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Once-Daily Intravenous Busulfan with Therapeutic Drug Monitoring Compared to Conventional Oral Busulfan Improves Survival and Engraftment in Children Undergoing Allogeneic Stem Cell Transplantation

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ABSTRACT

Because of intra- and interindividual variability, bioavailability, and pharmacokinetics of busulfan (Bu) in children, oral busulfan without therapeutic drug monitoring (TDM) is assumed to be associated with higher graft failure rates as well as higher toxicity (eg, veno-occlusive disease [VOD]). This study compares the outcome of hematopoietic stem cell transplantation (HSCT) of 2 groups: 1) 30 patients who received myeloablation with once-daily intravenous (i.v.) dose-targeted busulfan (BUdtIV) based on TDM and 2) 30 patients who received the current practice of untargeted oral busulfan (BUPO). Patients received a 3-hour infusion of Bu at a first dose of 120 mg/m^2 (age ≥1 year) or 80 mg/m^2 (<1 year), or BUPO 1 mg/kg 4 times daily. Both regimens were continued for 4 days. The target area under the curve (AUC) was defined as 17,500 μg*h/l. BUdtIV resulted in higher event-free survival (EFS) and survival rates compared to BUPO (EFS: 30% versus 83%, $P < .001$, survival: 53% versus 83%, $P = .016$). BUdtIV was associated with more cases of VOD. TDM was feasible in routine clinical practice. The results show that i.v. Bu using TDM is preferable over oral Bu in children undergoing allogeneic stem cell transplantation, especially in those at high risk for graft failure/relapse.

KEY WORDS

Busulfan • Allogeneic stem cell transplantation • Therapeutic drug monitoring

INTRODUCTION

Busulfan (Bu) is an alkylating agent that has been used since the 1950s. Currently, Bu is one of the most frequently used chemotherapeutic agents in high-dose preparative chemotherapy combination regimens. It can serve as an alternative to total body irradiation (TBI) in patients undergoing hemopoietic stem cell transplantation (HSCT) for various malignant and nonmalignant diseases [1,2,3].

Until recently, Bu was only available in an oral form and was given 4 times daily. Although Bu was shown to be effective when used in this regimen, the therapeutic potential of the oral drug has been compromised owing to unpredictable exposure, especially in children [2,4,5]. To reduce both intra- and interindividual variability of Bu pharmacokinetics (PK), i.v. formulations of Bu have recently been developed. In adults, i.v. Bu showed predictable and consistent pharmacokinetic profiles with acceptable toxicity [6-11]. In children, however, interindividual variance of PK profiles after i.v. dosing of Bu remained rather high [12].

Bu has a narrow therapeutic window: high Bu exposure is associated with toxicity, such as the development of veno-occlusive disease (VOD) [2,13-15], whereas underexposure to Bu was found to be associated with an increased incidence of graft rejection or relapse [16,17]. This appears to be even more important in diseases known to be associated with high graft failure/relapse rates resulting in a lower event-free survival (EFS), such as inborn error of metabolism (IEM), myelodysplastic syndrome (MDS) and hemophagocytic lympho-histiocytosis (HLH) [18-22] Dose targeting based on therapeutic drug monitoring (TDM) improved clinical outcome in pediatric HSCT recipients [3,5,23,24]. For these reasons, the use of an i.v. formulation of Bu combined with dose targeting based on TDM (BUdtIV) might have advantages over oral dosing without dose targeting (BUPO) in children.

Thus far, Bu (p.o. and i.v.) is mainly given 4 times daily [1,5,7,11,14,15,23-26]. However, once-daily dosing is possible and would be much more convenient for the patient and caregivers. In addition, it can be hypothesized that reducing the exposure period to Bu might be associated with reduced toxicity. The use of once-daily dosing could allow enzyme recovery of glutathione-S-reductase and glutathione-S-transferase between doses. As a result, no accumulation of Bu would occur. A decrease of the dosing frequency of Bu showed similar or decreased toxicity (VOD) in adults [27-29].

This study used a once daily i.v. dose of Bu and targeted for an "area under the curve" (AUC) of 17.500 μg*h/l (4263 μmol*min/l). This target was based on past literature (of 4 times daily dosing) [9,10,12,25,30]. Comparison was made between the outcome of HSCT in children after a myeloablative preparative chemotherapyregimen including BudtIV and the current practice of untargeted oral Bu.

MATERIALS AND METHODS

Study Population

In July 2003, this study's researchers replaced oral Bu with BudtIV based on TDM. The effects on outcome were studied after the inclusion of 30 patients with BudtIV. Outcomes were compared with the 30 most recent patients receiving oral Bu. BudtIV was gradually introduced, starting with nonmalignant indications known to be associated with high graft-failure rates (ie, inborn error of metabolism [IEM]), followed by immune deficiency patients and, finally, patients with malignancies. In December 2004, the first IEM patient received BUdtIV. In the University Medical Center Utrecht (UMCU), all patients undergoing HSCT are prospectively included in a research database. All patients who were treated with Bu (p.o. or i.v. combined with TDM) from July 2003 until March 2007, were included in this study. Patients were enrolled in the HSCT protocol and research protocol after giving their written informed consent.

