Association of busulfan exposure with survival and toxicity after haemopoietic cell transplantation in children and young adults: a multicentre, retrospective cohort analysis

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Summary

Background Intravenous busulfan combined with therapeutic drug monitoring to guide dosing improves outcomes after allogeneic haemopoietic cell transplantation (HCT). The best method to estimate busulfan exposure and optimum exposure in children or young adults remains unclear. We therefore assessed three approaches to estimate intravenous busulfan exposure (expressed as cumulative area under the curve [AUC]) and associated busulfan AUC with clinical outcomes in children or young adults undergoing allogeneic HCT.

Methods In this retrospective analysis, patients from 15 centres in the Netherlands, USA, Canada, Switzerland, UK, Italy, Germany, and Australia who received a busulfan-based conditioning regimen between March 18, 2001, and Feb 12, 2015, were included. Cumulative AUC was calculated by numerical integration using non-linear mixed effect modelling (AUC_{NONMEM}), non-compartmental analysis (AUC from 0 to infinity [$AUC_{0-\infty}$] and to the next dose [AUC_{0-t}]), and by individual centres using various approaches (AUC_{centre}). The main outcome of interest was event-free survival. Other outcomes of interest were graft failure or relapse, or both; transplantation-related mortality; acute toxicity (veno-occlusive disease or acute graft versus-host disease [GvHD]); chronic GvHD; overall survival; and chronic-GvHD-free event-free survival. We used propensity-score-adjusted Cox proportional hazard models, Weibull models, and Fine-Gray competing risk regressions for statistical analyses.

Findings 790 patients were enrolled, 674 of whom were included: 274 (41%) with malignant and 400 (59%) with non-malignant disease. Median age was 4·5 years (IQR 1·4–10·7). The median busulfan AUC_{NONMEM} was 74·4 mg×h/L (95% CI 31·1–104·6), which correlated with the standardised method $AUC_{0-\infty}$ (r^2=0·74), but the latter correlated poorly with AUC_{centre} (r^2=0·35). Estimated 2-year event-free survival was 69·7% (95% CI 66·2–73·0). Event-free survival at 2 years was 77·0% (95% CI 72·1–82·9) in the 257 patients with an optimum intravenous busulfan AUC of 78–101 mg×h/L compared with 66·1% (60·9–71·4) in the 235 patients at the low historical target of 58–86 mg×h/L and 49·5% (29·2–66·0) in the 44 patients with a high (>101 mg×h/L) busulfan AUC (p=0·011). Compared with the low AUC group, graft failure or relapse occurred less frequently in the optimum AUC group (hazard ratio [HR] 0·57, 95% CI 0·39–0·84; p=0·0041). Acute toxicity (HR 1·69, 1·12–2·57; p=0·013) and transplantation-related mortality (2·99, 1·82–4·92; p<0·0001) were significantly higher in the high AUC group (>101 mg×h/L) than in the low AUC group (<78 mg×h/L), independent of indication; no difference was noted between AUC groups for chronic GvHD (<78 mg×h/L *vs* ≥78 mg×h/L, HR 1·30, 95% CI 0·73–2·33; p=0·37).

Interpretation Improved clinical outcomes are likely to be achieved by targeting the busulfan AUC to 78–101 mg×h/L using a new validated pharmacokinetic model for all indications.

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Introduction

Allogeneic haemopoietic cell transplantation (HCT) is the standard treatment for various malignant and non-malignant disorders (eg, immunodeficiencies, inherited metabolic diseases, and haemoglobinopathies).[1] Busulfan is an alkylating drug routinely used in conditioning regimens before allogeneic HCT.[2] The pharmacokinetics of intravenous busulfan vary substantially among children,[3–7] and the optimum 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, such as mucositis, graft-versus-host disease (GvHD), and veno-occlusive disease (VOD) or sinusoidal obstructive syndrome, and transplantation-related mortality.[8–11] A low busulfan AUC has been associated with a higher probability of graft rejection or disease relapse.[12–14] Therefore, therapeutic drug monitoring to optimally

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Research in context

Evidence before this study

We searched PubMed on June 10, 2016, using the search term busulfan AND (exposure OR concentration) AND (bone marrow transplantation OR hematopoietic cell transplantation), with no language or date restrictions. We selected studies that related outcomes to intravenous busulfan exposures in adults and children, those with different dosing schedules of intravenous busulfan, and those that compared busulfan with different concomitant chemotherapies. We identified 14 studies, 13 retrospective and one randomised prospective study of intravenous busulfan in children and adults, which showed that higher exposure (expressed as cumulative area under the curve [AUC]) was associated with an increased risk of toxicity, including mucositis, graft-versus-host disease, veno-occlusive disease, and transplantation-related mortality, and a low busulfan AUC was associated with a higher probability of graft rejection or disease relapse. Findings from one study suggested that the maximum serum concentration, not the AUC, was associated with veno-occlusive disease. We identified four retrospective single-centre studies that established an optimum AUC of intravenous busulfan in children or young adults. The number of patients ranged from 56 to 102 and median follow-up was between 1 year and 3 years. Two were retrospective studies in which busulfan at a large range of exposures was combined with cyclophosphamide or melphalan, or both, for various indications, with 74–82 mg×h/L as the optimum AUC (for the busulfan, cyclophosphamide, and melphalan combination). The other two studies were a retrospective single centre and a prospective multicentre study in which busulfan was combined with fludarabine in patients with non-malignant drugs such as chronic granulomatous disease targeted to a busulfan AUC of 45–65 mg×h/L without comparisons with other exposures. Busulfan exposures were measured using numeric integration by a pharmacokinetic model in three studies and concentration at steady state from 0 h to 6 h after the first dose values were calculated using a trapezoidal rule in one study.

Added value of this study

Findings from our study showed that the way of calculating the AUC affects the optimum AUC. Validated population pharmacokinetic methods seem to be the most reliable way to calculate the AUC, allow for comparisons of busulfan AUCs between institutions, and help to facilitate prospective studies of individualised busulfan dosing strategies. A cumulative busulfan exposure of between 78 mg×h/L and 101 mg×h/L, combined with the non-alkylating drug fludarabine, predicted the highest event-free survival in children or young adults independent of indication and cell source. An increased risk of acute and chronic toxicity was noted at higher exposures, whereas an increased risk of graft rejection or disease relapse occurred at lower exposures.

