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ORIGINAL ARTICLE

Should busulfan therapeutic range be narrowed in pediatrics? Experience from a large cohort of hematopoietic stem cell transplant children

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Busulfan, the corner stone of hematopoietic stem cell transplantation regimens, has a narrow therapeutic window. Therapeutic drug monitoring (TDM)-guided dosing to reach the conventional area under the concentration–time curve (AUC) target range of 900–1500 μmol min/L is associated with better outcomes. We report our experience with busulfan TDM in a large cohort of children. The aims were to investigate the relevance of using a more restricted therapeutic range and investigate the association between busulfan therapeutic range and clinical outcome. This study includes 138 children receiving 16 doses of intravenous busulfan, with the first dose assigned based on weight and doses adjusted to a local AUC target range of 980–1250 μmol min/L. Busulfan TDM combined with model-based dose adjustment was associated with an increased probability of AUC target attainment, for both target range: 90.8% versus 74.8% for the conventional target range and 66.2% versus 43.9% for the local target range ($P < 0.001$). The median follow-up was 56.2 months. Event-free survival was 88.5%, overall survival was 91.5% and veno-occlusive disease occurred in 18.3% of patients. No difference was observed for clinical outcomes depending on the selected target range. Pharmacokinetic monitoring and individualization of busulfan dosage regimen are useful in improving target attainment, but using a restricted target range has no impact on clinical outcomes.

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is used for the treatment of various malignant and non-malignant hematological diseases.[1,2] Busulfan, an alkylating agent developed in the 1950s, is frequently included as part of a myeloablative therapy before HSCT. It has been increasingly used in conditioning protocols as an alternative to TBI, which is often poorly tolerated in children.[3–5] Busulfan has a narrow therapeutic index: low drug exposure is associated with increased risk of rejection and disease relapse in transplant recipients,[6,7] whereas high drug exposure is associated with toxicity (that is, veno-occlusive disease (VOD)), fatalities and overall poor outcome.[8–10] The exposure to busulfan is measured by the area under the concentration–time curve (AUC). Adjusting busulfan doses to achieve a target AUC has been shown to lead to improved patient outcome.[11] When busulfan is given four times a day, the conventional AUC_{0-6h} target range is 900–1500 μmol min/L, whatever the patient and disease characteristics. In the last decade, the use of oral busulfan, which was complicated by variable bioavailability, has been progressively replaced by that of the IV formulation.[12–14] However, data on the use of IV busulfan in children are limited and considerable inter-individual variability in busulfan exposure is still observed, especially in children.[15–19] Controlling patient exposure has become a major issue to improve efficacy and safety of busulfan-based conditioning. Busulfan therapeutic drug monitoring (TDM) is widely used to control the AUC target attainment and to guide dose adjustment if necessary. However, toxic events are still observed in patients, even when busulfan AUC is within the target range.[20–23] These observations question the rationale of the so-called therapeutic range of IV busulfan.

The aims of this study were to report our experience with busulfan TDM in a large cohort of children, investigate the relevance of using a more restricted therapeutic range and investigate the association between busulfan therapeutic range and clinical outcome.

MATERIALS AND METHODS

Patients

Data were retrospectively collected from medical records of patients who received IV busulfan in the Pediatric Hematology and Oncology Units of Lyon and Grenoble between June 2006 and August 2014, and underwent busulfan TDM. The information included patients' demographics, diagnoses, busulfan dosing history, busulfan plasma concentrations and clinical outcomes.

Depending on the underlying disease and recommended international treatment protocols, busulfan was used in combination with either cyclophosphamide, fludarabine, thiotepa, melphalan or etoposide. GvHD prophylaxis consisted in cyclosporine alone or combination with anti-thymocyte globulin in patients transplanted from unrelated donors. Anti-thymocyte globulin was also used in patients transplanted for sickle cell

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anemia or thalassemia, to prevent graft rejection due to previous allo-immunization.

