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GSTA1 Genetic Variants and Conditioning Regimen: Missing Key Factors in Dosing Guidelines of Busulfan in Pediatric Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Busulfan (Bu) is a key component of conditioning regimens used before hematopoietic stem cell transplantation (SCT) in children. Different predictive methods have been used to calculate the first dose of Bu. To evaluate the necessity of further improvements, we retrospectively analyzed the currently available weight- and age-based guidelines to calculate the first doses in 101 children who underwent allogenic SCT in CHU Sainte-Justine, Montreal, after an intravenous Bu-containing conditioning regimen according to genetic and clinical factors. The measured areas under the curve (AUCs) were within target (900 to 1500 μM/min) in 38.7% of patients after the administration of the first dose calculated based on age and weight, as locally recommended. *GSTA1* diplotypes linked to poor Bu metabolism (G3) and fludarabine-containing regimens were the only factors associated with AUC within target (OR, 4.7 [95% CI, 1.1 to 19.8, $P = .04$]; and OR, 9.9 [95% CI, 1.6 to 61.7, $P = .01$], respectively). From the 11 methods selected for dose calculation, the percentage of AUCs within the target varied between 16% and 74%. In some models G3 was associated with AUCs within the therapeutic and the toxic range, whereas rapid metabolizers (G1) were correlated with subtherapeutic AUCs when different methods were used. These associations were confirmed by clearance-prediction analysis, in which *GSTA1* diplotypes consistently influenced the prediction errors of the methods. These findings suggest that these factors should be considered in Bu dose prediction in addition to the anthropometric data from patients. Furthermore, our data indicated that *GSTA1* diplotypes was a factor that should be included in future population pharmacokinetic models, including similar conditioning regiments, to improve the prediction of Bu exposure after its initial dose.

INTRODUCTION

The bifunctional alkylating agent busulfan (Bu) is a key component of several conditioning regimens administered before stem cell transplantation (SCT). In children it is used as an alternative to total body irradiation with comparable event-free survival in patients with acute myeloblastic leukemia [1] and produced less developmental and cognitive impacts in long-term survivors [2,3]. Bu is also included in conditioning regimens before allogeneic SCT for acute lymphoblastic leukemia [4] and nonmalignant diseases [5,6] as well as in regimens used before autologous transplantation for high-risk neuroblastoma [7-9] and relapsed/refractory Hodgkin lymphoma [10].

Bu has a narrow therapeutic window with higher rates of relapse and rejection observed in patients with a low Bu exposure, and overexposure is associated with transplant-related toxicities such as acute graft-versus-host disease, sinusoidal occlusive syndrome, and death [11-15]. Therapeutic

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drug concentration monitoring is an important tool and allows the adjustments of doses to optimize treatment outcomes [13]. Bu exposure over time is expressed by the area under the curve (AUC), which has a proposed target AUC of 900 to 1500 μM.min for a 6-hour dosing schedule, although some debate exists about the optimal therapeutic range [15-17].

The intra- and interpatient pharmacokinetic (PK) variability of Bu after equivalent doses during conditioning regimens is high [18]. The clearance of high-dose oral Bu proved to be significantly higher in children than adults, especially in children under 4 years old [19-21]. The use of the i.v. form of Bu is intended to minimize this variability. However, a high fluctuation of PK parameters persists [22-24]. The only known metabolic pathway of Bu is its conjugation to glutathione, a reaction mainly catalyzed by the hepatic enzyme glutathione S-transferase, in particular by its isoform α-1 [25,26]. In addition to anthropometric measures, several groups have shown that part of the PK variability of Bu was explained by different metabolic potentials that result, to a certain extent, from genetic variations in the enzyme coding gene *GSTA1* [12,27-33].

In clinical practice a test dose can be administered to assess the Bu clearance, which is subsequently used to calculate dose (dose = measured clearance × desired AUC) [34]. However, the Bu dose is usually obtained from recommendations, most of which are based on population-based PK studies [35,36]. Several guidelines with different nomograms, algorithms, and internet-based calculation tools are also available using weight and/or age to calculate initial Bu doses [24,35-44]. A review [17] of those methods revealed a high coefficient of variation that showed simulated first-dose AUCs within the therapeutic target in 51% to 74% of cases. Based on previous associations between Bu PK variability and *GSTA1* variants, the current study aimed to evaluate the impact of this genetic factor on the performance of the currently available i.v. Bu dosing guidelines in children.

