Bone Marrow Transplantation (2014) **49,** 628–633

www.nature.com/bmt

ORIGINAL ARTICLE

Allogeneic hematopoietic SCT for adults AML using i.v. BU in the conditioning regimen: outcomes and risk factors for the occurrence of hepatic sinusoidal obstructive syndrome

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I.v. BU is frequently used in the conditioning regimen prior to allogeneic hematopoietic SCT (allo-HSCT); however, overall outcomes, incidence of hepatic sinusoidal obstructive syndrome (SOS) and its risk factors are not well known. With this aim, we performed a study on 257 AML adult recipients. Seattle Criteria were used for diagnosis and classification of SOS. The median age was 44 years. Donors were HLA-identical siblings in 60%, HLA-matched unrelated in 29% and HLA mismatched in 11%. Conditioning regimen was myeloablative in 84% (i.v. BU with CY was the most frequently used regimen) and it was reduced intensity in 16% (i.v. BU associated with fludarabine). Acute and chronic GVHD was observed in 28% and 44%, respectively. Two-year incidence of non-relapse mortality was 16 ± 2% and 2-year leukemia-free survival for patients in CR1, CR2 and non remission at HSCT were 55 ± 4%, 58 ± 7%, and 20 ± 5%, respectively. At 6 months, incidence of SOS was 7.8 ± 2%; and it was severe in eight patients (3%). Factors associated with the occurrence of SOS were: HLA-mismatched donor HSCT ($P = 0.002$) and patients transplanted in non-remission ($P = 0.002$). In conclusion, outcomes of HSCT using i.v. BU are encouraging in this setting, SOS incidence is low and it is influenced by the type of donor and disease status at the time of transplant.

Bone Marrow Transplantation (2014) **49,** 628–633; doi:10.1038/bmt.2014.7; published online 17 February 2014

Keywords: i.v. BU; allogeneic stem cell transplantation; AML; hepatic SOS

INTRODUCTION

Allogeneic hematopoietic SCT (allo-HSCT) is a potentially curative treatment for a wide range of hematologic malignancies but is associated with a high risk of treatment-related complications.[1,2] BU is an alkylating agent that has been used since the 1950s. Currently, high-dose BU combined with CY is one of the most frequently used chemotherapeutic agents in preparative chemotherapy combination regimens for patients with AML. It can serve as an alternative to TBI in patients undergoing HSCT for various malignant and nonmalignant diseases.[3–6]

Oral BU has an erratic and unpredictable absorption with wide inter- and also intra-patient pharmacokinetic (PK) variability.[6] A high area under the curve for BU plasma concentration vs time is associated with a high risk of regimen-related toxicity and, in particular, venoocclusive disease (VOD) of the liver[4,7–9] (more recently called sinusoidal obstructive syndrome (SOS)) and non-relapse mortality (NRM). Conversely, low BU concentrations are associated with a higher risk of graft rejection[3,4,10,11] and leukemia relapse.[11] Monitoring of BU levels and dose adjustments can allow better control of the dose administered and reduction of these risks, yet in many patients, this cannot be easily achieved.[6,8,12,13] I.v. BU has been introduced into clinical use[14–18] and its main advantage over oral BU is that the former can be easily administered to patients. Also it has been reported to decrease the incidence of hepatic SOS compared with oral BU, therefore decreasing morbidity and mortality after allo-HSCT.[15,19–23]

The pathogenesis of SOS is thought to involve chemotherapy and radiation-induced damage to the sinusoidal endothelium, resulting in endothelial injury, microthrombosis, subendothelial damage and cytokine activation.[24,25] Severe SOS is typically associated with multi-organ failure and high mortality.[25] Some well-established risk factors are younger age, hepatic inflammation, previous abdominal irradiation, hepatic fibrosis or cirrhosis, myeloablation, use of gemtuzumab ozogamicin, alternative donor transplantation and advanced status of the disease at the time of transplant.[19,26] However those factors were mainly observed in series of HSCT recipients after using TBI or oral BU in the conditioning regimen.[27–30] In fact, incidence and risk factor analysis for SOS after conditioning regimes containing i.v. BU has not been described in a large and homogenous group of patients. With this aim and also to study overall outcomes of using i.v. BU, we have analyzed 257 allo-HSCT recipients with AML using i.v. BU in the conditioning regimen.

PATIENTS AND METHODS

Data collection, inclusion criteria and definitions

European Blood and Marrow Transplant is a voluntary working group of 605 transplant centers. Participating centers are required to report all

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Received 15 November 2012; revised 26 September 2013; accepted 26 September 2013; published online 17 February 2014

transplantations, consecutively and the compliance is monitored by on-site audits. Inclusion was based on the following criteria: confirmed diagnosis of AML; age $\geqslant$18 years; transplants performed between 2000 and 2005 and conditioning regimen containing i.v. BU. HLA-matched unrelated donors were defined with no difference at HLA-A, -B, -C and -DRB1 (8/8), all others were considered HLA mismatched. A specific questionnaire was used to collect dose of i.v. BU, and the European Blood and Marrow Transplant questionnaire was built using the definition of SOS based on the Seattle criteria (definition described below). For the patients with SOS according to the Seattle criteria, a questionnaire was sent with the aim to classify the patients according to Baltimore criteria (definition below). The transplant centers were asked to meet the following definition of SOS: a diagnosis of SOS was made when at least two of the following events developed within 100 days of transplantation—sudden weight gain (2% of baseline body weight, defined as the weight preceding the first dose of BU), hyperbilirubinemia (total serum bilirubin $\geqslant$2 mg/dL or $>$34 mmol/L) and hepatomegaly or right upper quadrant pain of hepatic orgin.[19] The signs and symptoms must have developed in the absence of other explanations. SOS was scored as mild, moderate or severe on the basis of previously published criteria.[24] Patients classified as having mild SOS had transient jaundice and weight gain that caused no apparent adverse effects and were not treated. Patients classified as having moderate SOS had fluid retention requiring diuretics or liver pain requiring analgesics, but signs and symptoms of SOS were completely reversible by day 100 post transplantation; that is, total serum bilirubin returned to 2 mg/dL and weight returned to baseline. Patients classified as having severe SOS had jaundice and fluid retention that was not reversible before death or by day 100 post transplantation. As described by Richardson *et al.*,[31] severe SOS was also defined when SOS progressed to multi-organ failure. Baltimore criteria was also used retrospectively to classify patients with SOS. Baltimore criteria was defined as having hyperbilirubinemia (total serum bilirubin $>$2 mg/dL or $>$34 mmol/L), plus two or more of the following: painful hepatomegaly, ascites or weight gain ($>$5% of baseline body weight).