Patients received standard antiemetic drugs (ondansetron), gut decontamination and infection prophylaxis, prophylactic anticonvulsive therapy (clonazepam during busulfan conditioning and 1 more month for patients with sickle cell disease) and VOD prophylaxis[24] (ursodeoxycholic acid and defibrotide in children with the following risk factors: pre-existing hepatic disease, second myeloablative transplant, diagnosis of primary hemophagocytic lymphohistiocytosis or prior treatment with gemtuzumab ozogamicin).

All patients were confined and cared for in high-efficiency, particle-free, air-filtered, positive-pressure isolation bedrooms.

The approval of an ethical committee was not needed for this observational study, in accordance with French legislation (French Public Health Code: Article R1121-2).

Busulfan dosage regimen, drug assay and pharmacokinetic analysis

IV busulfan was administered four times a day for 4 days, as a 2-h infusion. Hence, each patient received a total of 16 busulfan IV infusions. The initial dose was calculated by using the manufacturer weight-based recommendations[18] presented in Table 1.

Before 2010, busulfan plasma concentrations were measured by HPLC with UV detection.[25] In 2010, the assay technique was changed and a cross-validated liquid chromatography coupled to tandem mass spectrometry method has been used since then. The liquid chromatography technique was derived from two methods.[25,26]

Two blood samples were drawn 0.5 and 2 h after the end of the first infusion of busulfan in each patient. These sampling times were chosen according to optimal sampling theories.[27] Afterwards, individual pharmacokinetic (PK) parameter values of a one-compartment model[28] were estimated by using maximum *a posteriori* Bayesian modeling program included in the USC*PACK software.[29] Individual PK parameter estimated were used to perform subsequent estimation of busulfan plasma concentrations, calculation of the AUC after the initial dose (AUC_{dose1}) and determination of the future doses necessary to achieve an average target AUC_{0-6h} for the rest of the regimen.

The AUC target range used in our center (980–1250 μmol min/L) was historically more restricted than the conventional range. We started busulfan TDM before the actual and conventional range became available, following studies from Pierre Fabre Laboratories.[11] At this date, some pharmacokinetic studies on busulfan supported the use of a target range.[9,30] We used this target range while busulfan was only available as an oral form[11] and continued then when the IV form became available.

A dose adjustment was performed if the predicted average individual AUC_{0-6h} over the entire therapy was outside the target range of 980–1250 μmol min/L. The predicted average AUC_{0-6h} was calculated as the total cumulative predicted AUC divided by the total number of doses ($n = 16$), based on the initial dose.

Busulfan plasma levels were measured only after the first dose, except in patients with thalassemia in whom concentrations were also measured on day 2, according to French recommendations.[31] In addition, a second busulfan monitoring was performed on day 2 in patients for whom a large dose adjustment (± 30% of the first dose) was necessary on the first day.

In order to assess the impact of busulfan TDM combined with model-based dose adjustment, we calculated and compared the AUC over the entire therapy based on the real, adjusted dosage regimen ($adjAUC_{16}$) and the simulated AUC if the initial dose would have been applied over the entire therapy, without any dose adjustment (AUC_{16}).

Table 1. Busulfan manufacturer dosing guidelines[18]

Patient weight (kg)	*IV busulfan dose (mg/kg/dose)*
< 9	1
9 to < 16	1.2
16 to < 23	1.1
23 to 34	0.95
> 34	0.8

Clinical evaluation

The main study endpoints were VOD-free survival at 1 month post transplantation and engraftment at months 1, 3 and 6. Chimerism ⩾95% was considered as full donor engraftment, donor chimerism ⩾10 and < 95% was considered as partial engraftment, and graft rejection was defined as donor chimerism < 10%. Event-free survival (EFS) and overall survival (OS) after HSCT were also evaluated for patients with a follow-up of at least 9 months. EFS was calculated from the time of transplant until death, relapse or graft failure, whichever occurred first. OS was the time between transplantation and death of any causes. VOD was diagnosed according to modified Seattle criteria.[32] Severity of VOD was assessed according to the system of Bearman.[33] VOD was the only toxicity endpoint studied.