METHODS

Patients

After approval by the local institutional review board, the medical charts of 148 pediatric patients who received i.v. Bu between April 2002 and April 2012 at CHU Sainte-Justine were retrospectively reviewed. All received i.v. Bu as part of conditioning regimen in preparation for autologous or allogeneic SCT. Signed informed consent was available for 114 patients. Twelve patients were excluded because of the absence of *GSTA1* genetic information, and 1 patient was excluded because the first-dose PK profile was unavailable. This analysis is part of an ongoing study registered at clinicaltrials.gov (NCT01257854). PK and clinical outcomes of a subgroup of 84 patients receiving exclusively myeloablative conditioning regimens have been previously reported [12]. Nine patients with chronic granulomatous disease received significantly higher Bu first-doses in comparison with doses routinely prescribed because they were participants in another study in which higher daily AUCs were suggested [5]. Consequently, those patients were not included in the measured AUC analysis; however, they were considered for predicted AUC and clearance evaluation.

Treatment Regimen

Bu (Busulfex; Otsuka Pharmaceuticals, Saint-Laurent, Montreal, Quebec, Canada) was administered i.v. in a 2-hour infusion every 6 hours. The first doses were prescribed based on age and total body weight as previously described [43]: 16 mg/m^2 in infants ≤ 3 months old; .8 mg/kg in children > 3 months old and <1 year old or ≥4 years old; and 1.0 mg/kg in children ≥ 1 and <4 years old. Weight adequacy was classified as previously reported for patients older than 2 years old [45].

PK Analysis and Genotyping

Samples were obtained from a central venous line, which was not used for Bu administration, immediately before and at 120, 135, 150, 180, 240, 300, and 360 minutes after the start of the infusion. The plasma Bu concentration was determined by using a modified HPLC assay [46]. The PK parameters were estimated by noncompartmental analysis using WinNonlin (Certara, version 3.1, Princeton, NJ). Peripheral mononuclear cells or saliva collected from all patients before Bu infusion were used for *GSTA1* genotyping, as previously described [47]. The diplotypes composition is presented in Supplementary Table S1. Patients were further grouped based on respective expected promoter activity of *GSTA1* [12] into rapid (G1), normal (G2), and poor metabolizers (G3), as shown in Figure 1.

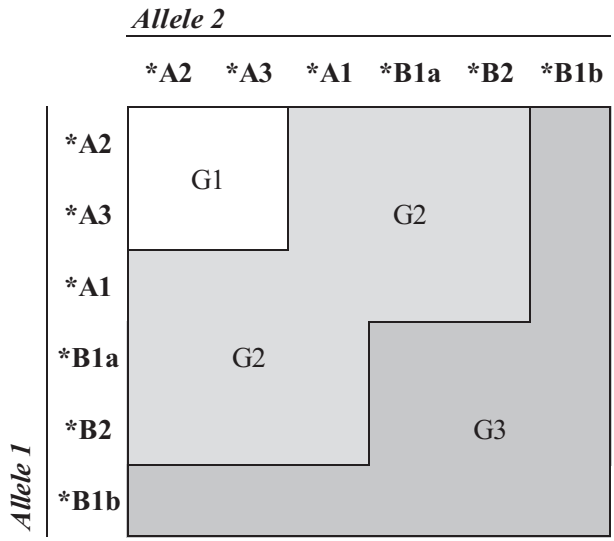

Figure 1. Grouping composition based on *GSTA1* diplotypes. G1 group contains homozygous patients for haplotypes associated with a rapid Bu metabolism, G3 contains homozygous for haplotypes associated with poor metabolism (**B*) and heterozygous **B1b*, and G2 contains diplotypes not classified as G1 or G3.