Transplantation Details, Conditioning Regimens and Supportive Care

Human leukocyte antigen-matching (HLA-matching) was based on high-resolution (HR)-typing for class I and class II (10 antigens) for family and unrelated bone marrow (BM) or peripheral blood stem cell (PBSC) donor. For cord blood (CB)-donors, lower resolution criteria were used (ie intermediate resolution for loci A and B and major histocompatibility complex, class II, DR beta 1 (DRB1) by HR-typing). A DPB1 mismatch was not taken into account. For the analyses, patients were divided into a matched and mismatched group. CB-grafts, which were identical according to the lower resolution criteria mentioned above, were regarded as matched (6 antigens).

Bu (Busilvex® Pierre Fabre Medicament, Boulogne, France) was given as an i.v. infusion during 3 hours once daily for 4 consecutive days. Patients older than 1 year received a first dosage of 120 mg/m^2; patients younger than 1 year received 80 mg/m^2. TDM was performed in these patients.

Bu seems to have an age independent correlation between body surface area (BSA) and clearance in children [12,31]. The clearance of drugs in newborns is highly dependent on the pace of development of the specific enzymes involved in metabolism or renal elimination. Data on the pace of maturation of glutathione S-transferase (the enzyme which metabolizes Bu) is lacking [16]. Generally, most enzyme pathways have matured after 1 year [32-37]. Therefore, the age of 1 year was chosen as a boundary between the 2 dosage regimens in this study and a dosing schedule based on BSA was used.

BUPO was given 1 mg/kg 4 times daily for 4 days (total dose 16 mg/kg). Children younger than 3 years received 1.25 mg/kg 4 times daily. No TDM was performed nor was dosing adjusted to a target AUC in the BUPO patients. All i.v. cystostatic medications were prepared by the pharmacy.

In general, a combination of Bu, cyclophosphamide (Cy), and melphalan (Mel) was used for myeloid malignancies (including MDS and infant acute lymphoblastic leukemia (ALL); Bu, Cy, and, in selected cases, fludarabine (Flu) for nonmalignant indications; and Bu, Cy and etoposide (VP16) for patients with HLH, as well as for some younger patients with ALL (<3 years). Patients with Fanconi anemia are more vulnerable to chemotherapy. Therefore, the patients with Fanconi anemia received a reduced dose and for the intravenous group they were targeted to a lower AUC (total 30,000 μg*h/l) in addition to a reduced dose of Cy (cumulative dose 40 mg/kg). Bu was given as first agent followed by Cy after at least 24 hours and Mel, Flu, or VP16. Unrelated donors received serotherapy

with either anti-thymocyte globulin (ATG)-rabbit (Genzyme) or alemtuzumab (Genzyme). Patients treated for ALL received ATG-rabbit (Fresenius). As supportive care, patients received antiemetic drugs; prophylactic anticonvulsive therapy (clonazepam) during Bu; and as antimicrobial prophylaxis, ciprofloxin, fluconazol and acyclovir. As graft-versus-host disease (GVHD) prophylaxis: cyclosporine was given in the matched sibling donors; cyclosporine (CSP) plus methotrexate (MTX) was given in the unrelated recipients; and CSP plus prednisolon in the CB recipients. Patients who received CB were treated with filgrastim (Amgen Europe B.V.) from day +7 until neutrophils were above 2000 /μL.

Target AUC and Therapeutic Drug Monitoring

The target AUC was defined as 17.500 μg*h/l (4263 μmol*min/l) per day. On the second day of treatment, dose adjustments based on AUC were performed before the second dose.

The analysis by high pressure liquid chromotography was based on the method previously described by Zwaveling et al [11] and analyzed using a limited sampling model established by Cremers et al [7,11]. Empirical Bayesian pharmacokinetic parameter estimates (clearance and volume of distribution) were generated using the pharmacokinetic software package MwPharm [38]. An one-compartment model was used, based on the literature [7,11]. The AUC was calculated from the expression dose/clearance and was based on 3 blood samples: 1, 2 and 3 hours after the end of infusion.

Treatment with i.v. Bu started at the first day at 9:00 AM. Blood samples were collected trough the lumen of the central catheter that was not used for Bu infusion and taken at 1, 2 and 3 hours after the end of the first infusion at day 1 and 4. In addition, a sample was taken at 24 hours after the start of the first infusion.

After the inclusion of 17 patients, a small change in the design of the dose targeting was introduced. If a dose adjustment >25% was suggested, blood samples at day 2 (and if necessary on day 3) were collected. If necessary, dose adjustments at day 3 and 4 were performed.

End Points for Evaluation of Therapy

Primary end points were EFS, and survival after HSCT with a follow-up of at least 6 months. EFS was defined as "alive and engrafted and not having a relapse." Secondary end points were VOD and GVHD grade 2 or higher, and the feasibility of TDM was studied.

VOD was diagnosed according to the modified Seattle criteria: at least 2 of 3 symptoms (painful hepatomegaly; unexplained weight gain of ≥5% from baseline; and hyperbilirubinemia, ie bilirubin ≥34 μmol/l) present before day 21 after HSCT and after exclusion of other possible causes. Severity of VOD was graded according to Bearman et al [39]. VOD was treated with defibrotide (25 mg/kg/day divided in 4 doses per day). No patient received defibrotide prophylactically.

VOD risk was defined according to criteria described in VOD-DF prophylaxis study [40] (eg, Bu and Mel in the conditioning, HLH, second ablative conditioning, osteopetrosis, pre-existent liver disease).

