Biol Blood Marrow Transplant 19 (2013) 1381–1388

Establishing a Target Exposure for Once-Daily Intravenous Busulfan Given with Fludarabine and Thymoglobulin before Allogeneic Transplantation

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Article history:
Received 27 May 2013
Accepted 2 July 2013

Key Words:
Busulfan
Exposure
Pharmacokinetics

A B S T R A C T

A combination of fludarabine (Flu) and daily i.v. busulfan (Bu) is well tolerated and effective in patients undergoing allogeneic hematopoietic stem cell transplantation. Although there is some evidence that Bu exposures exceeding 6000 μM/min may lead to excessive toxicity, there is little information on the effect of exposures below this level on outcomes. We studied Bu exposure, as measured by area under the concentration-time curve (AUC), in 158 patients with various hematologic malignancies in an attempt to identify an optimal range for targeted therapy. The preparative chemotherapy regimen comprised Flu 50 mg/m^2 on days -6 to -2 and i.v. Bu 3.2 mg/kg on days -5 to -2 inclusive. Graft-versus-host disease (GVHD) prophylaxis included methotrexate, cyclosporin A, and antithymocyte globulin. Patients with Bu exposures below the median AUC of 4439 μM/min were at increased risk for acute GVHD grade II-IV (hazard ratio [HR], 2.30; 95% confidence interval [CI], 1.19 to 4.49; $P = .014$). Those in the highest and lowest Bu exposure quartiles (daily AUC <3814 μM/min and >4993 μM/min) had an increased risk of nonrelapse mortality (subdistribution HR, 3.32; 95% CI, 1.46 to 7.54; $P = .004$), as well as worse disease-free survival (HR, 1.81; 95% CI, 1.09 to 2.99; $P = .021$) and overall survival (HR, 1.94; 95% CI, 1.12 to 3.37; $P = .018$). Bu exposures between 4440 and 4993 μM/min were accompanied by the lowest risk of both nonrelapse mortality and acute GVHD.

INTRODUCTION

Although i.v. busulfan (Bu) has more predictable pharmacokinetics than the oral form, there remains at least a 3- to 4-fold variation in exposure between patients given the same dose based on weight [1]. A combination of fludarabine (Flu) and daily i.v. Bu at myeloablative doses has proven well tolerated and effective in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT) [2-11]. With this regimen, there is some evidence that Bu exposures exceeding 6000 μM/min are excessive, but there is little information on the effect of exposures below this level on outcomes [12]. As part of a routine program of therapeutic dose monitoring (TDM), we measured Bu exposure, as expressed by the area under the concentration-time curve (AUC), in patients receiving a Flu/Bu regimen before HSCT. The present study examined whether different exposures below 6000 μM/min have any influence on clinical outcomes, with the goal of defining an appropriate range for targeted therapy.

PATIENTS AND METHODS

Patients

This study is a retrospective analysis of outcomes of 158 consecutive adults with hematologic malignancies treated with HSCT after a myeloablative Flu/Bu regimen between August 2000 and July 2011 for whom TDM was performed and Bu exposure was <6000 μM/min. Diagnoses and details of the HSCTs are presented in Table 1. Patients were divided into 4 quartile groups according to total Bu exposure. Seventeen patients with acute leukemia were excluded, because our standard regimen now includes 400 cGy total body irradiation (TBI) for these patients [2,13,14]. Distribution of risk factors was similar across the 4 groups apart from the proportion of patients with chronic myelogenous leukemia (CML) in chronic phase (CP) 1 (0% in group 4 vs 16% in groups 1 to 3; $P = .02$). Surviving patients were followed for a median of 51 months (range, 12 to 142 months).