Measured and Predicted PK

The first-dose measured $AUC_{0\text{-}INF}$ was classified as below, within, or above the target range (900 to 1500 μM/min). To evaluate the prediction accuracy, predicted $AUC_{0\text{-}INF}$ values were calculated from Bu every-6-hour doses obtained from the available dosing guidelines [24,35-44]. The respective equations used to calculate the predicted doses are presented in Supplementary Table S3. For McCune et al.'s method [38], the predicted doses were obtained from the web-based calculator available at www.nextdose.org. The AUCs were predicted for each patient and each guideline based on the measured clearance through the equation available in the Supplementary Material (Supplementary Table S2, equation 1). Subsequently, the predicted AUCs were afterward classified similarly to measured equivalents. The guidelines were named according to the first author of each study and are summarized in Table 1. For each guideline, the same age and/or weight limits used of each guideline were respected.

Evaluation of Clearance Prediction Evaluation

We evaluated the fitness of individual and group Bu clearance prediction obtained from the 4 best performing guidelines through the calculation of the individual absolute prediction error (AE%) and the mean relative prediction error of the models. The respective equations are available in supplementary Table S2 (equations 2 and 3, respectively).

Statistical Analysis

Demographics and baseline characteristics were described for all patients (median and range or proportion according to variable characteristics). The comparison between PK parameters was performed by using a Mann-Whitney test or Student's *t*-test, as appropriate. The *GSTA1* intergroup comparisons for the proportions of patients with AUCs below, within, or above the target AUC were performed using the Pearson chi-square test for univariate analysis and logistic regression for multivariate analysis. Finally, a univariate and multivariate linear model (analysis of variance) with Bonferroni adjustment for categorical variables was used to assess independent factors related to the measurements of AUC and clearance AE%. Statistical analyses were performed by using IBM SPSS statistics (version 24; IBM Corp., New York, NY).

Table 1
Model Characteristics

Dosing Guideline	Year of Publication	Target AUC (μM/min)	Bu Formulation	Dose frequency (h)	Parameters for Dose Calculation	No. of Patients (Development)	Age Range (yr)	FluCR
Long-Boyle	2015	Css: 600-900 ng/mL	i.v.	6	ABW and age	90	.1-24	Yes
Ansari	2014	Css: 600-900 ng/mL	i.v.	6	ABW and age	75	.1-20	No
Buffery*	2014	900-1400 (p.o. and i.v. adults) 925-1350 (i.v. children)	77% p.o.	6 (p.o.) 24 (i.v.)	ABW	144 (89 pediatric)	.5-58	NA
McCune	2014	1125	i.v.	6, 8, 12, 24	ABW, gender and PMA	1481 (133 available ABW)	.1-65.8	NA
Savic†	2013	1096	i.v.	6	ABW and age	149	.1-3.3	Yes
Bartelink	2012	1350	i.v.	6, 12, 24	ABW and age	245	.1-26	30% BuFlu(12%); BuCyFlu(11%); BuMelFlu(4%)
Paci	2012	1200	i.v.	6	ABW	115	0-12.3	No‡
Trame§	2011	1125 (ABW) 1150 (BSA)	54% p.o.	6 (p.o.) 24 (i.v.)	ABW or BSA	94	.4-18.8	NA
Wall	2010	900-1350	i.v.	6	ABW	24	0-18	Only BuCy
Booth	2007	900-1350	i.v.	6	ABW	24	.25-16.7	NA
Nguyen	2004	1125	i.v.	6	ABW	24	.45-16.7	Only BuCy

FluCR indicates Fludarabine-containing regimen; Css, steady-state concentration; ABW, absolute body weight; PMA, postmenstrual age; NA, not available; Cy, cyclophosphamide; Mel, melphalan; BSA, body surface area.
* Buffery et al.'s method is limited to patients of at least 10 kg of weight.
† Savic et al.'s method is conceived to patients having 12 kg or less.
‡ Paci et al. stated that patients received no other chemotherapy before Bu, although that BuFlu was used in rare cases.
§ Trame et al.'s publication indicated 2 calculation methods: ABW based and BSA based. Adapted from Zao et al. [17].