Acute GVHD (aGVHD) was diagnosed and graded according to Glucksberg et al [41]. Chimerism of >95% was regarded as having full donor. Donor chimerism of >10% and <95% was regarded as mixed.

Statistical Analysis

The associations between the variables and the end points were analyzed in univariable and multivariable logistic regression analyses. Dichotomous outcomes (eg, EFS: yes/no) were used as dependent and predictors as independent variables. Univariable predictors of outcome that were statistically significant ($P < .10$) were selected for multivariable logistic regression analysis. Results are expressed as odds ratios (OR) and corresponding 95% confidence intervals (95% CI). CIs not including 1 $P < -.05$) were considered statistically significant.

Time to event (primary end point) was analyzed with Kaplan-Meier curves. Significances were expressed as Log-Rank.

Because there was no randomization, the study employed "propensity score techniques" to adjust for prognosis in comparability (chance to get a certain treatment: BUPO versus BUdtIV) [42]. To calculate the propensity score, logistic regression analysis was used including the following variables: T cell depletion (TCD), stem cell source, HLA-matching, indication, and risk of VOD. The calculated propensity score was included in multivariable analysis for the primary endpoints to adjust for comparability. Statistical analyses were performed using SPSS version 12.1 (SPSS, Chicago, IL).

RESULTS

Patients' Characteristics

In total, 61 patients were included in the study between July 2003 and March 2007. Ages ranged between 2 months and 21 years; body weight ranged between 5 and 100 kg. Thirty patients received oral Bu and 31 patients received i.v. Bu with TDM as part of a myeloablative conditioning. For 1 patient in the BUdtIV group, TDM was not possible. The laboratory method to determine the concentration of the busulfan monsters was not available at that time. This patient was excluded from further analysis, as she did not meet our inclusion criteria. The patients in the BUdtIV and BUPO group were comparable

Table 1. *Patient Characteristics*

	BUPO	BUdtIV	*P*-Value
Median age at HSCT (range) in years	5 (1-16)	3.2 (0.2-21)	NS
Median follow up (range) in weeks	31 (1-212)	52 (10-134)	NS
	N (%)	N (%)	
Sex			
Male	19 (63)	14 (43)	
Female	11 (37)	16 (57)	NS
Indication¶			
Malignant	10 (33)	11 (37)	
Non-malignant	20 (67)	19 (63)	NS
HLA-disparity§			
Matched	19 (63)	13 (43)	
Mismatched	11 (37)	17 (57)	NS
Number of tx			
First tx	28 (93)	27 (90)	
Second tx	2 (7)	2 (7)	
Third tx	0	1 (3)	NS
TCD			
No	23 (77)	30 (100)	
Yes	7 (23)	0	.01
Donor			
Family	9 (30)	5 (17)	
Un-related	21 (70)	25 (83)	NS
Source			
BM	17 (58)	13 (44)	
CB£	5 (17)	16 (53)	
PBSC	8 (27)	1 (3)	.02
Conditioning			
Bu/Cy/Mel	6	7	
Bu/Cy/VP16	5	6	
Bu/Cy (+Flud)	16 (+3)	15 (+2)	
Risk on VOD			
No	16 (53)	13 (44)	
Yes	14 (47)	17 (57)	NS

TCD indicates Tcell depletion (CD3+ cell in graft ranged from 10^4 to 10^7/kg); HSCT, hemopoietic stem cell transplantation; VOD, Veno occlusive disease; HLA, human leukocyte antigen; BM, bone marrow; CB = cord blood; PBSC = peripheral blood stem cells, NS = not significant; CY, cyclophosphamide; Mel, melphalan; Flud, fludarabine.

¶Malignant disease was subdivided (BUPO vs. BudtIV) in ALL (5 vs. 4), MDS (4 vs. 5), Anaplastic Lymphoma (0 vs. 1), AML (1 vs. 1). Non-malignant was subdivided in immune deficiencies (7 vs. 7), including Hemophagocytic Lympho-Histiocytosis (HLH: 0 vs. 4), inborn errors of metabolism (9 vs. 9: including mucopolysaccharidosis, α-mannosidose, metachromatic leukodystrophy, GM1 gangliosidosis, purine nucleoside phosphorilase deficiency), Fanconi anemia (2 vs. 2), thalassemia (1 vs. 0) and Glanzmann (1 vs. 0).

§Matched was defined when either 10 of 10 molecularly typed allels were matched for bone marrow or PBSC or 6 of 6 for CB based on Rubinstein criteria. Within the mismatched BM/PBSC group (n = 8) 6 were 9/10 matched and 2 were 8/10 matched. Within the mismatched group (n = 20) 16 were matched 5/6 and 4 were matched 4/6.

£Median cell dose of the CBs used was: 7.8 x 10^7 NC/kg (range 2.7-20.0 x 10^7) and 4.5 x 10^5 CD34+/kg (range 1.1-10.0 x 10^5). All cord bloods were unrelated.

regarding age, sex, follow up time and indication for HSCT (Table 1). TCD (CD3+ cell in graft ranged from 10^4 to 10^7/kg) was used more often in the BUPO group and CB was used more often in the BUdtIV. However, univariable analysis of the data showed that these differences did not influence the primary, nor the secondary, outcomes (Table 2).