Treatment

The preparative chemotherapy comprised Flu 50 mg/m^2 on days -6 to -2 and i.v. Bu (Busulfex; Otsuka America Pharmaceutical, Princeton, NJ) 3.2 mg/kg (based on the lower of actual or adjusted ideal body weight) on days -5 to -2 inclusive. Supportive care was similar for all patients. No protective isolation was used [15]. Platelets were administered to maintain a platelet count >10 × 10^9/L, and RBCs were administered to maintain a hemoglobin level >80 g/L. Growth factors were not routinely administered. All patients received trimethoprim/sulfamethoxazole as prophylaxis for *Pneumocystis jirovecii*. Antibacterial prophylaxis was provided with ciprofloxacin 500 mg twice daily until 2003, after which time no antibacterial antibiotics were given routinely. All blood products were cytomegalovirus (CMV)-safe. A policy of surveillance for pp65 antigen or CMV polymerase chain reaction, followed by preemptive therapy with ganciclovir when indicated, was used when the donor and/or recipient were CMV antibody-positive. Routine monitoring for Epstein-Barr virus viral load was not done. The acute graft-versus-host disease (aGVHD) prophylaxis protocol included cyclosporin A (CsA) orally or i.v. twice daily to maintain blood levels between 150 and 400 mol/L. Methotrexate was given at 15 mg/m^2 i.v. on day 1 and at 10 mg/m^2 on days 3, 6, and 11. Folinic acid 5 mg i.v. or orally was started at 24 hours after each methotrexate dose and continued every 6 hours until 12 hours before the next dose. In addition, all patients were given rabbit antithymocyte globulin (ATG; Thymoglobulin; Sanofi, Paris,

Financial disclosure: See Acknowledgments on page 1385.

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1083-8791/$ – see front matter © 2013 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.07.002

Table 1
Patient and Transplant Characteristics

Characteristic	Group (Range of Bu Exposure (μM/min)				Total
	1 (2184-3813)	2 (3814-4439)	3 (4440-4993)	4 (4994-5995)	
Number	39 (100)	40 (100)	40 (100)	39 (100)	158 (100)
Patient age, yr, median (range)	50 (30-65)	52 (20-64)	50 (29-65)		51 (18-66)
Male patient, n (%)	25 (64)	25 (63)	27 (68)	30 (77)	107 (68)
Low-risk disease (CML CP), n (%)	5 (13)	7 (18)	7 (18)	0*	19 (12)
High-risk disease, n (%)	34 (87)	33 (83)	33 (83)	39 (100)	139 (88)
CML AP	1 (3)	2 (5)	1 (3)	3 (8)	7 (4)
CLL/SLL	9 (23)	10 (25)	10 (25)	3 (8)	32 (20)
Myelodysplastic syndrome	7 (18)	6 (15)	6 (15)	7 (18)	26 (16)
Multiple myeloma	0	0	1 (3)	5 (13)	6 (4)
Non-Hodgkin lymphoma	7 (18)	7 (18)	10 (25)	14 (36)	38 (24)
MF/MPD	8 (21)	5 (13)	2 (5)	3 (8)	18 (11)
Hodgkin disease	1 (3)	1 (3)	2 (5)	2 (5)	6 (4)
Waldenstrom macroglobulinemia	1 (3)	1 (3)	1 (3)	1 (3)	4 (3)
Hairy cell leukemia	0	1 (3)	0	0	1 (1)
T cell prolymphocytic leukemia	0	0	0	1 (3)	1 (1)
Donor, n (%)					
Matched related	26 (67)	24 (60)	17 (43)	23 (59)	90 (57)
Mismatched related	0	0	3 (8)	0	3 (2)
Matched unrelated	8 (21)	10 (25)	14 (35)	13 (33)	45 (28)
Mismatched unrelated	5 (13)	6 (15)	6 (15)	3 (8)	20 (13)
CMV antibody-positive recipient or donor, n (%)	27 (69)	31 (78)	26 (67)†	26 68)†	110 (70)†
Female-to-male HSCT, n (%)	13 (33)	13 (33)	10 (25)	13 (33)	49 (31)
Blood as stem cell source, n (%)	33 (85)	38 (95)	35 (88)‡	36 (92)‡	142 (90)
HCT-CI score ≥1, n (%)	12 (31)	12 (30)	11 (28)	10 (27)	45 (28)

AP indicates accelerated phase; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma with or without Richter's transformation.
* $P = .004$ for CML CP in group 4 versus groups 1, 2, and 3.
† One patient/donor with unknown status.
‡ Remainder bone marrow except for 1 cord blood in groups 2 and 3.