RESULTS

Observed PK Parameters and Influencing Factors

Patient characteristics are shown in Table 2. The overall median of administered Bu first doses was .81 mg/kg (range, .63 to 1.09), which resulted in a median $AUC_{0\text{-}INF}$ of 846 μM.min (range, 385 to 1751). After the exclusion of patients with chronic granulomatous disease, despite the similar Bu first doses, $AUC_{0\text{-}INF}$ and steady-state concentration were significantly higher and Bu clearance significantly lower in patients who received fludarabine-containing conditioning regimens (FluCR), as detailed in Table 3. In a linear multivariate analysis controlled for sex, with Bu dose scaled by weight and age, FluCR ($P = .02$) and *GSTA1* groups G2 and G3 ($P = .03$) were associated with higher $AUC_{0\text{-}INF}$, whereas malignant diseases were associated with lower $AUC_{0\text{-}INF}$ ($P = .03$).

After the first of dose of Bu, the target AUC was achieved in 38.7% of patients, whereas it was in the toxic range in 1% of patients. The fractions of AUCs within the target were 0%, 39.4%, and 66.7%, for G1, G2, and G3 patients, respectively ($P = .01$). Most patients administered FluCR achieved the AUC target range in comparison with other regimens (75% versus 30.1%, respectively; $P = .05$). In the multivariate logistic regression controlled for sex, disease, and Bu dose per kilogram and age, FluCR (odds ratio [OR], 9.9; 95% confidence interval [CI], 1.6 to 61.7; $P = .01$) and G3 (OR, 4.7; 95% CI, 1.1 to 19.8; $P = .04$) were associated with a higher probability of having a first AUC within the target range. The PK parameters of both regimens are summarized in Table 3.

Table 2
Patient Characteristics

Characteristics		No. of Patients (%)	
Gender	Male	56	(55.4)
	Female	45	(44.6)
Age	<1 yr	16	(15.8)
	1-4 yr	20	(19.8)
	>4 yr	65	(64.4)
Diagnosis	Malignancies	61	(60.4)
	AML	30	(29.7)
	ALL	10	(9.9)
	MDS	19	(18.8)
	Myeloproliferative syndrome	1	(1.0)
	Neuroblastoma	0	(.0)
	Other solid tumors	1	(1.0)
	Nonmalignancies	40	(39.6)
	Immunodeficiencies	19	(18.8)
	Hemoglobinopathy	10	(9.9)
	Hemophagocytic syndrome	5	(5.0)
	Metabolic diseases	6	(5.9)
Conditioning regimen			
	BuCy	75	(74.3)
	BuFlu	17	(16.8)
	BuCyVP16	7	(6.9)
	BuMel	2	(2.0)
Ethnic groups			
	White	80	(79.2)
	Black	11	(10.9)
	Other	10	(9.9)
Weight adequacy			
	Overweight	15	(14.9)
	Obesity	6	(5.9)
GSTA1 haplotype group			
	Rapid metabolizers (G1)	10	(9.9)
	Normal metabolizers (G2)	79	(78.2)
	Poor metabolizers (G3)	12	(11.9)
Median age, yr (range)	8.5 (.1 - 21)		

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; VP16, etoposide.

Table 3
Comparison of the Measured PK Parameters between Patients Receiving BuFlu or Other Regimens

	BuFlu (n = 9)		Other (n = 84)		
Parameter	Median	Range	Median	Range	*P*
Age at SCT, yr	13.3	.4-21.0	7.4	.1-19.9	.11
Dose, mg/kg	.79	.75-1.00	.8	.63-1.09	.48
Cmax, ng/mL	976	537-1338	846	537-1249	.21
Css, ng/mL	762	264-1021	555	302-1198	.03
$AUC_{0\text{-}INF}$, μM.min	1,113	385-1491	810	442-1751	.03
Clearance, mL/kg/min	2.9	2.3-7.7	4.2	1.8-7.2	.02

Cmax indicates maximal Bu concentration; AUC, area under the curve; Css, steady-state concentration.

Performance Evaluation of the Guidelines

Overall, doses calculated by different guidelines resulted in 49.5% of predicted AUCs within the target (range, 16% to 74%). G3 patients performed better: 66.7% of the predicted AUCs achieved the target (range, 41.7% to 100%). However, these patients also had the highest percentage (7.3%) of AUCs in the toxic range (range, 0% to 25%). In contrast, G1 patients had the highest percentage of AUCs below the target (60%; range, 30% to 100%) and no AUCs in the toxic range. The overall performances of the different methods and evaluation of the *GSTA1*-based groups are shown in Table 4.