Survival and Event Free Survival

In univariable analysis, BUdtIV was the only predictor for higher EFS and survival (OR = 11.7, $P <$ .001; OR = 4.38, P = .016) (Table 2 and Figure 1). In addition, after adjustment for "prognosis in comparability" (propensity scoring technique), BudtIV remained a predictor for higher EFS and survival (OR = 18.1, $P <$ .001; OR = 6.03 P = .016). Initial donor cell engraftment was found in 100% of patients treated with BUdtIV in comparison to 83% in the BUPO group. In the BUdtIV group, 2 patients became mixed chimeric after 2 months, resulting in graft-failure/relapse (HLH patient who received second transplant) or relapse (ALL patient). All patients who are alive have maintained full donor chimerism. In the BUPO group, 10 patients had graft failure (for which 7 were retransplanted) and 1 patient died early before engraftment. In the BUPO group, two patients who are alive have stable mixed chimerism.

In the BUPO group, graft failure was more frequently seen in the patients with nonmalignant disease: (1 thallassemia, 4 mucopolysaccharidose, 1 metachromatic leukodystrophy, osteopetrosis, variant Blackfan-Diamond anaemie, and common variable immunodeficiency. 9 of 20 patients with nonmalignant disease had graft failure versus 2 out of 10 in the malignant group (1 ALL, 1 MDS: OR = 4.10, CI 95% = 0.69-23.95, P =.12). On the other hand, TRM appeared to be higher in the malignant group, was 5 of 10, versus 5 of 20 in the nonmalignant group. The 2 patients in the BUdtIV group who had a graft failure, suffered from ALL and HLH.

In the BUdtIV group, 6 patients died (of whom 1 after second transplant): 1 from a relapse; 1 Epstein-Barr virus-associated posttransplant lymphoproliferative disease; 3 from multiorgan failure; and 1 from GVHD. In the oral Bu group, 14 patients died (of whom 5 after second transplant): 2 from relapse/disease progression; 2 from acute cardiac problems; 4 from multiorgan failure; 3 from GVHD; 2 from (viral) disease; and 1 from idiopathic pneumonia syndrome.

Treatment-related Toxicity

VOD occurred significantly more frequently in the BUdtIV group than in the BUPO group, as shown in Table 3 (OR = 3.76, P = .044) (Table 3a). The patients who developed VOD (all mild to moderate: Bearman grade 2), were successfully treated with defibrotide. VOD did not influence the primary end points. The number of patients who were classified as having an increased risk to develop VOD, was similar in both groups (Table 1). Of all patients, 51% had an

Table 2. *Univariate Predictors of Event-Free Survival and Survival*

	Event-Free Survival						Survival				
	N total	N	%	OR	95%-CI	p-value	N	%	OR	95%-CI	p-value
Overall	60	33	55				40	67			
Age	60			0.99	0.9-1.1	.773			0.92	0.8-1.0	.134
Sex											
Male	32	16	50	1			21	66	1		
Female	28	18	64	1.27	0.6-5.1	.267	20	71	1.31	0.4-3.9	.630
Indication											
Malign	21	13	62	1			10	48	1		
Non malign:	39	21	54	0.85	0.3-2.5	.773	28	71	1.94	0.6-5.9	.245
HLA-disp.											
Matched	32	19	59	1			22	68	1		
Mismatched	28	15	54	0.79	0.3-2.2	.789	19	66	0.96	0.3-2.9	.941
TCD											
No	53	32	60	1			36	53	1		
Yes	7	2	29	0.26	0.0-1.5	.130	5	71	1.18	0.2-6.7	.852
Donor											
Family	14	6	43	1			8	57	1		
Unrelated	46	28	61	2.1	0.6-7.0	.238	33	72	1.90	0.6-6.6	.308
Source											
BM	30	19	63	1			21	70	1		
CB	21	12	57	0.77	0.3-2.4	.656	15	71	1.07	0.31-3,66	.912
PBSC	9	3	33	0.29	0.1-1.4	.122	5	56	0.54	0.1-2.5	.424
Conditioning											
BUPO	30	9	30	1			16	53	1		
IVdtBU	30	25	83	11.7	3.4-40	<.001	25	83	4.38	1.3-15	.016
Acute-GVHD											
No	52	28	54	1			35	67	1		
Yes	8	6	75	2.57	0.5-14	.274	6	75	1.46	0.3-8.0	.665
VOD											
No	45	25	56	1			30	67	1		
Yes	15	9	60	1.20	0.4-3.9	.764	11	73	1.38	0.4-5.0	.632

OR indicates odd ratio; CI, confidence intervals; TCD, T-cell depletion; VOD, Veno occlusive disease; HLA disp, human leukocyte antigens disparity; GVHD, graft versus host disease; BM, bone marrow; CB, cord blood; PBSC, peripheral blood stem cells; N, amount; BUPO, oral busulfan; BUdtIV, An intravenous dose of busulfan, combined with drug targeting based on therapeutic drug monitoring.

