

Abbreviations: AUC, area under the curve; HR, hazard ratio; CI, confidence interval; EFS, event free survival; TRM, transplant related mortality; GEFS, cGVHD-free event-free survival; aGvHD, acute graft versus host disease; VOD, veno-occlusive disease. cGvHD, chronic graft versus host disease

* Acute toxicity was defined as aGvHD (grade II+) and VOD.

** Cumulative AUC categories 78 – 101 and > 101 mg*hr/L were merged because of too few observations.

*** Patients at risk of developing cGvHD at day 100: 136 (AUC < 78), 113 (AUC 78 – 101), 26 (AUC > 101).

**** GEFS was defined as EFS without presence of cGvHD.