France) 4.5 mg/kg i.v. in divided doses over 3 days. Each dose was given as a continuous infusion over 4 to 8 hours. To minimize reactions, the first dose was reduced to 0.5 mg/kg, and the next 2 doses were 2 mg/kg, with the final infusion given on the day of transplantation. Premedication included methyprednisolone 40 mg i.v. every 12 hours for 6 doses and benadryl 50 mg i.v. before each dose of ATG.

Therapeutic Dose Monitoring

A total of 123 patients were given a test dose of Bu at 2 to 5 days before the treatment dose, as described previously [16]. Fifteen patients received a test dose of Bu 12 mg over 20 minutes, 3 received a test dose of 0.8 mg/kg over 3 hours, and the remaining 105 received a test dose of 0.8 mg/kg at a rate of 80 mg/hour according to our current protocol. Nine patients (1 in group 2, 4 in group 3, and 4 in group 4) had a dose adjustment to target 5000 μM/min if the test dose predicted an exposure exceeding 5500 μM/min. This allowed a margin of error to ensure the therapeutic dose exposure remained below 6000 μM/min. No other patients had a dose adjustment based on the test dose. Total daily exposure was calculated to include one-quarter of the test dose exposure.

Engraftment

Daily blood counts were performed until discharge, with bone marrow aspirations done at 3 months for surviving patients and thereafter as clinically indicated. Granulocyte engraftment was defined as a count of $>0.5 \times 10^9$/L for 3 consecutive days. The platelet count needed to exceed 20×10^9/L without transfusion for 7 days.

GVHD

aGVHD was graded according to standard criteria [17]. Grading was performed by physicians at onset and during treatment, with later confirmation and recording by data managers. aGVHD was treated with prednisone or methylprednisolone initially while continuing CsA. First-line therapy for steroid-resistant aGVHD was ATG 2 mg/kg every other day for 2 to 4 doses while CsA was withheld. Chronic GVHD (cGVHD) was treated with prednisone with or without CsA, with the introduction of other agents if response was incomplete.

Statistical Analysis

Continuous data are presented as median and range, and categorical data are presented as count and percentage. Bu exposures were compared using a 2-tailed *t*-test. The survivorship function of time-to-event data was estimated using the product-limit method and compared using the log-rank test. For analysis of time to nonrelapse mortality (NRM), patients were right-censored at time of relapse, and for analysis of onset of cGVHD, patients were right-censored at the time of death, donor lymphocyte infusion, or second HSCT. Clinical endpoints of relapse, NRM, disease-free survival (DFS), and overall survival (OS) were recorded at 3 years from HSCT. Univariate analysis was performed with GraphPad Prism (GraphPad Software, San Diego, CA).

Cox multivariate proportional hazards regression was used to identify factors associated with time to death, aGVHD, cGVHD, and the composite outcome of death or relapse. The standard hazard ratio (HR) and associated 95% confidence interval (CI) was used to measure the strength of the association. For the competing endpoints of relapse and NRM, multivariate competing-risks regression was used, and the subdistribution hazard ratio (SHR) with 95% CI was used to describe the association, given that the standard proportional hazards approach has been shown to be methodologically inadequate when outcomes are competing [18]. All multivariate analyses were performed using Stata version 11.2 (StataCorp, College Station, TX). For all analyses, P values $<.10$ are reported as trends, and P values $<.05$ are considered statistically significant.

RESULTS

Bu Exposure

The median Bu exposure of all patients was 4439 μM/min (range, 2184 to 5995 μM/min), with first (25%) and third (75%) quartiles of 3824 and 4994 μM/min, respectively. Mean exposure was 4373 ± 835 μM/min. Compared with the remaining patients, those with myelofibrosis/myeloproliferative disorder (MF/MPD) had lower exposures (mean, 3960 ± 236 μM/min versus 4426 ± 68 μM/min; $P = .03$), and those with non-Hodgkin lymphoma had higher exposures (mean, 4619 ± 134 μM/min versus 4289 ± 76 μM/min; $P = .04$). Although there were no patients with CML CP in group 4, compared with 19 of 119 in groups 1 to 3 ($P = .004$), there was no difference in mean AUC between the patients with CML CP and other patients.