Statistical analysis

The Kruskal–Wallis test was used to assess the possible differences between pharmacokinetic parameter values between the subgroups. The Student's *t*-test was used to assess the significance of bias and precision. Differences of proportions between groups were assessed using χ^2 Pearson's test. Association between busulfan exposure and endpoints (EFS, OS and VOD) were analyzed using univariate and multivariate Cox proportional hazards regression models, including the following:

- Body weight, stratified in five groups, corresponding on weight-based dosing[18]: < 9, 9–16, 16–23, 23–34 and > 34 kg;
- Disease, stratified in four groups (because of the small number of some of them): hematological malignant disease, hemoglobinopathies, solid malignant tumor and non-malignant disease. We also tested specific diseases known as 'VOD high risk': thalassemia and SCID;
- Use of defibrotide prophylaxis;
- Conditioning: test of each of the used drugs;
- Type of graft: genoidentical allograft, phenoidentical allograft or autologous;
- Stem cell sources: bone marrow, cord blood and peripheral blood stem cells;
- First dose AUC value: in or out the AUC target range (conventional and local);
- Total AUC value: in or out the AUC target range (conventional and local);
- Busulfan dose adaptation during the conditioning.

OS, EFS and VOD-free survival were estimated with the Kaplan–Meier method, and compared using a log-rank test. All statistical analyses were performed by using SPSS for Windows (version 9.0, SPSS, Chicago, IL, USA).

RESULTS

Patient characteristics

A total of 138 patients treated between June 2006 and August 2014 were included in the study. Four children received two HSCT (three non-engraftments and one relapse post HSCT). Finally, 142 HSCT with busulfan-based conditioning were analyzed. Table 2 summarizes patient, disease and transplantation characteristics. Nineteen children received defibrotide (25 mg/kg/day) as VOD prophylaxis, because of individual risk factors.[24]

TDM of busulfan

Pharmacokinetic parameters. Busulfan PK parameter values calculated by Bayesian estimation are summarized in Table 3. PK parameter distributions were not homogeneous according to weight ($P < 0.001$).

Achievement of target AUC and dose adjustment. The mean estimated AUC without any dose adjustment (AUC_{16}) was 1115 ± 268 μmol/L min (range: 627–2341 μmol/L min). It was outside the conventional and local ranges in 25.2% and 56.1% of patients, respectively. A dose adjustment was performed after the first two doses of busulfan in 78 patients (56.1%). The dose was increased in 42 patients (mean increase: 15.8%, range: 2.0%–48.1%), whereas it was decreased in 36 patients (mean decrease: 18%, range: 1.7%–53.3%). Infants are statistically more

represented in the group with adjusted dose (19.8% vs 9.2%, *P = 0.038*). Patients with a dose decrease were statistically younger and were with a lower weight (4.5 ± 4.3 years, 18.0 ± 13.1 kg) compared with patients with a dose increase (8.2 ± 5.9 years, 28.3 ± 18.4 kg, $P < 0.05$). Types of pathologies were equally displayed between the two groups.