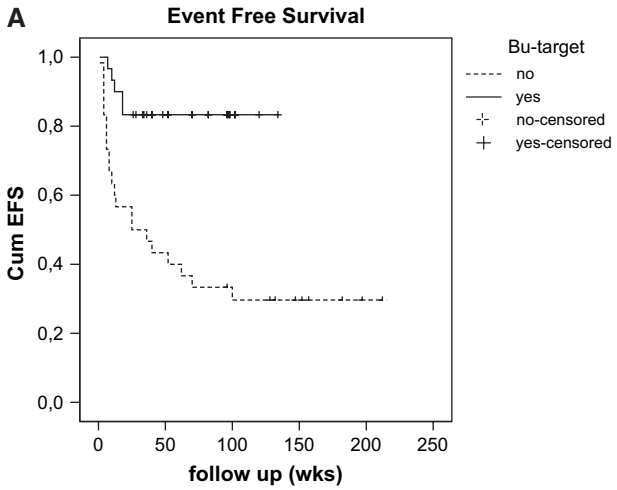
increased risk to develop VOD. Of this high-risk group within BUdtIV, 32% developed VOD, whereas 17% of patients without risk factors, developed VOD. 6 of 7 patients (87%) developed VOD after a conditioning regimen of BUdtIV in combination with cyclophosphamide and Mel, whereas only 5 of 23 (22%) other patients in the BUdtIV group developed VOD (OR = 3.62, CI 95% = 0.98-13.42, $P = .054$)

No significant difference in GvHD between the two Bu groups was found (Table 3b). Patients with nonmalignant disease showed a lower incidence of GVHD than patients treated for malignant diseases (OR = 0.15, $P = .028$). Multivariable analysis that patients who develop VOD, also more frequently developed acute GVHD (OR = 12.7, $P = .008$).

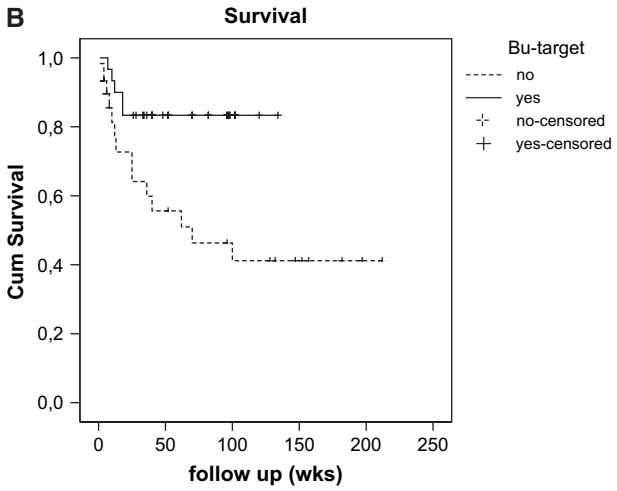
Therapeutic Drug Monitoring

The results of dose targeting based on TDM are shown in Table 4. Figure 2 shows a representative concentration-time curve of BUdtIV. In all but 1 patient, the AUC was determined after the first dose. For one patient, the laboratory method to determine the concentration of the busulfan monsters was not available at that time. As mentioned above, this patient was excluded from analysis. In all but 1 patient, trough concentrations were below the limit of detection (<50 μg/l). One patient showed a trough concentration 24 hours after the first dose of 90 μg/l. In 10 patients, no dose adjustment was needed; in 19 patients a dose decrease was made and in 1 patient a dose increase was made.

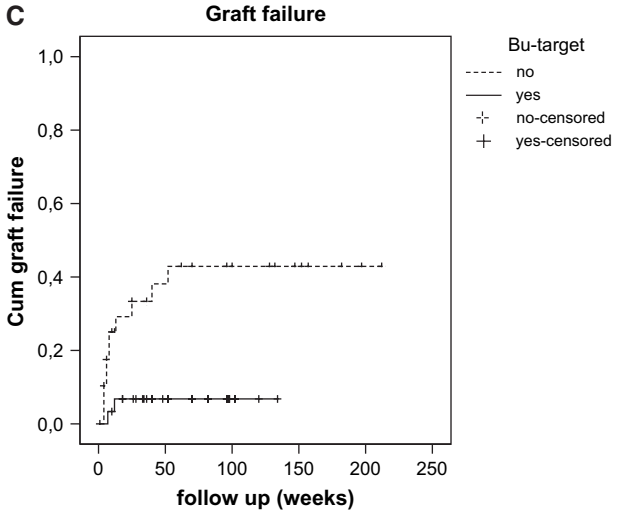
In patients UPN 12 and 17, the dose was reduced >25% on the AUC of day 1. The analysis at day 4 of these 2 patients showed that the AUC had declined more than expected. The AUC at day 4 was 30% lower than the target AUC. These patients showed a cumulative AUC of 60.158 μg*h/l, in comparison to a median of 79,614 μg*h/l recorded in the other patients. These 2 patients became mixed chimeric after 2 months resulting in graft-failure and relapse. A third patient (with a combined immunodeficiency), whose AUC at day 4 was out of range, received a total AUC of 65,600 μg*h/l after a dose reduction of 20%. This patient is alive and engrafted (with chronic GVHD [cGVHD]). After this observation, the dose adjustment policy was changed: when a dose adjustment



Event free survival (Log-rank = 0.0001)



Survival (log-rank = 0.0074)



Graft failure (Log-rank = 0.0030)

Figure 1. Kaplan-Meier Curve of event free survival (a), survival (b) and graft failure (c) after HSCT with iv-targeted-Bu versus oral Bu.

>25% was suggested, further dose adjustments based on TDM at day 3 and 4 were performed.