Implications of all the available evidence

No studies have reported on a comparison of the various methods used to calculate the AUC, which makes comparison and interpretation difficult. This multicentre study is, to our knowledge, the largest reported (~700 children and young adults) to include raw pharmacokinetic data, which made it possible to compare different AUC calculation approaches. Using this harmonised method in either treatment protocols or study protocols is expected to result in more predictable outcomes.

individualise the dose of intravenous busulfan is often done in children undergoing allogeneic HCT. Various targets (eg, a cumulative AUC of 58–86 mg×h/L, or an AUC_{0-6} [ie, AUC calculated from 0 h to 6 h after busulfan administration] per dose of 900–1350 μM×min or the concentration at steady state from 0 h to 6 h after the first busulfan administration or after several administrations [steady state] of 600–900 ng×m/L) have been published.[3,12,14,15] Additionally, several methods to estimate the AUC are used to individually optimise doses (eg, numeric integration or trapezoidal rule, AUC from 0 to infinity [$AUC_{0-\infty}$] or to the next dose [$AUC_{0-\tau}$], and concentration at steady state from 0 h to 6 h). Additionally, only a few small, retrospective studies have been done to establish the optimum AUC of busulfan in children or young adults.[14,16–18] Findings from recent studies in adults and children suggest that a busulfan AUC of $AUC_{0-\infty}$ 6000 μM×min/day×4 (equivalent to a cumulative AUC of 100 mg×h/L) achieves optimum efficacy.[10,11,14] However, the optimum target is likely to vary with age, diagnosis, concomitant drugs 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 optimum efficacy and prevent severe toxicity.

We therefore aimed to assess the relationship between busulfan exposure and clinical outcomes. To achieve this, we recalculated all cumulative busulfan AUCs by numerical integration using non-linear mixed-effects modelling methods NONMEM (AUC_{NONMEM}) and non-compartmental analysis ($AUC_{0-\infty}$ and $AUC_{0-\tau}$), based on raw concentration–time data and AUC values estimated by site-specific preferences for routine therapeutic drug monitoring. We subsequently did a retrospective analysis to relate exposure measures of busulfan to various allogeneic HCT outcomes.

Methods

Study design and patients

In this analysis, we included all patients (no age limits) who received their first allogeneic HCT with intravenous busulfan as part of the conditioning regimen at 15 paediatric transplantation centres in the Netherlands, USA, Canada, Switzerland, UK, Italy, Germany, and Australia between March 18, 2001, and Feb 12, 2015, and from whom raw concentration–time data were available.

Busulfan dosing was done as described in the appendix (p 13). Patients received transplants according to site-specific HCT protocols. The minimum follow-up for surviving patients was 6 months. Although analysed in retrospect, clinical data were collected by the individual institutes prospectively and registered to clinical databases. Patients provided written informed consent before the start of the HCT procedure in accordance with the Declaration of Helsinki.

Procedures

All laboratories used validated methods to quantify busulfan in plasma, according to Good Laboratory Practices. Cross validation of the methods between centres has been done previously.[20]

For patient care, busulfan exposures were calculated by individual centres using various approaches (AUC_{centre}, appendix p 13). Briefly, non-compartmental analysis was done using a log-linear trapezoidal rule with the individual raw time–concentration data in R software (R.3.2.0). The AUC was divided by the used dose and multiplied by the cumulative dose. If there were several sampling days, the mean clearance derived by the cumulative AUCs for each individual were calculated and a cumulative AUC was calculated by multiplying mean clearance by cumulative dose. When the AUC was measured at steady state, the next dose level (AUC_{0-tau}) was calculated. The AUC_{0-tau} and the concentration at steady state for four times daily dosing were derived using the equation (AUC / tau)×1000. To better understand differences in exposure when estimates for AUC are derived using these different methods, we first compared AUCs estimated by the individual centres (AUC_{centre}) with the most commonly used approach: measurement of $AUC_{0-\infty}$ by non-compartmental analysis using the individual raw concentration–time data. The optimum approach to estimate AUCs for this analysis was identified using validated population pharmacokinetic models. Therefore, exposures were re-estimated using non-linear mixed effect modelling AUC_{NONMEM}, as described in the appendix (pp 2–3).[4,5,21] We calculated the median difference, correlation, and r^2 between the estimates by AUC_{NONMEM} and those with $AUC_{0-\infty}$, $AUC_{0-\tau}$, and concentration at steady state from 0 h to 6 h by linear regression (appendix pp 2–3).

Outcomes and effect modifiers

The main outcome of interest was event-free survival, defined as survival from HCT to last contact whereby graft failure, relapse of disease, or death were regarded as events. All surviving patients were censored at day of last contact. Duration of follow-up was the time from allogeneic 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, transplantation-related mortality, acute toxicity, chronic GvHD, overall survival, and chronic-GvHD-free event-free survival. Transplantation-related mortality was defined as death unrelated to underlying disease. Acute toxicity was defined as moderate or severe VOD or sinusoidal obstructive syndrome (graded according to Bearman),[22] or acute GvHD grade II–IV (diagnosed and graded according to Glucksberg and colleagues).[23] Chronic GvHD (extensive or limited) was classified according to the Shulman criteria.[24]

Predictors of outcome were patient-specific variables (age at transplantation, sex, and cytomegalovirus status), malignant or non-malignant disease, malignant underlying disease by baseline remission (first complete remission or more than one complete remission at time of transplantation), donor-related factors (cell source and HLA disparity [match or mismatch]), cytomegalovirus status of donor, conditioning regimen (one alkylating drug versus two or three), cumulative busulfan AUC, use of serotherapy, and year of transplantation (before 2006 or 2006 onwards). Non-malignant disease was defined as having a diagnosis of primary immune deficiencies, bone marrow failure, inherited metabolic diseases, and haemoglobinopathies. Non-malignant disease was categorised by risk of graft failure: standard risk (combined immunodeficiency, severe combined immune deficiency, haemophagocytic lymphohistiocytosis, and chronic granulomatous disease) or high risk (inherited metabolic diseases and haemoglobinopathies). HLA matching was based on high-resolution typing for class I and class II (ten alleles) for bone marrow or peripheral blood stem cell donors. For cord blood donors, intermediate-resolution criteria were used on six loci (low resolution for loci HLA-A, HLA-B, and HLA-DRB1 by high-resolution typing). One or more allele or antigen mismatches was considered a mismatch. GvHD prevention was defined as either ex-vivo T-cell depletion of the graft or any immunosuppressive treatment given after allogeneic HCT.

Statistical analysis

The exposure–response models were built as described in the appendix (pp 1–4). We used the PROC SURVEYSELECT procedure in SAS (version 9.3) to randomly split the datasets, using the simple random sampling option,[25] into two sets: two thirds of the data (training dataset) were used for model development and the other third of the data were used as a validation dataset to validate the event-free survival optimum AUC and covariate relationships. We assessed busulfan AUC association in a stepwise fashion. First, we assessed busulfan AUC as a categorical variable split into three subgroups (cumulative busulfan AUC <78 mg×h/L, 78–101 mg×h/L, and >101 mg×h/L). The independent effect of busulfan AUC and all other predefined patient-specific characteristics (ie, effect modifiers) on the outcomes of interest, stratified by HCT centre, was assessed by stratified multivariable Cox regression.