Table 2. Patient characteristics

Patient age (year), mean/median/range	6.5/5.0/0.17–21
Patient weight (kg), mean/median/range	23.6/17.7/3.2–87
No. of patients < 9 kg (%)	23 (16.2%)
Diagnosis, no. of patients (%)	
Hematological malignant diseases	72 (50.7)
Non-malignant diseases	30 (21.1)
Solid malignant tumors	17 (12.0)
Hemoglobinopathies	23 (16.2)
AML	43 (30.3)
ALL	13 (9.2)
SCID	12 (8.5)
Thalassemia	12 (8.5)
Neuroblastoma	11 (7.7)
Sickle cell anemia	11 (7.7)
MDS	8 (5.6)
Lymphohistocytiosis	4 (2.8)
SAA	3 (2.1)
Lymphoma	3 (2.1)
Ewing sarcoma	3 (2.1)
Others	19 (13.4)
HLA compatibility, no. of patients (%)	
Matched related	57 (40.1)
Mismatched related	2 (1.4)
Matched unrelated	18 (12.7)
Mismatched unrelated	47 (33.1)
Autologous	18 (12.7)
Stem cell sources, no. of patients (%)	
Bone marrow	92 (64.8)
Cord blood	29 (20.4)
PBSC	21 (14.8)
Conditioning regimen, no. of patients (%)	
Bu Cy	79 (55.6)
Bu Cy Mel	2 (1.4)
Bu Cy VP16	4 (2.8)
Bu Cy Thio	5 (3.5)
Bu Flu	22 (15.5)
Bu Flu Cy	1 (0.7)
Bu Flu Thio	11 (7.7)
Bu Thio	3 (2.1)
Bu Thio VP16	1 (0.7)
Bu Mel	14 (9.9)

Abbreviations: Bu = busulfan; Cy = cyclophosphamide; Flu = fludarabine; MDS = myelodysplastic syndrome; Mel = melphalan; SAA = severe aplastic anemia; Thio = thiotepa; VP16 = etoposide.

In the overall population ($n = 142$), the mean predicted AUC after model-based dose adjustment ($adjAUC_{16}$) was 1105 ± 163 µmol/L min (range: 651–2122 µmol/L min). Ninety-four patients had $adjAUC_{16}$ within our local AUC target range (66.2%), 24 had $adjAUC_{16}$ below (16.9%) and 24 had $adjAUC_{16}$ above (16.9%). One hundred and twenty-nine patients had $adjAUC_{16}$ within the conventional AUC target range (90.8%), 11 had $adjAUC_{16}$ below (7.8%) and 2 had $adjAUC_{16}$ above (1.4%). There was no significant difference in the characteristics of patients having adjAUC16 below or above the target range (data no shown).

Table 4 and Figure 1 show the proportion of AUC_{dose1}, AUC_{16} and $adjAUC_{16}$ within the target range. Busulfan TDM combined with model-based dose adjustment was associated with an increased probability of AUC target attainment, for both target range: 90.8% versus 74.8% for the conventional target range, 66.2% versus 43.9% for the local target range ($P < 0.001$, χ^2 Pearson's test). These results are similar for the children who weighed < 9 kg. Of note, the proportion of adjAUC16 within the local target range was significantly lower in patients with thalassemia (75%) than in patients with other diseases (92%, $P = 0.047$).

Clinical outcomes

The median follow-up was 56.2 months (range: 9–106 months). EFS was 88.5%, VOD-free survival was 81.7% and OS was 91.5%.

Data on engraftment are summarized in Table 5. Non-engraftment occurred in six children:

- Five non-malignant diseases: two SCID, one severe aplastic anemia, one adrenoleukodystrophy and one lymphohystiocytosis.
- One hematological malignant disease: juvenile myelomonocytic leukemia.

Mixed chimerism occurred in 19 children (at month 6):

- Eleven hemoglobinopathies (6 thalassemia and 5 sickle cell diseases).
- Seven non-malignant diseases (three SCID, one congenital neutropenia, one adrenoleukodystrophy, one metabolic disease and one diamond blackfan anemia).
- One chronic myelomonocytic leukemia.

All received a graft from a mismatched unrelated donor (four cord blood and two bone marrow). VOD occurred in 26 patients (18.3%). Data on VOD are summarized in Table 6. Severity of VOD was mild, moderate and severe in 6 (4.2%), 18 (12.7%) and 2 (1.4%) patients, respectively (3 patients who experienced VOD received defibrotide for VOD prophylaxis). AML was the most frequent disease in patients with VOD (31%). The average time of VOD onset was 14 days (minimum 1 and maximum 29). Two children died from VOD (none of them received prophylactic defibrotide). No busulfan-related neurological toxicity was observed.