Engraftment

There was no difference in time to engraftment across the 4 groups (Table 2). In the first quartile, there were 3 cases of

Table 2
Granulocyte and Platelet Engraftment

Group (n)	Granulocytes			Platelets		
	Granulocyte Engraftment, Days		Failure to Engraft, n	Platelet Engraftment, Days		Failure to Engraft (in the Presence of Granulocyte Engraftment), n
	Median	Range		Median	Range	
1 (39)	16	11-26	2 graft failures, second HSCT, days 68 and 79	18	0-45	1, second HSCT, day 72
2 (40)	15	10-25	1 graft failure (faulty product), second HSCT, day 21	18	0-54	
3 (40)	15	10-32	1 NRM, day 32	18	0-33	1 NRM, day 31
4 (39)	15	10-22		18	0-60	3 NRM, days 19, 40, and 68

graft failure (1 case of platelets alone) treated with a second HSCT, compared with 0 in the other 3 quartiles ($P = .01$). Two of the affected patients had MF/MPD, and 1 patient had chronic myelomonocytic leukemia. Other cases of failed engraftment were attributed to a faulty product (n = 1) or other causes of NRM (n = 5).

GVHD

Incidence data for aGVHD and cGVHD are provided in Table 3 and Figure 1. Patients with Bu exposures below the median had more than twice the risk of developing grade II-IV aGVHD (HR, 2.30; 95% CI, 1.19 to 4.49; $P = .014$) compared with patients with higher Bu exposures, accounting for differences in risk group, donor matching, cell type, CMV status of donor or recipient, female-to-male HSCT, and a Hematopoietic Cell Transplantation Comobodity Index (HCT-CI) $\geq$1. A similar observation was made for grade III-IV aGVHD; however, the difference failed to reach statistical significance (HR, 2.25; 95% CI, 0.85 to 5.97; $P = .102$). There was no difference in the time to cGVHD across the quartiles.

NRM

At 3 years, NRM varied between 13% and 34% across the 4 groups (Table 4). Patients in the highest and lowest quartiles had a signifantly higher risk of NRM compared with patients with intermediate Bu exposures (SHR, 3.32; 95% CI, 1.46 to 7.54; $P = .004$) after adjustment for risk group, donor matching, cell type, CMV status, female-to-male HSCT, and HCT-CI $\geq$1. Moreover, the 3-year rate of NRM was significantly higher among those with the highest and lowest Bu exposures (outside a range of 3814 to 4993 μM/min) compared ot those with intermediate exposures (31% versus 15%; $P = .02$, Figure 2C). Table 5 lists the causes of nonrelapse death.

Relapse

Relapse rates did not appear to be affected by Bu exposure (Table 4 and Figure 3) even after adjustment for other risk factors.

Table 3
GVHD

Group (n)	aGVHD Grade II-IV, %	aGVHD Grade III-IV, %	cGVHD, %
1 (39)	40	18	65
2 (40)	39	18	65
3 (40)	16	5	59
4 (39)	22	11	61
1 and 2 (79)	39*	18†	65
3 and 4 (79)	19*	8†	60

* $P = .008$.
† $P = .07$.

DFS and OS

Patients in the middle 2 quartiles had better DFS (HR, 1.81; 95% CI, 1.09 to 2.99; $P = .021$) and OS (HR, 1.94; 95% CI, 1.12 to 3.37; $P = .018$) than those in the highest and lowest quartiles (Table 4 and Figure 2A and 2B), after adjustment for risk group, donor matching, cell type, CMV status, female-to-male HSCT, and HCT-CI $\geq$1.