DISCUSSION

This study compared the outcomes of HSCT in children, after a preparative chemotherapy-regimen including i.v. Bu with TDM with the current practice of untargeted oral Bu (BUPO). TDM after a once daily dose of intravenous Bu (BUdtIV) resulted in a significantly better survival and EFS, in comparison to BUPO. Also, after adjustment, using propensity score techniques to adjust for prognosis in comparability, this association remained significant. BUdtIV was associated with VOD mainly in patients receiving Bu plus mel in their conditioning. Patients who developed VOD, also developed acute GVHD more frequently. In addition, TDM of Bu in clinical practice was feasible.

A randomized, blinded, controlled trial would be the best instrument to test the 2 therapies. Practically this was not possible for this study within the UMCU. The use of hard clinical endpoints diminished assessor bias as much as possible. In addition, the inclusion of patients was performed in the same period for both treatment groups. All patients were treated similarly, regarding indication associated conditioning regimens and supportive care given. Patient characteristics in both groups were similar, with the exception of two variables: TCD was used more often in the BUPO group and CB was used more often in the BUdtIV. These differences, however, did not influence the primary, nor the secondary, outcomes. Probably the relatively high CD3+ dose after TCD, is the reason why TCD did not influence the end points. By using propensity scoring techniques, adjustments were made for prognosis in comparability (ie, the chance to get a certain treatment: BUPO vs BUdtIV). The results from this analysis suggest that groups were comparable. However, the wide range of diseases, preparative regimens, and cell sources utilized in this relatively small cohort of children, remains a limitation in this study.

Theoretically, BUdtIV has advantages over BUPO: 1) the AUC of i.v. Bu is more predictable, due to the variability in oral absorption of Bu, which differ in children from 20% to 100%, and the circumvention of the first-pass hepatic effect after an i.v. dose [43]; 2) the possibility of its administration once daily, whereas BUPO may only be administered every 6 hours, mainly because only 2 mg tablets are available. A once daily dose is much more convenient for both caregivers and patients. Fewer infusions are needed which means: there is less burden for the patient; it is easier for the nursing personnel; and there is a smaller chance of administration errors. Possibly, single administration leads to a better penetration of poorly vascularized parts of the body, resulting from a higher

Table 3a. *Univariate Predictors for the Development of VOD and Acute GVHD*

		VOD					GVHD				
	N total	N	%	OR	95% CI	*P*-Value	N	%	OR	95% CI	*P*-Value
Overall	60	14	23				8	13			
Age	60			1.05	0.4-1.2	.422			1.06	0.9-1.2	.370
Sex											
Male	32	7	22	1			3	9	1		
Female	28	8	28	1.43	0.4-4.6	.551	5	18	2.10	0.4-9.7	.342
Indication											
Malign	21	8	38	1			6	298	1		
Non malign	39	7	18	0.40	0.1-1.3	.128	2	5	0.15	0.03-0.8	.028
HLA-disp.											
Matched	32	10	31	1			7	22			
Mismatched	28	5	18	0.48	0.1-1.6	.237	1	4	0.13	0.02-1.2	.067
TCD§											
No	53	14	26	1			7	13	1		
Yes	7	1	14	0.46	0.05-4.2	.495	1	14	1.10	0.1-11	.937
Donor											
Family	14	5	35	1			2	145	1		
Unrelated	46	10	21	0.50	0.1-1.8	.295	6	13	0.90	0.2-5.0	.905
Source											
BM	30	8	27	1			6	20	1		
CB£	21	4	19	0.64	0.2-2.5	.53	0	0	0.23	0.03-2.1	.194
PBSC	9	3	33	1.38	0.3-6.8	.69	2	22	1.37	0.2-8.7	.74
Conditioning											
BUPO	30	4	13	1			3	10	1		
IVdtBU	30	11	37	3.76	1.04-14	.044	5	17	1.80	0.4-8.3	.452
Acute-GVHD											
No	52	9	17	1							
Yes	8	6	75	14.3	2.5-83	.003					
VOD											
No	45						2	4	1		
Yes	15						6	40	14.3	2.5-83	.003

peak concentration [44]. As mentioned in the introduction, the lower exposure period to Bu in a once daily schedule might be associated with reduced toxicity. In addition to i.v. administration of Bu, dose targeting might further have optimized the outcome. In light of the more predictable kinetics with i.v.administration, TDM of i.v. Bu seems therefore to be more rational than dose targeting of oral Bu. Secondly, it was easier to perform TDM on a once daily dosing schedule than on a 4 times daily schedule, since no

Table 3b. *Multivariable Predictors for the Development of VOD and Acute GVHD*

Multivariate analysis	VOD			GVHD		
	OR	95% CI	*P*-Value	OR	95% CI	*P*-Value
BUdtIV	4.07	0.9-18	.060			
Acute-GVHD	15.4	2.4-99	.004			
VOD				12.7	1.9-84	.008
Indication				0.23	0.03-1.6	.131
Match				0.19	0.02-2.0	.165

OR indicates odds ratio; CI, confidence interval; TCD, T-cell depletion; VOD, Veno occlusive disease; HLA disp, human leukocyte antigenes disparity; GVHD, graft versus host disease; BM, bone marrow; CB, cord blood; PBSC, peripheral blood stem cells; BUPO, oral busulfan; BUdtIV, intravenous once daily busulfan, combined with drug targeting based on therapeutic drug monitoring.