Table 3. Pharmacokinetic parameter values in children receiving IV busulfan

	Mean ± s.d.					
	All (n = 142)	*< 9 kg (n = 23)*	*9 to < 16 kg (n = 42)*	*16 to < 23 kg (n = 24)*	*23 to 34 kg (n = 18)*	*> 34 kg (n = 35)*
Clearance (L/h/kg)	0.23 ± 0.06	0.24 ± 0.07	0.25 ± 0.05	0.23 ± 0.06	0.24 ± 0.05	0.20 ± 0.04
Volume of distribution (L/kg)	0.62 ± 0.07	0.62 ± 0.07	0.64 ± 0.06	0.62 ± 0.05	0.63 ± 0.08	0.60 ± 0.06
Elimination half-life (h)	1.97 ± 0.43	1.88 ± 0.49	1.84 ± 0.33	2.02 ± 0.54	1.86 ± 0.24	2.19 ± 0.42
AUC_{dose1} (µmol/L min)	1007 ± 215	951 ± 195	1117 ± 217	1078 ± 229	912 ± 141	900 ± 154
$adjAUC_{16}$ (µmol/L min)	1105 ± 163	1085 ± 151	1132 ± 149	1137 ± 237	1027 ± 124	1107 ± 139

Table 4. Achievement of AUC target range

	% Achievement of target AUC (% below; % above)	
	Conventional target range 900–1500 µmol min/L	*Local target range 980–1250 µmol min/L*
AUC_{dose1}		
Overall ($n=142$)	67.6 (29.5, 2.9)	43.9 (43.2, 12.9)
<9 kg ($n=23$)	56.2 (43.8, 0)	34.8 (52.2, 13)
AUC_{16}[a]		
Overall ($n=142$)	74.8 (18.0, 7.2)	43.9 (30.2, 25.9)
<9 kg ($n=23$)	69.6 (30.4, 0)	26.1 (47.8, 26.1)
$adjAUC_{16}$[b]		
Overall ($n=142$)	90.8 (7.8, 1.4)	66.2 (16.9, 16.9)
<9 kg ($n=23$)	82.6 (17.4, 0)	56.4 (21.8, 21.8)

Abbreviation: AUC = area under the concentration-time curve. [a]Comparison of AUC_{16} and $adjAUC_{16}$ for conventional and local target range (overall and <9 kg). [b]χ^2 Pearson's test: statistically significant ($P<0.001$).

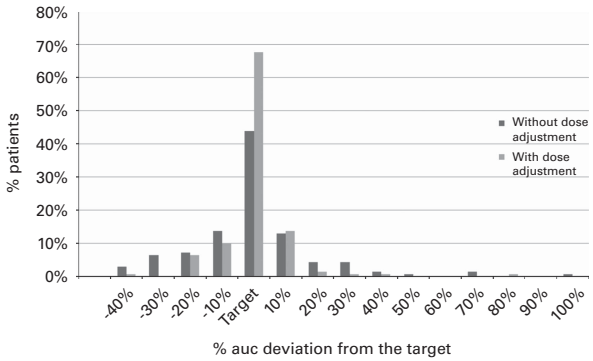

Figure 1. Mean total AUC deviation from the local target range (980–1250 µmol min/L).

Relationship between busulfan exposure and clinical outcome

Tables 5 and 7 show the achievement of target AUC in relation to various clinical outcomes.

No difference in percentage of target attainment rate (local or conventional) was observed among chimerism groups (full, partial or rejection) and study periods (1, 3 and 6 months).

No difference in $adjAUC_{16}$ was observed according to VOD (1127 ± 134 and 1100 ± 171 µmol min/L, $P=0.57$) or full engraftment (1117 ± 144 and 1110 ± 256 µmol min/L, $P=0.87$). Among the patients with no full donor engraftment at 1 month ($n=22$), only 2 (9.1%) had an $adjAUC_{16}$ <900 µmol.min/L. All patients with VOD had an $adjAUC_{16}$ <1500 µmol min/L.