In the multivariate analysis including age, sex, disease, conditioning, and *GSTA1*-based groups, G3 patients a had probability of achieving AUC within the target by using the absolute body weight–based Trame et al.'s method [44] (OR, 7.7; 95% CI, 1.4 to 40.8; $P = .02$) or Wall et al.'s method [24] (OR, 10.9; 95% CI, 2.1 to 56; $P = .004$). Patients with benign disease experienced an increased probability of achieving the target AUC using Wall et al.'s method (OR, 3.6; 95% CI, 1.3 to 9.7; $P = .01$) or Booth et al.'s method [35] (OR, 3.3; 95% CI, 1.3 to 8.5; $P = .01$). Girls were more likely to have an AUC within the target range than boys when doses were calculated by the methods of Bartelink et al. [37] (OR, 3.2; 95% CI, 1.1 to 8.9; $P = .02$), Buffery et al. [42] (OR, 4.6; 95% CI, 1.6 to 13.3; $P = .005$), or Booth et al. [37] (OR, 2.6; 95% CI, 1.0 to 6.5; $P = .048$).

In contrast, after a first dose calculated by Wall et al.'s method (OR, 6.9; 95% CI, 1.6 to 30.2; $P = .01$) and body surface area–based Trame et al.'s method (OR, 6.9; 95% CI, 1.6 to 29.2; $P = .01$) in patients from the G1 and G2 subgroups and calculated by Paci et al.'s method [41] (OR, 17.4; 95% CI, 1.5 to 207) in patients from the G1 subgroup had a higher probability of having AUC below the target. Lower AUCs were seen more frequently in boys, when doses were calculated by Long-Boyle et al.'s [39] (OR, 3.9; 95% CI, 1.0 to 9.4; $P = .04$) or Paci et al.'s methods (OR, 2.9; 95% CI, 1.1 to 7.8; $P = .03$); patients with malignant disease were also more likely to have AUCs below the target by using Long-Boyle et al.'s (OR, 3.9; 95% CI, 1.2 to 12.9; $P = .03$) or Wall et al.'s (OR, 3.2; 95% CI, 1.2 to 8.4; $P = .02$) methods. Younger patients had a higher probability of AUCs below the target when the doses were calculated by Wall et al.'s ($P = .02$) or Long-Boyle et al.'s ($P < .001$) methods.

Predicted AUCs in the Toxic Range

Using Bartelink et al.'s model, G3 patients were found to be at higher risk of AUCs in the toxic range (OR, 9.9; 95% CI, 1.1 to 88.8; $P = .04$), whereas patients receiving FluCR (OR, 9.7; 95% CI, 1.6 to 60.7; $P = .02$) and older patients ($P = .004$) experienced at a higher risk of toxic AUC when doses were calculated using Long-Boyle et al.'s model.

Clearance Prediction Error

The 4 selected methods [37-39,41] returned a global error from –20.8% to 4.6%. In univariate analysis *GSTA1* groups presented a consistent association with the prediction errors of all methods, except for Long-Boyle et al.'s (Table 5).

In the multivariate analysis including age, disease, sex, and conditioning regimen, *GSTA1* groups were revealed to be the only significant factor that influenced McCune et al.'s [38] ($P = .005$) and Paci et al.'s AE% ($P = .02$). For Bartelink et al.'s method, age was associated with AE% ($P = .03$) in addition to *GSTA1*-based groups ($P = .004$). For Long-Boyle et al.'s method, only age ($P < .001$) was strongly related to AE%.