DISCUSSION

Our results indicate that there may be a therapeutic window with better OS, DFS, and NRM at a daily Bu exposure between 3814 and 4994 μM/min following the study regimen. This conclusion is consistent with early studies of oral Bu with cyclophosphamide. In those studies, however,

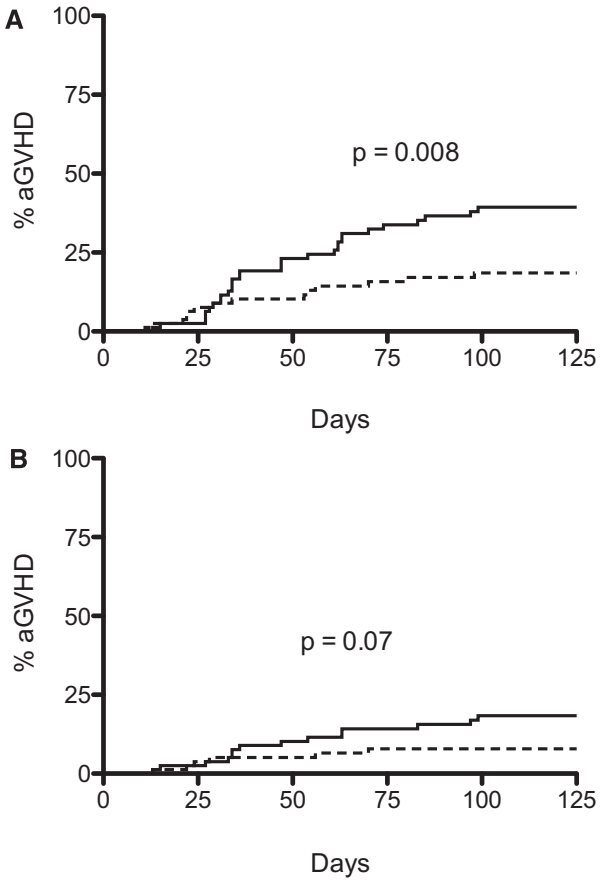

Figure 1. Kaplan-Meier plots of grade II-IV aGVHD (A) and grade III-IV aGVHD (B). Groups 1 and 2 (solid line) had a Bu AUC below the median, whereas groups 3 and 4 (dotted line) had a Bu AUC above the median.

Table 4
OS, DFS, NRM, and Relapse

Group (n)	OS, %	DFS, %	NRM, %	Relapse, %
1 (39)	61	54	32	21
2 (40)	72	64	17	22
3 (40)	71	70	13	19
4 (39)	56	40	34	38
1 and 4 (78)	61	47*	31*	29
2 and 3 (80)	79	67*	15*	21

* $P = .02$.

higher Bu exposures were associated with more regimen-related toxicity and lower Bu exposures were associated with more relapse and graft failure—outcomes that could be predicted on theoretical grounds [19-21]. Likewise, Andersson et al. [22] reported improved outvcomes in patients with CML receiving i.v. Bu every 6 hours with cyclophosphamide, with a per-dose AUC between ~950 and 1520 μM/min. Our conclusions are similar, but remain difficult to interpret completely.

Our finding of an increased incidence of aGVHD with lower Bu exposure was somewhat unexpected. More aGVHD was seen in both quartiles 1 and 2 and less in quartiles 3 and 4, so the observation seems unlikely to be an artifact. Whether an unbalanced representation of different diagnoses in the 4 groups could have contributed to this finding is hard to say, given the relatively small numbers involved. Experimental evidence suggests that more intensive conditioning will lead to more aGVHD via mechanisms involving cytokine release [23]. In general, reduced-intensity and nonmyeloablative conditioning is followed by less aGVHD compared with fully myeloablative regimens. More specifically, Andersson et al. [22], using a Bu-cyclophosphamide regimen for CML, found an increase in aGVHD and other toxicities with higher i.v. Bu exposures.

We have no evidence that Bu exposure is directly or indirectly associated with the activity of other components of the GVHD prophylaxis regimen. It is conceivable that lower exposure to Bu could allow the persistence of more host dendritic cells, which are known to be relatively resistant to cytotoxic agents and to contribute to aGVHD in animal models [24]. In a study of 109 patients with acute myelogenous leukemia (AML) treated with our Flu/Bu regimen and 400 cGy TBI, Lewis et al. [25] reported no significant

Table 5
Primary Causes of Nonrelapse Death

Group (n)	Death before Day 100 (n)*	Death after Day 100 (n)*	Total Deaths
1 (39)	Graft failure (1) GVHD-related (1) ARDS (1)	Graft failure (2) GVHD-related (5) Infection/second malignancy (1)	11
2 (40)		PTLPD (1) GVHD (4) Infection (1)	6
3 (40)	GVHD related (2) Infection/multiorgan failure (1) Cerebral hemorrhage (1)	Secondary graft failure/PTLPD (1)	5
4 (39)	Infection (4) PTLPD/infection (1) GVHD-related (1)	GVHD-related (4) Infection (1) ARDS (1)	12

ARDS indicates adult respiratory distress syndrome; PTLPD, post-transplantation lymphoproliferative disease.