See Online for appendix

	Patients (n=674)
Demographics	
Age (years)	4·5 (0·1–30·4)
Year of transplantation	2008 (2000–2015)
Sex	
Male	425 (63%)
Female	249 (37%)
Cytomegalovirus status of recipient	
Negative	332 (49%)
Positive	270 (40%)
Data missing	72 (11%)
Indication	
Malignant disease	274 (41%)
Acute myeloid leukaemia	118 (18%)
MDS	61 (9%)
Acute lymphatic leukaemia	31 (5%)
JMML	26 (4%)
CML	17 (3%)
Non-Hodgkin lymphoma	8 (1%)
Infant acute lymphatic leukaemia	5 (1%)
Hodgkin's disease	4 (1%)
Solid tumour	3 (<1%)
Biphenotypic acute leukaemia	1 (<1%)
Non-malignant disease	400 (59%)
Metabolic	123 (18%)
Haemoglobinopathy	75 (11%)
Combined immunodeficiency	61 (9%)
Severe combined immunodeficiency	43 (6%)
Haemophagocytic lymphohistiocytosis or X-linked lymphoproliferative disease	36 (5%)
Chronic granulomatous disease	29 (4%)
Congenital bone marrow failure	20 (3%)
Severe aplastic anaemia	7 (1%)
Common variable immune deficiency	3 (<1%)
Autoimmune	2 (<1%)
Bone marrow failure	1 (<1%)
Number of complete remissions before transplantation in patients with malignant disease	
One	69/274 (25%)
More than one	41/274 (15%)
Data missing	60/274 (22%)
Not applicable (MDS, JMML, and CML)	104/274 (38%)
Donor-related factors	
HLA disparity	
Matched	373 (55%)
Mismatched	251 (37%)
Data missing	50 (7%)
Source	
Bone marrow	311 (46%)
Umbilical cord blood	208 (31%)
Peripheral blood stem cell	144 (21%)
Peripheral blood stem cell and bone marrow combined	3 (<1%)
Data missing	8 (1%)

(Table 1 continues in next column)

	Patients (n=674)
(Continued from previous column)	
Cytomegalovirus status of donor	
Negative	380 (56%)
Positive	219 (32%)
Data missing	75 (11%)
Conditioning regimen	
Number of alkylating drugs	
1	252 (37%)
2	352 (52%)
3	70 (10%)
Graft-versus-host disease prophylaxis or ex-vivo T-cell depletion	
No	0 (0%)
Yes	659 (98%)
Graft-versus-host disease prophylaxis	620 (92%)
Ex-vivo T-cell depletion	39 (6%)
Data missing	15 (2%)
Serotherapy*	
No	134 (20%)
Yes	483 (72%)
Data missing	57 (8%)
Busulfan dosing regimen	
Once daily	267 (40%)
Four times daily	324 (48%)
Other	83 (12%)

Data are median (range) or number (%). Some percentages do not add up to 100 because of rounding. CML=chronic myeloid leukaemia. JMML=juvenile myelomonocytic leukaemia. MDS=myelodysplastic syndrome. *Defined as the use of alemtuzumab or anti-thymocyte globulin.

Table 1: **Characteristics of the study population at baseline**

Second, we assessed busulfan AUC as a continuous variable, by fitting propensity-adjusted models using exponential, gamma, log-logistic, log-normal, and Weibull survival models to the outome data. Third, we used Fine-Gray curves to visualise the cumulative incidence of each outcome (appendix p 4). We used the final AUC event-free survival model to estimate the event probability with the lowest probability of an event, allowing for 10% deviation in the event probability. Fourth, we used a continuous model to predict optimum busulfan AUC in relation to outcomes of interest. To establish whether type of disease (malignant or non-malignant or patients with non-malignant disease with high risk of graft failure) affected the optimum AUC of busulfan for a patient, we did several subset analyses (appendix p 5). Pharmacokinetic and pharmacodynamics analyses were done using the regression analysis of survival data (PHREG) and procedures to estimate the parameters by maximum likelihood (LIFEREG) methods. All analyses were done in SAS (version 9.3).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or

writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

790 patients (324 [41%] with malignant and 465 [59%] with non-malignant disease) were enrolled (appendix p 1). For 89 patients (11%) no raw concentration–time profile could be provided because at the centres they attended drug measurements were outsourced and estimated AUCs were reported to the institutes (appendix p 1); these patients were excluded from analyses. 27 patients (3%) were excluded because they received a second transplantation. In the remaining 674 patients who were included in analyses, the median age at allogeneic HCT was 4·5 years (range 0·1–30·4; IQR 1·4–10·7; table 1) and median follow-up was 1·5 years (IQR 0·5–3·6). The graft source was bone marrow in 311 patients (46%), umbilical cord blood in 208 (31%), and peripheral blood stem cells in 144 (21%; table 1). The most frequently used conditioning regimen was busulfan and cyclophosphamide (n=352 [52%]) followed by busulfan and fludarabine (n=252 [37%]) and busulfan, cyclophosphamide, and melphalan (n=70 [10%]). Busulfan was given once daily in 267 patients (40%) and in 324 patients (48%) it was given four times per day. At 13 (87%) of 15 centres, dose adjustments of busulfan were done with routine therapeutic drug monitoring and using various approaches to calculate busulfan exposures (appendix p 13).

Cumulative AUCs provided by the individual centres estimated by various different methods are listed in the appendix (p 13). Nine institutes (60%) used trapezoid $AUC_{0-\infty}$, three (20%) used $AUC_{0-\tau}$, and the other three (20%) used numeric integration by pharmacokinetic models. All centres used centre-specific sampling schemes, the first 12 used log-linear or linear trapezoidal rules during and after infusion; one institute used a test dose to estimate the cumulative exposures; in some institutes samples were repeated on one of the subsequent dosing days; and each institute differed in how to account for variability in exposure over time. The median $AUC_{0-\infty}$ estimated using the raw data in the current analysis was 3·6% higher than the AUC estimated by the individual centres (95% CI –27·7 to 127·3; figure 1A). Owing to large variability in estimation methods and sampling practices, cumulative AUCs estimated by the individual institutes showed a poor correlation compared with the standardised $AUC_{0-\infty}$ calculation method (figure 1A; r^2=0·35).