Table 4
Performance Comparison Among Different Guidelines Across the *GSTA1*-Based Groups

	Dose (mg/kg)		Overall n (%)						G1 n (%)					
Guidelines	Mean	SD	Below		Within		Above		Below		Within		Above	
McCune	1.05	.14	19	(18.8)	75	(74.3)	7	(6.9)	4	(40.0)	6	(60.0)	0	(.0)
Bartelink	1.05	.21	22	(23.7)	66	(71.0)	5	(5.4)	3	(30.0)	7	(70.0)	0	(.0)
Paci	.98	.15	32	(34.4)	58	(62.4)	3	(3.2)	5	(50.0)	5	(50.0)	0	(.0)
Long-Boyle	1.08	.11	27	(26.7)	60	(59.4)	14	(13.9)	4	(40.0)	6	(60.0)	0	(.0)
Nguyen	.96	.15	37	(39.8)	54	(58.1)	2	(2.2)	5	(50.0)	5	(50.0)	0	(.0)
Buffery*	.91	.16	31	(42.5)	41	(56.2)	1	(1.4)	2	(40.0)	3	(60.0)	0	(.0)
Savic*	1.03	.10	12	(50.0)	12	(50.0)	0	(.0)	5	(100.0)	0	(.0)	0	(.0)
Wall	.87	.10	54	(58.1)	38	(40.9)	1	(1.1)	8	(80.0)	2	(20.0)	0	(.0)
Booth	.87	.13	53	(57.0)	38	(40.9)	2	(2.2)	7	(70.0)	3	(30.0)	0	(.0)
Trame(ABW)	.76	.16	80	(79.2)	20	(19.8)	1	(1.0)	8	(80.0)	2	(20.0)	0	(.0)
Trame(BSA)	.75	.17	84	(83.2)	16	(15.8)	1	(1.0)	9	(90.0)	1	(10.0)	0	(.0)
				(46.7)		(49.5)		(3.8)		(60.0)		(40.0)		(.0)

	G2 n (%)						G3 n (%)					
Guidelines	Below		Within		Above		Below		Within		Above	
McCune	15	(19.0)	59	(74.7)	5	(6.3)	3	(20.0)	10	(66.7)	2	(13.3)
Bartelink	19	(26.8)	49	(69.0)	3	(4.2)	0	(.0)	10	(83.3)	2	(16.7)
Paci	26	(36.6)	43	(60.6)	2	(2.8)	1	(8.3)	10	(83.3)	1	(8.3)
Long-Boyle	21	(26.6)	47	(59.5)	11	(13.9)	2	(16.7)	7	(58.3)	3	(25.0)
Nguyen	30	(42.3)	40	(56.3)	1	(1.4)	2	(16.7)	9	(75.0)	1	(8.3)
Buffery*	27	(47.4)	29	(50.9)	1	(1.8)	2	(18.2)	9	(81.8)	0	(.0)
Savic*	7	(38.9)	11	(61.1)	0	(.0)	0	(.0)	1	(100.0)	0	(.0)
Wall	43	(60.6)	27	(38.0)	1	(1.4)	3	(25.0)	9	(75.0)	0	(.0)
Booth	41	(57.7)	28	(39.4)	2	(2.8)	5	(41.7)	7	(58.3)	0	(.0)
Trame (ABW)	65	(82.3)	13	(16.5)	1	(1.3)	7	(58.3)	5	(41.7)	0	(.0)
Trame (BSA)	68	(86.1)	10	(12.7)	1	(1.3)	7	(58.3)	5	(41.7)	0	(.0)
		(48.5)		(47.7)		(3.8)		(26.0)		(66.7)		(7.3)

SD, standard deviation; Trame (ABW), based on absolute body weight; Trame (BSA), based on body surface area.
* Buffery et al.'s guideline included patients ≥ 10 kg (n = 81) and Savic et al.'s ≤12 kg (n = 24).

Table 5
Mean of Prediction Mean Error (ME%) Across *GSTA1*-Based Groups

Model	ME%	95% CI		*P*
		Lower	Upper	
McCune (n = 101)	−3.3	−7.5	−.9	<.001
G1	−21.8	−32.2	−11.4	.02*
G2	−3.4	−7.9	−1.0	Reference
G3	12.7	−2.0	27.4	.03*
Bartelink (n = 101)	−20.8	−24.1	−17.5	<.01
G1	−30.2	−40.5	−20.0	.38
G2	−21.8	−25.4	−18.1	Reference
G3	−6.7	−16.0	2.7	.01*
Paci (n = 101)	−19.3	−23.8	−15.8	<.01
G1	−29.9	−40.6	−19.1	.25
G2	−19.8	−23.6	−16.0	Reference
G3	−7.2	−17.0	2.6	.06
Long-Boyle (n = 101)	4.6	−2.2	11.4	.13
G1	−13.2	−34.4	8.1	.34
G2	5.1	−2.5	12.7	Reference
G3	16.1	−3.3	35.5	.89

P values from analysis of variance test. ME% between groups compared with Bonferroni coefficient interval adjustment as fixing G2 as the reference group.
* Significant values ($P < .05$).