* Includes identified opportunistic infection when death was GVHD-related.

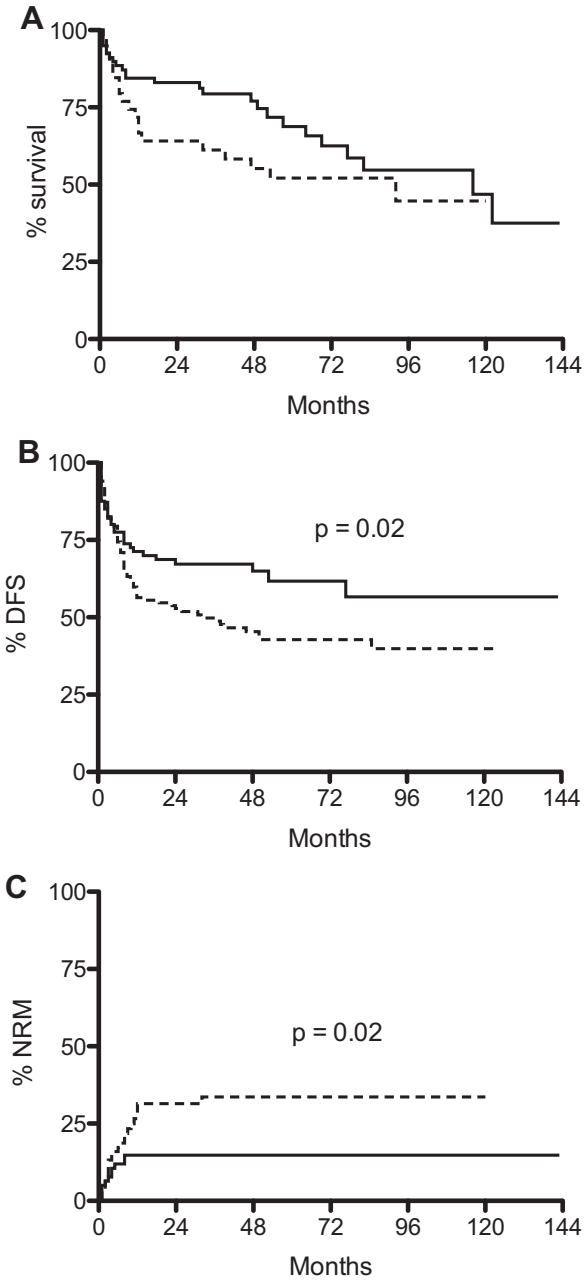

Figure 2. Kaplan-Meier plots of OS (A), DFS (B), and NRM (C). Groups 1 and 4 (dotted line) had a Bu AUC outside the range of 3814 to 4993 μM/min, whereas groups 2 and 3 (solid line) had a Bu AUC within that range.

difference in aGVHD with exposures above or below the median. This discrepancy would be consistent with the added effect of TBI on suppression of host dendritic cells in patients with the lower Bu exposures. It may be that our findings are relevant only with the conditioning and GVHD prophylaxis specific to this regimen, and that the diseases involved (AML versus other) may influence the outcome.

Examining the causes of NRM does not clearly explain the relationship of NRM to Bu exposure. The only cases of graft failure occurred in quartile 1, but the diseases treated also may have contributed to poor engraftment. The higher incidence of aGVHD in groups 1 and 2 is not reflected in aGVHD as the primary cause of NRM.