Final estimates of the NONMEM model used to estimate individual AUCs of all raw pharmacokinetic data (except data from the University of California, San Francisco [UCSF], CA, USA, because for this dataset these specific raw concentration–time data were modelled previously)[4] are shown in the appendix (p 14). The median busulfan AUC calculated by numerical integration using

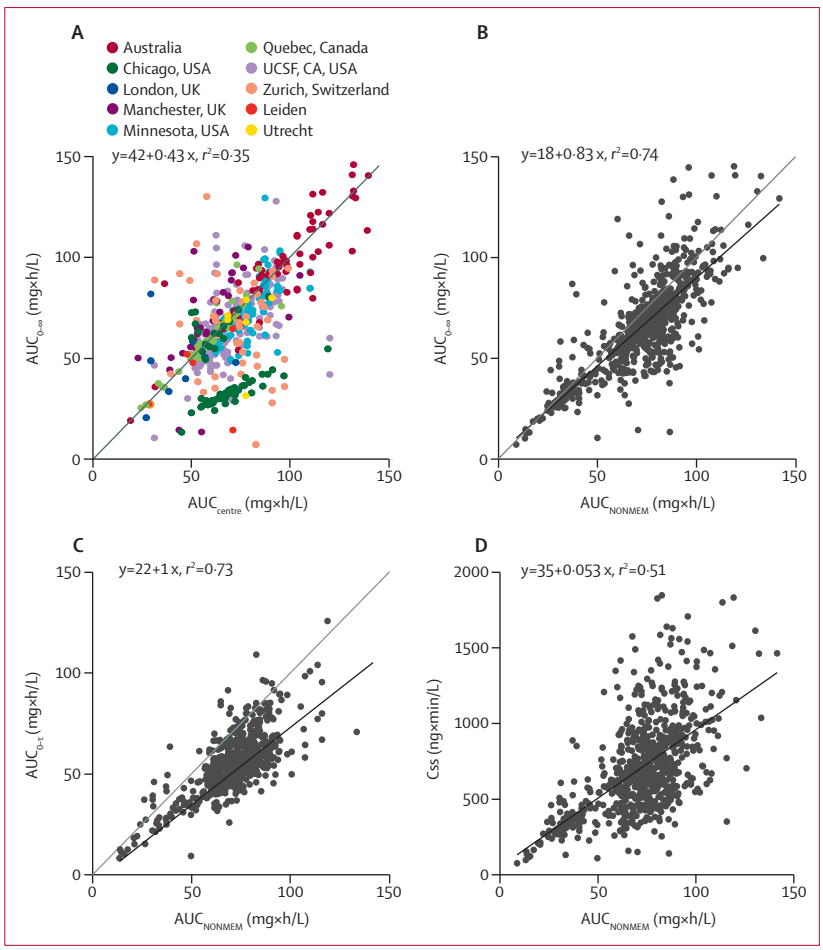

Figure 1: **Correlations between areas under the curve**
(A) Correlation between AUCs derived by individual centres (AUC_{centre}) and by non-compartmental analysis of the raw data ($AUC_{0-\infty}$), and between AUCs derived by NONMEM (AUC_{NONMEM}) and (B) non-compartmental analysis ($AUC_{0-\infty}$) and, in those with four times daily dosing, (C) $AUC_{0-\tau}$ and (D) Css. Individual cumulative AUCs (dots), line of identity (grey line), and the linear regression line (black line) are shown. Calculations show the correlation and r^2 between the AUC estimates. AUC=area under the curve. Css=concentration at steady state with four times daily dosing. UCSF=University of California, San Francisco.

NONMEM was 74·4 mg×h/L (95% CI 31·1–104·6). NONMEM plots of individual predicted concentrations and observed concentrations versus time show that the predictions by NONMEM decreased variability because of sampling errors and measurement errors (data not shown). Additionally, trapezoidal AUC under-predicted the actual AUC, which is better captured using AUC_{NONMEM} (appendix p 3). Also, the models captured the increased exposure at days 2–4 in all patients, as shown by the estimate of the clearance at days 2–4 in the pharmacokinetic model (appendix p 14). $AUC_{0-\infty}$ calculated using the raw data correlated well with AUC_{NONMEM} (r^2=0·74), but under-predicted the AUC by 8·3% (95% CI –34·6 to 17·8, figure 1B). $AUC_{0-\tau}$ led to a more pronounced under-prediction of –24·6% (95% CI –47·1 to –0·64) compared with AUC_{NONMEM}. Concentration at steady state from 0 h to 6 h (r^2=0·51) and $AUC_{0-\tau}$ (r^2=0·73) showed the poorest correlation