The rates of mixed chimerism (34.0% vs 1.1%, $P<0.01$) and rejection (9.4% vs 1.1%, $P=0.02$) were higher in patients with non-malignant diseases than in patients with malignancies.

In multivariate Cox regression analysis, only one risk factor, body weight <9 kg, significantly influenced the onset of VOD (relative risk = 3.3, $IC_{95\%}$ 1.1–10.6). No correlation was found between AUC (AUC_{dose1} and $adjAUC_{16}$) and VOD.

Twenty-eight patients had an adjAUC16 within the bounds of the conventional and out of the bounds of the local AUC target range (that is, AUCdiff = 900–980 µmol min/L or 1250–1500 µmol-min/L). No difference was observed between these patients and patients with an adjAUC16 within the local AUC target range for OS ($P=0.87$) and VOD-free survival ($P=0.85$).

Table 5. Engraftment: percentage of donor chimerism at day 30, 60 and 90 post transplantation and corresponding percentage of target attainment

	Engraftment		
	Day 30	*Day 60*	*Day 90*
Patients (n)	*116*	*98*	*91*
% Full donor chimerism	80.2	81.6	80.2
Conventional/local PTA[a]	93.5/66.7	92.5/67.5	93.2/68.5
% Mixed donor chimerism	14.6	18.4	19.8
Conventional/local PTA[a]	88.2/70.6	88.9/66.7	83.3/61.1
% Rejection	5.2	0	0
Conventional/local PTA[a]	80.0/60.0	—	—

Abbreviation: PTA = percentage of target attainment for AUC_{16}. [a]The χ^2 Pearson's test: there was no significant difference in the percentage of target attainment.

Relapses occurred in 15.7% of patients with malignancies. There was a higher probability of relapse for patients with an adjAUC16 below the conventional AUC target range (<900 µmol min/L, 42.9%) than for patients with an adjAUC16 within the conventional AUC target range (13.4%, $P=0.04$).

DISCUSSION

The conditioning regimen before HSCT aims at facilitating engraftment and eradicating malignant disease, but it may be associated with toxicity. Indeed, busulfan has a narrow therapeutic index. In addition, busulfan displays large inter-individual PK variability. This variability is partly explained by several factors such as age, underlying disease, liver status and genetic polymorphisms of glutathione *S*-transferase.[17,20,34–36] Since the early 2000s, the use of the intravenous formulation of busulfan has been associated with less inter- and intra-individual variability in busulfan exposure than the use of the oral formulation. However, this study confirms that significant variability is still observed with IV busulfan, which need to be controlled in order to prevent under- and overdosing.

Controlling drug exposure allows optimizing myeloablation and managing toxicity: based on a large experience in adults and adolescents, a standard therapeutic AUC range of 900–1500 µmol min/L/dose has been defined.[7–10,37] Low drug exposure has been associated with increased risk of graft rejection or relapse,[6,7] whereas high drug exposure has been associated with increased toxicity.[8–10] Achieving this target seems to provide a higher rate of successful engraftment and a lower incidence of VOD. Nguyen *et al.*[18] have shown that IV busulfan clearance correlates with childrens' body weights, but in a nonlinear manner. This finding led to the development of a weight-based dosing nomogram in children who has then been approved in Europe.[18] Simulations based on the nonlinear weight-based dosing strategy from Nguyen and colleagues predicted a 75% probability of achieving the target AUC range of 900–1500 µmol min/L/dose, which was significantly better than various other dosing methods. Since then, several clinical studies have confirmed this finding, with proportion of AUC target achievement ranging from 71.2% to 78% when using this dosing strategy.[19,22,38,39] In our study, if no dose adjustment had been performed, it was predicted that the mean AUC_{16} would have been in the conventional target range in 74.8% of patients, which is also consistent with previous published studies. This means that one out of four patients might experience busulfan under- or overdosing if the recommended dosage regimen is used over the entire therapy, without any dose adjustment.