DISCUSSION

This study shows that the first dose of Bu varied significantly based on the available guidelines. These results agreed with those of Zao et al. [17], who reported performances from 51% to 74% (compared with 16% to 74% in the present results), although patients in the Zao et al. cohort were more frequently in the toxic range (6% to 30% versus 0% to 15% in our cohort). Some variables were already included in dosing methods, such as age. However, other variables not usually considered in Bu dose estimates, such as baseline disease (malignant or not), components of the conditioning regimen, sex, and genetic background, appeared to interfere with the guidelines' performance. Methods such as McCune et al.'s [38], Bartelink et al.'s [37], Paci et al.'s [41], and Long-Boyle et al.'s [39] presented a better overall performance than the age- and weight-based dosing method used in our center. Nevertheless, some groups of patients did not follow the overall prediction even when best-performing guidelines were used.

Bu is mainly metabolized by glutathione conjugation, which is dependent on glutathione S-transferase, the enzyme responsible to catalyze the reaction [26]. Glutathione S-transferase α-1, encoded by the *GSTA1* gene, is the most important isoform [25] involved in Bu metabolism. Single nucleotide variations in the promoter region of the *GSTA1* gene and the derived haplotypes have been proven to result in different levels of enzyme expression, which were associated with differences in Bu PK [12,28,30,33,48,49].

Similarly, the present analysis showed that the recipient's *GSTA1* genetic background was associated with measured AUC. More than half of the patients treated in our center presented a measured AUC below the target, and this proportion was independently related to *GSTA1* diplotype group (67% in G1/G2 and 36% in G3 patients). Likewise, in predicted AUCs, *GSTA1* diplotypes appeared as an independent factor associated with toxic (G3) or subtherapeutic (G1) AUCs when particular models were used for the dose calculation.

Although consistent data exist on *GSTA1* genotype as a prediction for Bu PK, as reviewed by Huezo-Diaz et al. [50], it was only recently successfully introduced into an adult population PK model. Choi et al. [30] suggested that patients heterozygous for the *GSTA1* haplotype **B* (**B1b*, **B1a*, and **B2*; see Supplementary Table S1) have 15% lower clearance than the other haplotype (**A*) and required a reduced dose. Because of the rarity of haplotype **B* in the Asian population [28], no homozygous patients were included in that model. In our dataset, which comprised 79% of white patients, homozygous **B* individuals were also represented by G3 group (12% of our cohort). Previous reports from our group identified G3 patients were at higher risk of Bu toxicities after non-FluCR, partially because of a 20% lower Bu clearance in those patients [12,33]. The present analysis was concordant with these findings: When the best-performing methods were used to calculate the Bu dose, higher rates of AUCs in the toxic range were predicted in G3 patients (8.3% to 25%) in comparison with G2 (2.8% to 13.9%) and G1 (no AUCs in the toxic range). In contrast, G1 patients (homozygous **A2* or **A3* or a heterozygous compound of these two haplotypes), which were previously recognized as rapid metabolizers [12,33], showed consistent underestimation, up to 35%, when using the same dosing methods. This demonstrated that all variability of Bu PK cannot be explained by anthropometric data, even if more elaborate population PK models are used in attempt to predict individual Bu clearance [37-39,41].