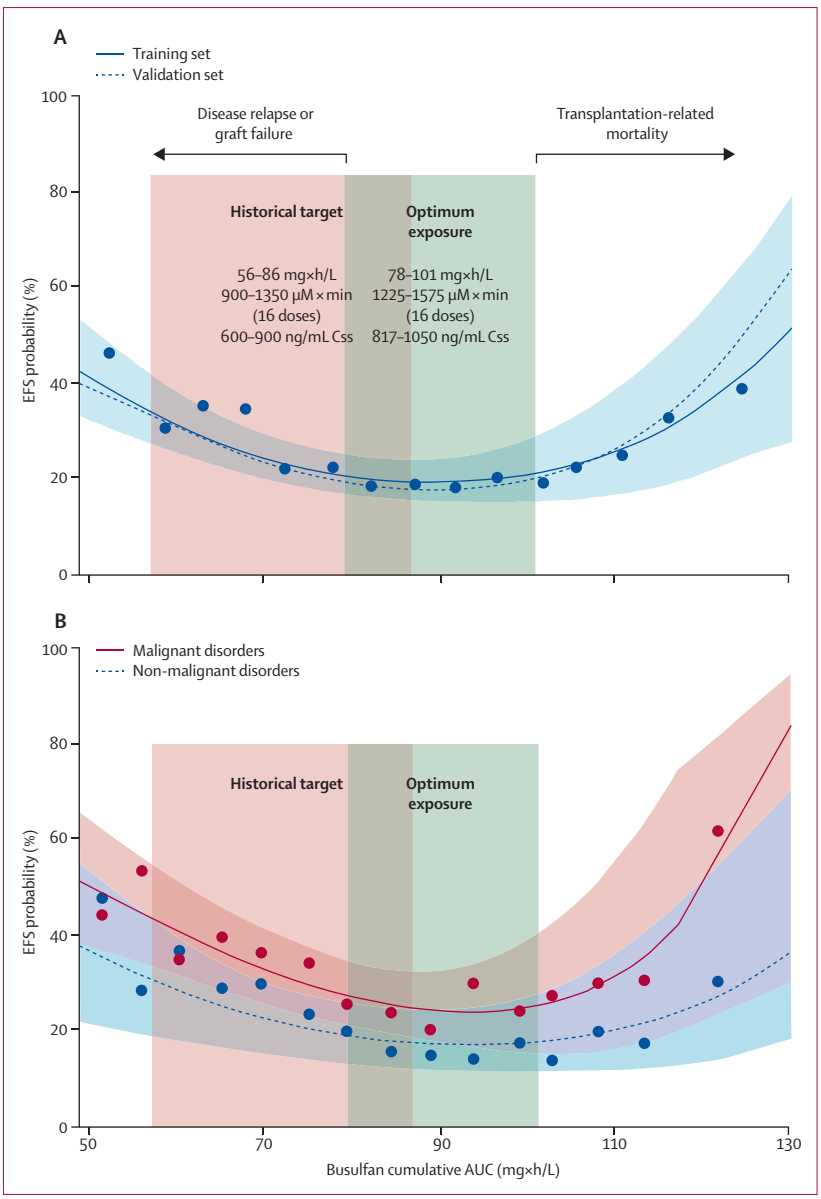

Figure 2: **Polynomial Weibull models of the association between busulfan cumulative area under the curve and event-free survival**

(A) The polynomial Weibull model of the association between busulfan cumulative AUC and EFS (using uncensored data) is able to reproduce the central tendency, because all raw EFS data of the Δ 5 mg×h/L AUC groups (dots) in the training (blue solid line) and internal validation datasets (blue dashed line) fall within the 95% CI of the model predicted association between busulfan cumulative AUC and EFS. (B) The busulfan cumulative AUC and EFS polynomial Weibull 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. The red shaded rectangles show the historical target, as defined in previous studies.[13,15,26,27] The green shaded rectangles show the target defined in the present study. Shaded areas represent 95% CIs. AUC=area under the curve. Css=concentration at steady state. EFS=event-free survival.

(figure 1C and 1D). AUCs and concentration at steady state from 0 h to 6 h values estimated by non-compartmental analysis were low if measured on one occasion only versus several occasions, after prolonged infusion times, after a longer period between infusion and the first sample, and when limited sampling schemes were used (data not shown). For these reasons, AUC_{NONMEM} was used to associate busulfan exposure with outcomes.

Estimated event-free survival after allogeneic HCT was 72·6% (95% CI 69·8–74·9) after 1 year and 69·7% (66·2–73·0) after 2 years. At 2 years, estimated probability of graft failure was 6·2% (95% CI 3·9–8·7), transplantation-related mortality 25·3% (21·0–29·8), and relapse 20·1% (16·4–24·7). In the multivariable adjusted Cox regression models, busulfan cumulative AUC (hazard ratio [HR] 0·64, 95% CI 0·47–0·87; p=0·0036), malignant disease (1·72, 1·20–2·47; p=0·0033), the addition of a third alkylating drug in the conditioning regimen (1·60, 1·00–2·57; p=0·049), HLA mismatch (1·70, 1·03–2·95; p=0·031), and transplantation before 2006 (0·77, 0·63–0·95; p=0·013) were independent predictors that negatively affected event-free survival (appendix p 15); donor source, cytomegalovirus status of recipient, cytomegalovirus status of donor, busulfan dosing regimen, serotherapy, sex, age, HLA disparity, baseline malignant disease remission, and use of serotherapy did not affect event-free survival.

We randomly included 449 patients in the training set and 225 patients in the validation set. To identify the optimum exposure, we fitted multivariable models correlating exposure with event-free survival. Since most events took place early after allogeneic HCT and the number of events decreased over time, a Weibull model with decelerated hazard best described the baseline (appendix p 17). We used a fourth-order polynomial model to describe the association between cumulative AUC and event probability (1–event-free survival) (figure 2A; appendix p 17). Plots of model predictions versus observed events in the validation dataset show that the model could predict outcomes in new patients and the optimum exposure identified using the validation set was within the 95% CI of the originally defined optimum (figure 2A; table 2). The Weibull model produced an optimum cumulative AUC of 90 mg×h/L (range 78–101 mg×h/L, allowing for 10% deviation in the event probability); figure 2A). Figure 3 shows the event-free survival for the optimum exposure compared with the commonly used historical busulfan target or an exposure above the optimum exposure or below the historical target. The 2-year event-free survival was 77·0% (95% CI 72·1–82·9) at the optimum busulfan AUC of 78–101 mg×h/L compared with 52·3% (39·4–62·1) at an AUC of less than 58 mg×h/L, 66·1% (60·9–71·4) at the historical target of 58–86 mg×h/L, and 49·5% (29·2–66·0) at an AUC above 101 mg×h/L (p=0·025; figure 3). Compared with the low AUC group (<78 mg×h/L), the optimum AUC decreased the probability of graft failure or disease relapse (HR 0·57, 95% CI 0·39–0·84; p=0·0041), whereas a high AUC (>101 mg×h/L) increased the risk of transplantation-related mortality (2·99, 1·82–4·92; p<0·0001; figure 4A; appendix p 15). This finding was similar in patients with malignant (median follow-up 1·0 years, IQR 0·3–3·0) and non-malignant disease (median follow-up 2·0 years, IQR 0·7–3·8; appendix

pp 6, 7). Our previously published model-based dosing nomogram[20] can be used to reach a target AUC of 90 mg×h/L (range 78–101; appendix p 18).

Additionally, we designed 12 models to assess how other patient-specific variables could affect the exposure–event-free survival relationship (table 3). In the training dataset, 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 the curve event-probability or the optimum busulfan AUC between malignant and non-malignant disease (figure 2B), and the optimum busulfan AUC did not depend on the number of alkylating drugs (appendix p 8).

In a subset analysis, event-free survival differed significantly between combined immunodeficiency, severe combined immune deficiency or haemophagocytic lymphohistiocytosis, chronic granulomatous disease, or common variable immunodeficiency disorders and other non-malignant diseases (HR 0·44, 95% CI 0·22–0·88; p=0·021), but the optimum busulfan AUC did not differ (appendix p 9). Also, when severe combined immune deficiency was analysed separately, the optimum AUC remained the same for all groups (data not shown).