Table 6. Patients' characteristics with VOD

ID	*Age*	*BW (kg)*	*Indication*	*Disease classification*	*Conditioning*	*DF prophylaxis*
1	12.0	87	AML	Hematological malignant disease	Bu Cy ATG	N
2	5.0	15.1	ALL	Hematological malignant disease	Flu Bu ATG	N
3	2.0	11.3	JMML	Hematological malignant disease	Bu Cy Mel	N
4	3.0	15.1	Thalassemia	Hemoglobinopathie	Bu Cy ATG	N
5	3.0	13.1	ALL	Hematological malignant disease	Flu Bu Thio	N
6	0.0	7.9	ALL	Hematological malignant disease	Bu Cy VP16	N
7	2.0	15.4	AML	Hematological malignant disease	Bu Cy ATG	N
8	1.0	7	JMML	Hematological malignant disease	Bu Cy ATG	N
9	19.0	54.4	AML	Hematological malignant disease	Bu Cy	N
10	15.0	55.6	CML	Hematological malignant disease	Bu Cy	N
11	13.0	28.7	Sickle Cell	Hemoglobinopathie	Bu Cy ATG	N
12	0.0	8	AML	Hematological malignant disease	Bu Cy	N
13	21.0	60	AML	Hematological malignant disease	Bu Cy ATG	N
14	12.0	28.9	SCID	Non-malignant disease	Flu Bu ATG	N
15	9.0	36.7	Thalassemia	Hemoglobinopathie	Bu Cy ATG	N
16	14.0	39.8	AML	Hematological malignant disease	Bu Cy ATG	N
17	1.0	7.7	Neuroblastoma	Solid malignant tumor	Bu Mel	N
18	2.0	10	Neuroblastoma	Solid malignant tumor	Bu Mel	Y
19	2.0	10.175	AML	Hematological malignant disease	Bu Cy ATG	N
20	1.0	7.67	LH	Non-malignant disease	Bu Cy VP16	Y
21	13.0	40	AML	Hematological malignant disease	Bu Cy Thio ATG	N
22	10.0	25.8	MDS	Hematological malignant disease	Flu Bu Thio ATG	N
23	1.0	5.8	ALL	Hematological malignant disease	Bu Cy Mel	N
24	2.0	11.8	ALL	Hematological malignant disease	Flu Bu Thio	N
25	3.0	14.8	Sickle cell	Hemoglobinopathie	Bu Cy ATG	N
26	0.0	4.56	LH	Non-malignant disease	Bu Cy VP16	Y

Abbreviations: ATG = antithymocyte globulin; BW = body weight; Cy = cyclophosphamide; DF = defibrotide; Flu = fludarabine; LH = lymphohystocytiosis; MDS = myelodysplastic syndrome; Mel = melphalan; N = no; Thio = thiotepa; VOD = veno-occlusive disease; VP16 = etoposide; Y = yes.

Table 7. Achievement of target AUC_{16} according to the outcome

Event	*% Achievement of target AUC*		
	Yes	*No*	P-*value*
Full engraftment at 1 month	93.5	86.4	0.439
VOD	88.5	91.4	0.413

Abbreviations: adjAUC_{16} = conventional target range; AUC = area under the concentration–time curve; VOD = veno-occlusive disease.

It has been suggested that the ability to achieve the busulfan AUC target range can be improved by using TDM. In the study of Malär *et al.*,[21] 62% of patients achieved the AUC target interval of 9000–12 000 ng/mL/h (busulfan was administered twice daily as a 4-h infusion) using the above weight-based nomogram and TDM (dose adjustment was assessed after the first dose), whereas only 26% of patients had achieved the AUC target range after the first dose. In two other studies, TDM performed between the first and the ninth dose was associated with more patients with AUC within the target range (increase from 19%[40] to 22%). Dose adjustment based on TDM results was necessary to achieve the target AUC range in 40%–68% of patients.[21,23,35,40–42] In our study, the influence of TDM combined with model-based dose adjustment on the attainment of the conventional AUC target range was very significant. The proportion of target attainment for adjAUC_{16} was 90.8%, compared with only 74.8% if the manufacturer dosing schedule would have been applied throughout the therapy. We found a similar impact of TDM on the proportion of patients achieving AUC_{16} within the local target range (66.2% vs 43.9%).