We also showed that the conditioning regimen influenced Bu PK parameters. Patients administered FluCR presented Bu first-dose AUCs 37% higher and clearance 30% lower than those who received other regimens. This resulted in more patients administered such treatments with AUCs within the target after the first dose of Bu (77.8% versus 34.5% for other regimens). Some studies in adults using oral [51] or i.v. Bu [52,53] have shown 10% to 30% lower Bu clearance after 3 to 4 days of Flu exposure when compared with protocols using combinations of Bu and cyclophosphamide. Similar interpatient variability was observed in adults [53] and children [23] administered FluCR. However, this Bu–Flu interaction was not confirmed in studies where Bu PK was assessed earlier during Flu exposure [54,55]. In our cohort all patients received Bu on the same day, immediately after Flu. Although the difference was not significant, age may be a factor of the conditioning regimens (13.3 versus 7.4 years, $P = .11$), which could explain the lower clearance of apparently older patients in the Flu group. Because only Bu first doses were analyzed, no conclusion can be drawn about a late decrement in Bu clearance over Flu exposure. However, an interaction between the drugs cannot be excluded.

Flu has a different metabolic pathway in comparison with Bu. It is a purine analogue that is quickly phosphorylated in erythrocytes, endothelial cells, and large organs to 9-β-D-arabinosyl-2-fluoroadenine (F-ara-A) by 5′-nucleotidase. Intracellular phosphorylation by deoxycytidine kinase converts F-ara-A to its active form, F-ara-ATP, which is ultimately incorporated into the DNA and blocks cell division [51,56]. The activation of Flu and the elimination pathway does not seem to overlap with Bu metabolism, but it is unknown if Flu exposure interferes in Bu elimination. FluCR is known to be an important component of reduced-toxicity conditioning regimens [57] and it sometimes selected instead of Bu and cyclophosphamide in patients with a higher risk of toxicity. Although rare, complications such as sinusoidal occlusive syndrome [52] and nonrelapse mortality [58,59] have also been reported with FluCR and are clearly related to the intensity of Bu exposure. In addition to the influence of *GSTA1* genotypes on baseline Bu clearance, this potential Bu–Flu interaction becomes a concern in poor-metabolizer patients, because the risk of complications is theoretically increased, even when fludarabine-based reduced-toxicity

conditioning regimens are used. This association could not be assessed in our cohort because it included only one 13-year-old boy from G3 group who received FluCR. His first AUC was within the target, close to the upper limit (1492 μM.min), after a Bu dose of 1 mg/kg.

Age interfered with the performance of some models, which most likely demonstrated the suboptimal control of this well-known influencing factor of Bu clearance in the original models [60,61]. Girls were more often within the target AUC, but no other data seem to support this finding. Differences in fat mass composition, which are described and included in McCune et al.'s prediction model [38], may be responsible for this finding.

An improvement in the first AUC prediction based on patients characteristics that would preclude Bu therapeutic drug concentration monitoring is far from clinical reality. PK variability and clinical outcomes appear to be only partially explained by the currently known anthropometric and even genetic factors. Although data are still missing to support the notion that patients with target AUC after the first dose are at lower risk of Bu toxicity, the collection of information on several factors that influence this variability may provide an improved assessment of each patient undergoing SCT; it would be possible to tailor the treatment to the profile of the patient. In this context the incorporation of *GSTA1* diplotypes into Bu dosing algorithms could be the first step toward a genetically based personalization of the conditioning regimen. Depending on patient's *GSTA1* diplotype group, Bu first-dose tailoring can be estimated from doses obtained from currently available weight- and/or age-based guidelines. However, the inclusion of the *GSTA1* diplotype groups as a covariate in a novel pharmacogenetics-based population PK model appear to be a more attractive option.

An important interindividual variability and a potential decrease in Bu clearance in association with Flu is a phenomenon that should be more deeply investigated, despite the apparent absence of a direct pharmacologic interaction. It is noteworthy that, as shown in Table 1, although most articles analyzed in the present study have included FluCR in their training dataset, subgroup analyses of different conditioning regimens are not available. The potential drug–drug interaction and detrimental effect on Bu poor metabolizers is still not fully understood and requires further study.

In conclusion, we found that factors such as the *GSTA1* genotype and the association of Flu in the conditioning regimens were significantly related to the performance of different guidelines currently used to prescribe the first dose of Bu. Improvement in the current guidelines, or a new model in which these factors are considered, is necessary to ensure a better first-dose personalization, especially in patients recognized to be at a higher risk of SCT complications.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2017.07.022.

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