At day 100, the estimated probability of acute toxicity was 22·9%, of VOD was 9·1%, and of grade 2–4 acute GvHD was 15·3%. Estimated probability of chronic GvHD (limited and extensive) at 2 years was 8·9%. 2-year event-free survival in patients with non-malignant disease with standard risk of graft failure and treated with bone marrow or peripheral blood stem cells at an historical target AUC of 45–65 mg×h/L[16] was 71%, whereas at the optimum of 78–101 mg×h/L it was 81%. Compared with a low AUC (<78 mg×h/L), a cumulative AUC above the optimum exposure (>101 mg×h/L) was associated with an increased risk of acute toxicities (HR 1·69, 95% CI 1·12–2·57; p=0·013) and transplantation-related mortality (2·99, 1·82–4·92; p<0·0001), but not with chronic GvHD (<78 mg×h/L *vs* ≥78 mg×h/L HR 1·30, 95% CI 0·73–2·33; p=0·37; table 2; figure 4). The use of three alkylating drugs was another independent predictor for acute toxicity (HR 2·12, 95% CI 1·17–3·85; p=0·013; appendix p 16) and transplantation-related mortality (2·33, 1·01–5·96; p=0·048; appendix p 15). Additionally, risk of acute toxicity was reduced in patients who received transplantation from 2006 onwards compared with those who received transplantation before 2006 (HR 1·28, 95% CI 1·00–1·64; p=0·048; appendix p 16). The lowest probability of acute GvHD, VOD, and chronic GvHD was noted in the single alkylating drug group (appendix pp 11, 12).

The estimated probability of chronic-GvHD-free event-free survival was 66·8% at 1 year and 62·6% at 2 years after allogeneic HCT. The shape of the event-probibility curve and the optimum busulfan AUC related to overall survival (78–101 mg×h/L HR 0·71, 95% CI 0·53–0·94; p=0·016) and chronic-GvHD-free event-free survival (0·57, 0·44–0·73; p<0·0001) was similar to the cumulative AUC event-free survival relationship (table 2). The validation dataset showed the same association between cumulative AUC and all outcomes of interest (table 2).

	Training dataset (n=449)				Validation set (n=225) HR (95% CI)
	Number of patients	Number (%) of events	HR (95% CI)	p value	
1–event-free survival*					
<78 mg×h/L	280	95 (34%)	1		1
78–101 mg×h/L	141	32 (23%)	0·64 (0·47–0·87)	0·0036	0·61 (0·37–0·97)
>101 mg×h/L	28	14 (50%)	1·21 (0·73–2·00)	0·45	1·04 (0·44–1·69)
Graft failure or relapse					
<78 mg×h/L	280	62 (22%)	1		1
78–101 mg×h/L	141	20 (14%)	0·57 (0·39–0·84)	0·0041	0·46 (0·27–0·92)
>101 mg×h/L	28	5 (18%)	0·41 (0·14–1·17)	0·094	0·41 (0·21–1·35)
Transplantation-related mortality					
<78 mg×h/L	280	22 (8%)	1		1
78–101 mg×h/L	141	7 (5%)	1·07 (0·61–1·89)	0·82	1·05 (0·49–2·23)
>101 mg×h/L	28	5 (18%)	2·99 (1·82–4·92)	<0·0001	2·43 (1·42–6·26)
Acute toxicity†					
<78 mg×h/L	280	88 (31%)	1		1
78–101 mg×h/L	141	52 (37%)	1·14 (0·88–1·47)	0·32	1·13 (0·64–1·87)
>101 mg×h/L	28	17 (61%)	1·69 (1·12–2·57)	0·013	1·57 (0·81–3·12)
Chronic GvHD‡					
<78 mg×h/L	280	12 (4%)	1		1
78–101 mg×h/L§	141	11 (8%)	1·30 (0·73–2·33)	0·37	1·02 (0·59–3·12)
>101 mg×h/L§	28	1 (4%)	..		..
1–overall survival*					
<78 mg×h/L	280	79 (28%)	1		1
78–101 mg×h/L	141	28 (20%)	0·71 (0·53–0·94)	0·016	0·66 (0·31–1·23)
>101 mg×h/L	28	10 (36%)	1·03 (0·63–1·68)	0·92	1·21 (0·59–2·59)
1–chronic GvHD-free event-free survival*¶					
<78 mg×h/L	280	101 (36%)	1		1
78–101 mg×h/L	141	36 (26%)	0·57 (0·44–0·73)	<0·0001	0·45 (0·37–0·99)
>101 mg×h/L	28	15 (54%)	1·38 (0·90–2·12)	0·14	1·40 (0·81–2·84)

AUC=area under the curve. GvHD=graft versus host disease. HR=hazard ratio. VOD=veno-occlusive disease. *The probability of an event. †Defined as acute GvHD (grade II+) and VOD. ‡Patients at risk of developing chronic GvHD at day 100: 136 for AUC <78 mg×h/L, 113 for AUC 78–101 mg×h/L, and 26 for AUC >101 mg×h/L. §Categories merged because of too few events. ¶Defined as event-free survival without the presence of chronic GvHD.

Table 2: **Multivariate Weibull models showing the association between busulfan cumulative area under the curve and clinical outcomes**

Discussion

This study was done to identify the optimum therapeutic window for busulfan in children or young adults undergoing allogeneic HCT, to improve survival and reduce toxicity. We show that the optimum busulfan AUC_{NONMEM} of 78–101 mg×h/L predicted higher event-free survival in children or young adults compared with lower and higher exposure groups. No other variables, such as disease and cell source, affected the optimum AUC of busulfan. Graft failure or relapse occurred less frequently in the optimum AUC group than in the low AUC group, whereas acute toxicity and transplantation-related

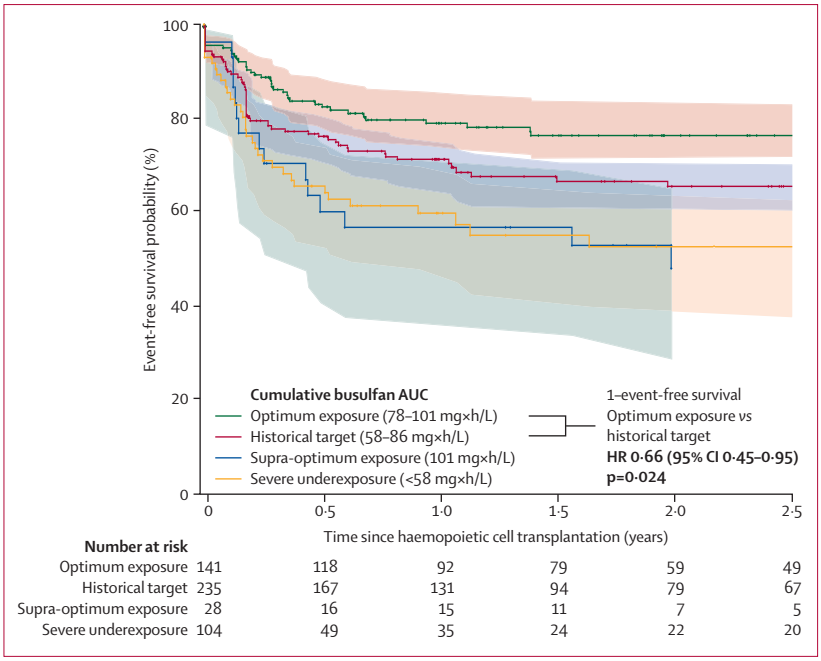

***Figure 3*: Kaplan-Meier plots of event-free survival in three busulfan exposure groups**
Event-free survival is shown stratified by historical busulfan cumulative AUC, the new target (optimum AUC, defined in the current study), AUC above the new target, and AUC below the historical target. Observed event-free survival (solid lines) with 95% CIs (shaded areas; Fine-Gray risk regression analysis) are shown. AUC=area under the curve. HR=hazard ratio.