Table 4. *Busulfan Pharmacokinetics*

	Median	Mean	95% CI
AUC after first dose (μg*h/l)	20,211	20,946	18,839-23,054
AUC after 4th dose (μg*h/l)	20,313	20,313	17,236-23,237
Adjusted dose (mg/m^2)	101.0	99.7	91.7-107.7
Adjusted dose (mg/m^2) in children < 1 year (n=6)	75.6	80.7	73.9-87.4
Adjusted dose (mg/kg)	4.21	4.10	3.78-4.88
Total AUC (μg*h/l)§	79,270	79,940	77,443-82,437
Total dose/day (mg/m^2)	104.6	103.1	96.1-110.1
Total dose (mg/m^2) in children < 1 year (n=6)	76.3	81.8	75.6-88.0

The analyses of the two Fanconi anemia patients are excluded. These patients received one third of the dose of other patients.
AUC indicates area under the curve; Sd, standard deviation.
§In these AUC calculations, 2 patients are included, who received a >25% dose reduction based on the AUC of day 1. The analysis at day 4 of these patients showed that the AUC had declined more than expected (AUC day 4 were 11,390 and 12,594 μg*h/l). Both patients had a graft failure after 2 months. After exclusion these patients, the mean total AUC is: 79,614 μg*h/l (CI 95%: 77,073-81,264 μg*h/l).

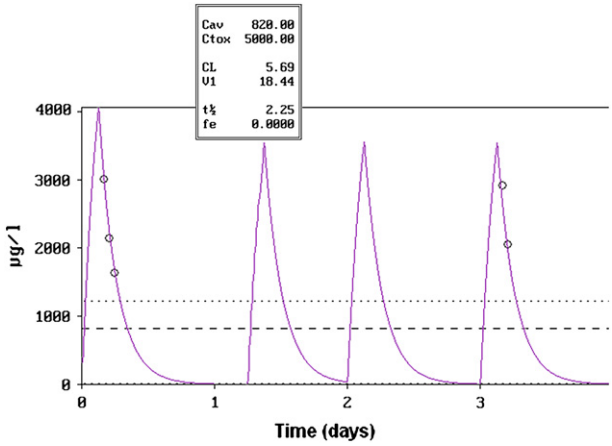


Figure 2. Example of blood concentrations in a patient.

accumulation of Bu occured in this schedule. Additionally, individualization of BUPO dosing based on TDM could have been jeopardized by problems inherent to the use of the oral form, like vomiting, incomplete absorption, and unpredictable bioavailability between administrations. These problems occur especially in the very young. This study included many young patients, 22 patients were younger than 3 years.

In most previous studies, i.v. Bu (like oral Bu) was administered 4 times daily. Results from the studies using the same AUC per day, divided in 4 doses, were in line with the results of this study: approximately 90% of patients had complete donor cell chimerism versus 100% in our study; and EFS ranged from 65% to 85% as compared to 83% in our study [9-11]. The patient populations, however, were different. Our study included a high percentage (52%; IEM, MDS, HLH) of indications known to be associated with high graft-failure/relapse rates as compared to 5% and 40% in other studies [9,11].

Patients with non-malignant disorders (eg IEM) in general have history of allograft resistance. In the BUPO group, the patients with non-malignant diseases, noted a large incidence of primary or secondary graft failures (45%), in line with other studies [1], while a trend to less graft failure was seen in the malignant indications in this study. In the BudtIV group, on the other hand, no difference in graft-failure/relapse was seen (only 2 / 30). This study shows that i.v. Bu using TDM improved mainly the outcome of patients with a high risk for graft failure. These results are in line with other studies in which similar patients with similar indications were treated (eg, in patients with Hurler's syndrome); BUdtIV also showed improved EFS in comparison to BUPO (EFS: 53% versus 87%) [45] as well as with the results reported by the Leiden University Medical Center (a comparable pediatric HSCT ward). They treated another 14 patients with the same BUdtIV protocol as this study's protocol (AUC targeted; once daily). These patients (7 malignant {6 MDS, 1 ALL} and 7 nonmalignant) showed similar results: 79% (11/14) EFS and overall survival (OS), 100% engraftment, no relapses/graft failures and 42% VOD (mainly in the Bu/Cy/Mel group).

Because of the narrow therapeutic window, the target AUC seems to be important for reduction of toxicity and graft-failure. This was once again indicated by a recent study using a once daily dose of i.v. Bu in children [12]. This study used a target AUC of 15,600 µg*h/l (3800 µmol*min/l) for a similar distribution of indications as in our study. In this study by Zwaveling et al, only 55% of patients were alive and engrafted (EFS) after first procedure, compared to >80% in this study. However, EFS depends on many factors, which makes precise comparison difficult.