Bleyzac *et al.*[11] showed that TDM combined with model-based adjustment of busulfan dose regimens could improve clinical outcome in HSCT children. In our study, OS was 91.5%. In other studies, with a smaller number of children than our cohort, OS was lower (from 70% to 83%[40,42,43]). Only one study reported a rate of OS as high as ours (91% at day +100).[35] Less than 5% of allografted patients had no engraftment. This is consistent with previous studies that reported engraftment of 90%–100%.[17,22,40,43] Our results confirm the importance of TDM to enhance engraftment. Moreover, in our center, almost all children have reached the conventional AUC target range, because TDM is realized very soon, so that dose adjustment can be performed early if necessary (third dose). As literature review has clearly demonstrated, we can consider the AUC target range as clinically relevant despite these results.

In our clinical practice, an AUC target range narrower than the conventional one has been used for years. It was assumed that such a target range could optimize the graft outcome. However, no difference related to clinical outcomes was observed between patients within the local AUC target range and those within the AUCdiff target range (that is, 900–980 or 1250–1500 µmol min/L). This suggests that a more restrictive AUC target range around the median seems not to be relevant to improve clinical outcomes.

In this study, 18.3% of patients experienced VOD, but only 7.7% of those VOD were severe. These results are consistent with those recently published.[21–23,35,39,40,42] Age and/or weight have been found to be risk factors of VOD.[21,44,45] PK and pharmacodynamics of drugs in infants can differ widely between children and adults, owing to developmental changes in physiological and metabolic processes, which may alter drug disposition.[46,47] In the study of Nguyen *et al.*,[18] only four patients weighed < 9 kg (16.7%) and none weighed < 7.1 kg. This raises questions about the adequacy of the recommended dose in this group of very young children. The simulation study analysis[18] demonstrated that this fixed dose enabled only 54% of infants to achieve the target AUC range (vs 75% of target achievement in children > 10 kg). Other studies have confirmed these poor results, which are probably due to a higher inter-individual PK variability of busulfan in infants

compared with older children.[19,20,22,38,48] Savic *et al.*[50] proposed a model-dosing strategy based on age for infants and children weighing $\leqslant 12$ kg, to achieve a target of 900–1250 µmol min/L.[49] According to their model, a 1.7-fold increase in busulfan clearance would occur between the ages of 6 weeks and 2 years. Busulfan is largely metabolized by glutathione *S*-transferase. The maturation of glutathione *S*-transferase enzyme activity seems to be the main determinant of this age-dependent clearance change in the first 2 years of life.[46,49,51] In our study, we found that children < 9 kg are approximately three times more likely to develop VOD.

To adequately manage the risk of VOD, the relevance of AUC as marker of this toxicity may be questioned. In previous clinical studies using the IV formulation of busulfan in children, no relationship could be found between busulfan AUC and the occurrence of VOD.[19–23,39,40,42] Our study results are in agreement, as all patients with VOD showed an AUC < 1500 µmol min/L. However, even if no statistically significant correlation was found between VOD incidence and AUC (% of target range achievement or mean AUC), we have to be cautious with these results because of the little proportion of VOD in our cohort. Other criteria than AUC as potential predictor of VOD should be explored.

CONCLUSION

In conclusion, our findings indicate that TDM allows increasing the proportion of AUC within the target range and that all children should benefit from TDM. However, using a restricted busulfan AUC target range has no superior impact on clinical outcomes compared with using the conventional AUC target range. It could be due to the lack of AUC relevance, especially for VOD. Another more specific PK criterion should be studied to predict efficacy and safety.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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