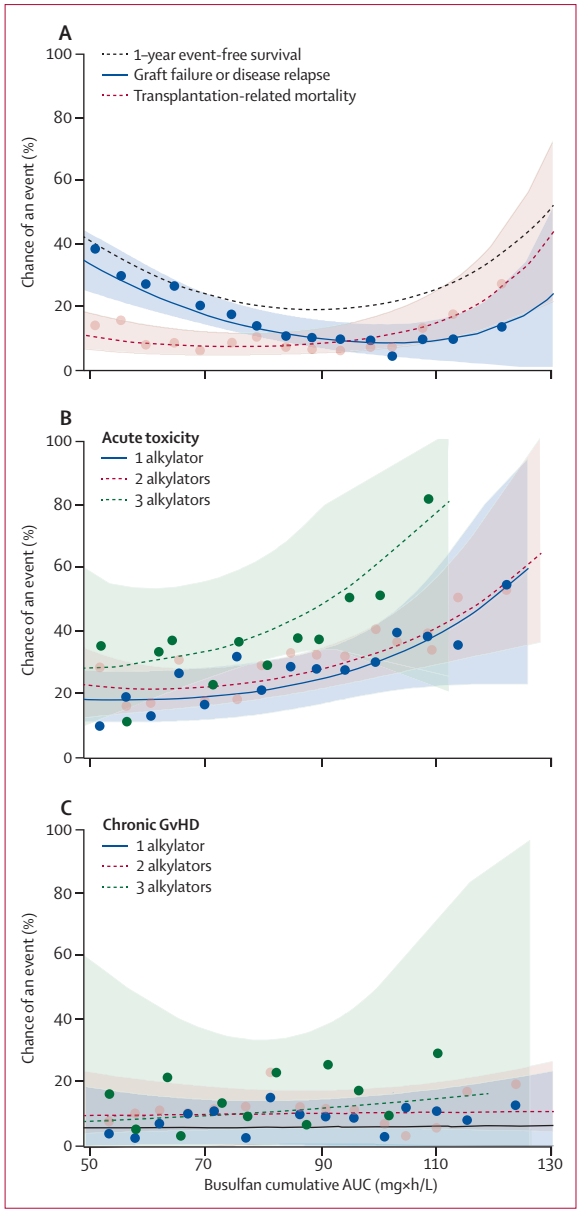

***Figure 4*: Polynomial Weibull models of the association between busulfan cumulative area under the curve and outcomes of interest**
Associations between AUC and (A) graft failure or disease relapse and transplantation-related mortality (using uncensored data), and (B) acute toxicity (at 6 months after haemopoietic cell transplantation) and (C) chronic GvHD, with toxicities stratified by number of alkylating drugs, are shown. Shaded areas are 95% CIs. AUC=area under the curve. GvHD=graft-versus-host disease.

mortality were significantly higher in the high AUC group than in the optimum AUC group. With the limitations of a retrospective cohort study taken into account, our data suggest that optimising the target for cumulative busulfan exposure has a significant effect on survival chances.

Our data suggest that standardisation of the approach to AUC estimation among transplant centres is important. AUC estimations vary when derived using different calculation approaches (based on population pharmacokinetic models or traditional non-compartmental analysis). Results of calculations based on traditional non-compartmental analysis vary when using different pharmacokinetic sampling schemes (limited or intensive), infusion times, and specific equations used to calculate AUC for the first dose or at steady state ($AUC_{0-\infty}$ or $AUC_{0-\tau}$). Using a population approach by NONMEM to calculate AUC_{NONMEM} reduces the need to plan specific sampling strategies and better estimates the cumulative AUC because it takes into account the exact time of infusion, accounts for errors in sampling and analysis, and uses individual clearance to calculate exposures. Additionally, the models capture the increased exposure at days 2–4 in all patients. Using non-compartmental analysis, the latter effect can only be identified in patients when sampling occurs over several days. The variability in estimates derived using different approaches suggests that for future studies, harmonisation of the pharmacokinetic-estimation approach is important. This harmonisation will also allow for better comparisons of busulfan AUCs between institutions and help to facilitate prospective studies of individualised busulfan dosing strategies. Furthermore, it would reduce the number of blood samples needed for AUC estimation, and would lead to better harmonisation in clinical trial design.[28] Population pharmacokinetic models, based on published models, are accessible for clinical use (eg, InsightRX, DoseMe, NextDose, and a model by Limoges University Hospital Laboratory of

For **InsightRX** see http://www.insight-rx.com

For **DoseMe** see http://doseme.com.au

For **NextDose** see http://www.nextdose.org

For the **Limoges University model** see https://pharmaco.chu-limoges.fr/PORTAIL_PK/

Pharmacology [Limoges, France]). Using model-based dosing combined with these pharmacokinetic tools, implementation of the new harmonised approach of dose targeting busulfan (ie, therapeutic drug monitoring and modification of busulfan dosing to achieve optimum exposure) is feasible in clinical practice worldwide. Busulfan therapeutic drug monitoring is standard practice in the USA and used often in Europe because it is recognised as essential to optimise outcomes (ie, to reduce toxicity and maximise efficacy). The European Society for Blood and Marrow Transplantation–European School of Haematology guidelines recommend a target AUC of 90 mg×h/L for myeloablative exposure.[29] This target has also been used in study protocols for gene therapy (eg, metachromatic leukodystrophy),[30] in a randomised controlled trial comparing treosulfan with pharmacokinetic targeted busulfan in patients undergoing HCT for non-malignant diseases (EudraCT number 2013-005508-33), and in a cord blood expansion trial (NCT02715505). Thus, dose targeting of busulfan is feasible and the use of optimum busulfan exposure will potentially change practice.