In contrast to what this study's hypothesis, i.v. Bu was associated with more cases of VOD. Also, in comparison to a similar studies, (ie once daily, same AUC) in adults this incidence was high: 37% of the children in this study versus 8% or 1% of the adult patients in other studies developed VOD [27,28]. This difference might be explained by the fact that other criteria (the Jones criteria) were used to define VOD [46]. In a pediatric study using once daily i.v. Bu an incidence of VOD of only 6% was found [12]. This study, however, used a lower target AUC. Because a high AUC of Bu is correlated to a higher risk to develop VOD [2,13-15], these results cannot be compared to our results. The studies with Bu divided over 4 doses per day with the same target AUC per day, showed an incidence of VOD of 0% to 25% [9-11]. Only the study of Zwaveling et al showed a similar incidence of VOD (25% of patients) and used similar criteria to define VOD as in our study [11]. An alternative reason for the high incidence of VOD could be that our study population was at quite high risk for the development of VOD (50% at risk based on conditioning/indication). The higher frequency of VOD was mainly observed in the patients also receiving Mel as part of a myelo-ablative conditioning. 87% of patients who used BUdtIV and Mel developed VOD. Mel probably makes the conditioning more toxic and the patients more vulnerable to develop VOD. In most other studies, other than the study of Zwaveling et al patients did not receive Mel [9,11,27,28]. In patients at risk of developing VOD, reports show that 30-40% of patients develop this side effect [40]. This incidence is similar to our results (excluding those patients who received Bu + Mel). Another explanation for the higher incidence of VOD might be the higher peak levels achieved with once daily dosing. Possibly the peak level rather than the exposure over time is more critical for the development of VOD. However, a recent study in adults by Ryu et al. [29] in which adult patients were randomized between the same dose of Bu divided in 4 times daily and a once daily dose, showed no major differences in effect nor side effect [47].

The high incidence of VOD shown in this study's patients is of concern. However, VOD did not influence the primary endpoints and was relatively easy to manage with the use of defibrotide. Because of the high engraftment rate in this patients group with "high-risk patients" for graft-failure, we believe that the higher incidence of VOD after once daily i.v. Bu dosing, seems to be an acceptable side effect.

Because VOD was seen more often in the Bu/Cy/Mel group, it might be speculated that this conditioning is too toxic with a total AUC of approximately 80,000μg*h/l for Bu. In the past, Mel was added to Bu (oral without TDM), Cy in the MDS group, mainly because of the higher graft-failure rates and because Mel showed to be a valuable drug in AML [48]. MDS sometimes transforms into AML. With an optimized Bu exposure using intravenous Bu with TDM, addition of Mel may not be necessary to overcome graft rejection or relapses in patients with MDS. Dose de-escalation studies (either reduce the target AUC of Bu or the dose Mel) might be necessary. Randomized studies comparing once daily dosed Bu versus the conventional 4 daily dosed Bu targeting for the same AUC, might be of interest to get answer on various questions raised above.

The occurrence of GVHD did not differ significantly between the two groups. GVHD was seen more frequently in patients with malignant diseases. This is probably associated with the amount of immune suppression after HSCT, as lower levels of immunosuppression were acceptable to allow some graft versus leukemia (GVL) effect. (CSP target trough concentration is generally 0.1-0.15 mg/l in malignant diseases and 0.2-0.25 mg/l in non-malignant disease and depends on risk qualification.) An association between VOD and GVHD was found in this as well as in other studies [39,49]. Probably both the treatment of Bu and alloreactivity contribute to the development of VOD.

The results of day 1 showed a variation in pharmacokinetics of Bu between individuals. The results show that TDM was and remains needed. The variation in PK, especially when large adjustments are made, indicates that TDM remains necessary in this population, at least until a better predictive model is available. There is some concern regarding the pharmacokinetics as a one-compartment model in this study. This model was based on earlier studies in which the dose of Bu was lower than the dose used in this study [2,11,50]. Pharmacokinetic data of Bu of a dose of 120 mg/m^2 were not available beforehand. A deviation of the model was shown in 2 patients who received a dose reduction of >25% after TDM. A further analysis of the data will clarify this inconsistency. These 2 patients developed graft failure. The total AUC in these patients was approximately 60.000 μg*h/l. As shown in the study of Zwaveling et al. a total AUC of 62.400 μg*h/l. was associated with early graft rejection in 25% of the patients [12]. Excluding these 2 patients, the mean total AUC was 79,614 μg*h/l. This AUC was significantly higher than the target of 70,000 μg*h/l (17,050 μmol*min/l). The results of this study are therefore associated with this relatively high total AUC.

The patients younger than 1 year received a smaller dose per m^2 in comparison to patients older than 1 year. This dosing schedule remained similar after TDM. Further research into the pharmacokinetics of Bu will be performed in order to increase the knowledge of the optimal doses of Bu in patients of all ages as well as to study whether distinctive indication groups, like patients with malignant diseases or recipients of HLA-matched grafts could receive a lower targeted AUC, in order to optimize EFS and to minimize side effects.

In conclusion, this study showed that once daily dosing of i.v. Bu, after dose adjustment to a total AUC of 79,614 μg*h/ (19,395 μmol*min/l), was associated with higher survival and EFS rates in a group of patients known to be associated with high graft failure/relapse rates. The higher incidence of VOD (in 37% of patients) might be a concern, although VOD was easy to treat. Melphalan as a risk factor in developing VOD in combination with Bu, will be studied into more detail for instance with dose de-escalation of Mel. Randomized studies and distinction between subgroups such as malignant and nonmalignant diseases, would increase our knowlegde of i.v. Bu in childern. Although once-daily dosing with TDM was feasible and convenient for caregivers, additional studies are needed to fine tune the pharmacokinetic/pharmacodynamic model to be used, resulting in an optimal dose and a better prediction of the target AUC for all pediatric age categories. These results strongly suggest that i.v. Bu, using TDM is preferable over oral Bu, in children undergoing allogeneic SCT, especially in those at high risk for graft failure/ relapse.

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