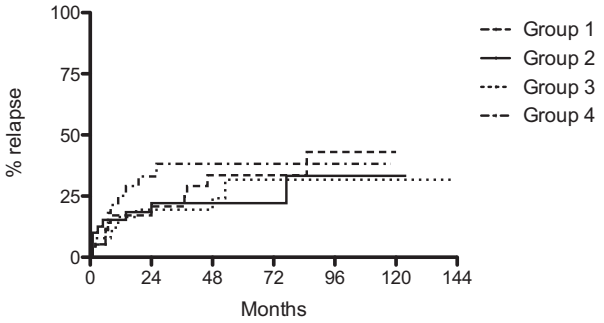

Figure 3. Kaplan-Meier plots of relapse.

Analysis of relapse is likely to be complicated by heterogeneity of diagnoses and some differences in exposures by disease. Moreover, the expectation that relapse should decrease with increased conditioning intensity is based in large part on studies of patients with AML, a condition not included in this analysis. We also know that relapse may be influenced by a graft-versus-malignancy (GVM) effect. Conceivably, a trend to less relapse with higher Bu exposures could be masked by a tendency for patients with lower exposures to experience more GVM, although GVM is usually attributable to cGVHD rather than aGVHD.

A previous study found significantly increased NRM at daily Bu exposures >6000 μM/min [12]. Below this level, no trend toward different levels of NRM was seen when the data were analyzed by, for example, quartiles as in the present study. The earlier patient group included those with acute leukemia receiving 400 cGy TBI in addition to Bu/Flu. TBI did not influence NRM in that study. When patients with AML were treated with additional TBI, NRM was significantly higher with Bu exposures above the median [25]. The discrepancies between these 2 studies might be explained by the smaller numbers of patients with and without AML in the initial series plus, perhaps, effects of TBI and/or the disease under investigation on outcomes.

We found that Bu exposures were lower in patients with MF/MPD and higher in those with non-Hodgkin lymphoma. Whether this finding relates simply to the disease per se, or also to other factors, such as previous chemotherapy, we cannot say. There appeared to be no impact of disease on NRM, although the numbers of individual diagnoses could be too low to allow detection of such an influence.

Our analysis did not examine outcomes over the whole range of exposures achieved when patients are given Bu doses based on weight, because of our data suggesting that exposures above 6000 μM/min may be harmful. However, only 9 patients had a dose adjustment to bring exposure below this level, and the conclusion that optimum exposure may be below 5000 μM/min is unlikely to be affected.

Other investigators have suggested that the safety threshold for daily Bu exposure with a Bu/Flu combination may be as high as 7500 μM/min [8]. This conclusion was arrived at by adjusting exposures on days 3 and 4 to successively higher levels rather than through a retrospective analysis. Whether the different conclusions are related to study design or the small numbers of patients experiencing high exposures in both studies is uncertain. There have been relatively few attempts at determining the optimum i.v. Bu exposure in diseases other than CML. Andersson et al. [26] reported less disease progression in patients with advanced AML with exposure targeted to 6000 μM/min compared with those with a fixed dose providing a median exposure of ~5000 μM/min. Toxicity was not increased with the higher exposure [26]. Others have reported that daily exposures below 6000 μM/min were well tolerated and effective in acute leukemias and, with rituxan, in lymphoma [9-11]; however, those studies used arbitrary (albeit rational) targets of Bu exposure rather than determining what an optimum range of exposure might be.

In conclusion, this study confirms the importance of TDM of i.v. Bu. Targets for Bu AUC may vary with other specific cytotoxic and immunosuppressive components of the conditioning protocol and also according to the disease being treated. Thus, it is difficult to state more than tentative conclusions until more data are available. Some of the shortcomings of this analysis could be overcome by prospective studies randomizing patients to receive different, targeted Bu exposures where the groups are balanced for diagnosis. For now, with the current regimen used under the conditions studied here, it seems reasonable to target daily exposure between 4440 and 4993 μM/min to minimize NRM and the morbidity of aGVHD.

ACKNOWLEDGMENTS

The authors thank the nursing staff at Foothills Hospital, Tom Baker Cancer Centre, and the Cross Cancer Institute for their care of these patients.

Financial disclosure: J.R. has received honoraria and grant support from Otsuka Pharma. There are no other conflicts of interest to report.

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