The optimum busulfan AUC_{NONMEM} of 78–101 mg×h/L is in line with previous publications showing that a high busulfan AUC predicts acute toxicity and transplantation-related mortality[8–10] and a low busulfan AUC leads to graft rejection or disease relapse.[12–14] Our data show that most children and young adults will experience a suboptimum busulfan AUC when using the lower, currently used, historical target of 58–86 mg×h/L.[13,15,26,27] Studies done primarily in the US adult population used a higher target cumulative busulfan AUC (100 mg×h/L) either in combination with cyclophosphamide or fludarabine, similar to the optimum exposure identified in this study.[10,11] Since the optimum exposure range is small and higher than current practice, and because of high inter-patient variability in busulfan pharmacokinetics,[28] therapeutic drug monitoring of busulfan is essential to achieve this narrow optimum exposure. The 95% CIs of the models suggest that there is still some unexplained variability in outcomes. Therefore, the optimised AUC should be considered with caution when applying the results to a patient, especially those with factors that may affect the AUC, such as a high comorbidity score.

The exposure–event-free survival association was not affected by any variable, similar to findings from previous studies in adults.[10,11] In line with the higher event-free survival in this study are findings from a recent retrospective study in adults who received fludarabine added to high-dose busulfan (12·8 mg/kg) that showed improved event-free survival compared with low-dose busulfan (6·4 mg/kg) because of a lower risk of relapse.[31] However, lower exposure is suggested to be sufficient in specific diseases; for example, Güngör and colleagues[16] reported in a prospective study that busulfan at a cumulative AUC of 45–65 mg×h/L combined with fludarabine resulted in a 2-year event-free survival of 89% in patients with chronic granulomatous disease who received bone marrow or peripheral blood stem cell transplantations.[16] In this study,[16] understanding what the

	Training dataset (n=449)			Validation set (n=225) Median optimum AUC (mg×h/L)
	Optimum AUC target (±10%*) (mg×h/L)	p value model	p value optimum *vs* other stratum	
All patients	90 (78–101)	0·011	..	86 (74–99)
Malignant underlying disease				
No	88 (75–101)	0·035	..	89 (77–97)
Yes	94 (82–103)	0·094	0·87	84 (73–94)
Malignant underlying disease by baseline remission				
First complete remission	97 (80–110)	0·49	..	81 (70–94)
More than one complete remission	91 (79–107)	0·61	0·91	89 (79–99)
HLA disparity				
Matched	87 (77–96)	0·35	..	84 (70–99)
Mismatched	94 (77–107)	0·095	0·89	87 (75–97)
Cytomegalovirus status of recipient				
Negative	92 (81–103)	0·11	..	86 (75–98)
Positive	88 (79–95)	0·13	0·91	86 (73–99)
Cytomegalovirus status of donor				
Negative	87 (80–98)	0·14	..	88 (76–100)
Positive	93 (81–101)	0·24	0·93	84 (71–98)
By donor relationship				
Matched related donor	87 (77–95)	0·032	..	90 (85–101)
Mismatched related donor	90 (86–100)	0·45	0·93	84 (71–97)
Matched unrelated donor	87 (71–103)	0·086	0·89	85 (69–93)
Mismatched unrelated donor	98 (83–112)	0·18	0·73	86 (73–98)
Number of alkylating drugs				
1	92 (76–102)	0·10	..	85 (75–98)
2	88 (80–100)	0·12	0·89	88 (77–101)
3	92 (84–96)	0·22	0·93	88 (73–100)
Age at HCT				
<2 years	94 (77–106)	0·032	..	82 (68–94)
2–5 years	84 (70–96)	0·11	0·80	89 (73–100)
5–12 years	93 (85–103)	0·13	0·88	83 (74–94)
>12 years	92 (80–99)	0·20	0·89	89 (72–101)
HCT source				
Umbilical cord blood	90 (80–100)	0·28	..	88 (79–99)
Bone marrow or peripheral blood stem cells	89 (79–98)	0·41	0·79	83 (74–96)
Year of transplantation				
Before 2006	89 (81–98)	0·043	..	86 (70–96)
2006 or later	93 (79–106)	0·054	0·33	86 (75–99)
Busulfan dosing regimen				
Once daily	89 (79–99)	0·70	..	85 (71–94)
Four times daily	93 (82–102)	0·53	0·81	87 (74–95)
By serotherapy				
No	88 (70–102)	0·33	..	90 (82–101)
Yes	92 (73–104)	0·15	0·88	82 (73–95)

AUC=area under the curve. HCT=haemopoietic cell transplantation. *Allowing for 10% deviation.

Table 3: **Multivariate Weibull models showing the optimum busulfan cumulative area under the curve target for event-free survival**

AUC would be when analysed in a harmonised way is important. In our cohort, 2-year event-free survival in patients with non-malignant disease with standard risk of graft failure and treated with bone marrow or peripheral blood stem cells at an AUC of 45–65 mg×h/L was 71%, whereas at 78–101 mg×h/L it was 81%, suggesting that further optimisation in these patients is likely to be possible, but this finding needs prospective validation. Because 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.

In view of the retrospective nature of this study, we acknowledge there might be other covariates not assessed in our analysis, such as generalised improvements in care after allogeneic HCT, GvHD prophylaxis, or the clinical status and risk of comorbidities (such as those defined by the Center for International Blood and Marrow Transplant Research risk) of the patient before transplantation because this might have affected decision making. These factors are likely to have contributed to clinical outcomes. Also, a small number of patients received defibrotide as VOD prophylaxis, most within a prophylaxis trial,[32] mostly in the busulfan, cyclophosphamide, and melphalan combination. Use of defibrotide might have affected the endpoint of VOD and potentially resulted in underestimation of the risk of VOD. Other limitations are that for some variables, such as minimal residual disease status before allogeneic HCT, comorbidity score, and GvHD prophylaxis regimen, doses and exposures of each individual drug and anti-thymocyte globulin exposure before and after HCT[33] might have affected the outcomes, but could not be included in this retrospective analysis. Using a large sample from 15 different HCT centres and by applying propensity-adjusted analyses, we adjusted for possible group selection of patients with low and high busulfan AUC. However, a randomised controlled trial in a specific disease group would probably be the best way to confirm this higher and narrow optimum exposure to busulfan.

In conclusion, the use of a new, harmonised, and validated approach to measuring busulfan exposure can be used to target a new, optimum cumulative busulfan exposure in children or young adults undergoing allogeneic HCT. If this new approach is adopted, we expect higher survival chances with lower toxicity in these patients. Busulfan targeted to the optimum cumulative busulfan exposure combined with the non-alkylating drug fludarabine further optimises the balance between efficacy and toxicity.

Contributors

IHB, AL, CCD, RMS, JRL-B, and JJB participated in the research design. IHB, AL, RMS, PJS, CEN, and JRL-B performed or gave advice on the pharmacokinetic–pharmacodynamics models. All authors collected and interpreted the data and wrote and reviewed the manuscript.

Declaration of interests

CCD has received personal fees from Jazz Pharmaceuticals. RJK is co-owner and employee of InsightRX, a company developing dose optimisation software for hospitals. All other authors declare no competing interests.

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