Other end points definitions

Other outcomes studied were: (i) NRM, defined as all causes of nonleukemic deaths; (ii) relapse incidence was defined on the basis of morphological evidence of leukemia in BM or other extramedullary organs; (iii) leukemia-free survival (LFS) was defined as time interval between the transplant and the first event (either relapse or death in CR); and (iv) OS.

Statistical analysis

Cumulative incidence curves were used to estimate SOS, NRM, relapse incidence considering death without SOS, relapse or progression as competing events and Gray test was used for comparisons.[32] Probabilities of OS and LFS used the Kaplan–Meier estimate.[33] The log-rank test was used for univariate comparisons for the variables considered. Variables considered to study risk factors for SOS were: recipient age at time of transplantation, donor characteristics (type, age, gender and gender compatibility, HLA compatibility, CMV serology before transplant), transplant characteristics (type of conditioning regimen, dose of i.v. BU, use of antithymocyte globulin, year of transplant, disease status at the time of transplant, source of stem cells). All factors found to influence the outcomes in univariate analysis with a *P*-value $<$0.10 were included in the multivariate model. The influence of SOS on NRM, OS and LFS was analyzed as a time-dependent covariate. All tests were two sided. Statistical analysis was performed using SPSS software (Version 18.0, SPSS, Chicago, IL, USA) and R package 2.7.1 (R Development Core Team, Vienna, Austria).

Table 1. Patient, disease, donor and transplantation characteristics

No. of patients	n = *257*
Median patient age (range)	44 years (17–67)
Patient gender	
Male	153 (53%)
Median year of HSCT	2004 (2000–2005)
Status of transplantation n (%):	
CR1	134 (52%)
CR2	48 (18%)
Advanced phase	75 (30%)
Median WBC at diagnosis (10^9/L)	12.1 (0.4–536)
Cytogenetics risk n (%):	
Good	21 (11%)
Intermediate	148 (77%)
Poor	25 (12%)
Donor type n (%):	
HLA-identical sibling	153 (60%)
HLA-mismatched related donor	2 (1%)
HLA-matched unrelated donor	75 (29%)
HLA-mismatched unrelated donor	27 (10%)
Stem cell source n (%):	
BM	46 (18%)
Peripheral blood	206 (80%)
Cord blood	5 (2%)
Median donor age (range)	39 years (1–75)
Donor gender	
Male	142 (56%)
Female to Male	55 (22%)
Positive CMV serology prior HSCT:	
Recipient's	188 (75%)
Donor's	149 (62%)
GVHD prophylaxis, n (%):	
CsA + MTX	107 (42%)
CsA + MMF	11 (4%)
T-cell depletion *in vivo*	89 (35%)
T-cell depletion *in vitro*	41 (15%)
Unknown	10 (4%)
Conditioning regimen:	
Myeloablative	216 (84%)
I.v. BUCY	138 (64%)
I.v. BUCY + VP16	23 (10%)
I.v. BUFLU	55 (26%)
Reduced intensity	41 (16%)
I.v. BUFLU	38 (93%)
I.v. BUCY	3 (7%)

Abbreviations: I.v. BUCY = intravenous busulfan + cyclophosphamide; I.v. BUFLU = intravenous busulfan + fludarabine; MAC = myeloablative; MMF = mycophenolate mofetil; PB = Peripheral blood; RIC = reduced-intensity conditioning; VP16 = ectoposide.

RESULTS

Patients' characteristics

Data of 257 adult AML patients undergoing i.v. BU-based conditioning before allo-HSCT are reported. The main clinical characteristics of the patients are reported in Table 1. The median age was 44 years (17–67), 53% of the patients were male and the median transplant year was 2004 (2000–2005). In all, 134 patients (52%) were transplanted in CR1, 48 (18%) in CR2 and two (1%) in CR3 and the remaining 30% in non-remission. Intermediate-risk AML was observed in 77% of patients (as per cytogenetic criteria), whereas 11% and 12% had good risk and poor risk disease, respectively. Median follow-up was 26 months (range 1–70 months). Conditioning regimen was myeloablative chemotherapy (MAC) in 84% and reduced-intensity conditioning (RIC) in 16% of the allo-HSCT. Of MAC-treated patients (considering the dose of i.v. BU more than 6.4 mg/kg), conditioning regimen consisted of i.v. BU and CY in 64%, 10% received BU and CY together with VP16 and 26% of the patients were treated with i.v. BU and fludarabine. The median total i.v. BU dose in the MAC was 12.6 mg/kg (range: 6.4–15). Almost all the allo-HSCT patients (93%) receiving RIC conditioning were treated with BU and fludarabine regimen. The median total i.v. BU dose in RIC was (5.9 mg/kg). Donors were 60% HLA-identical siblings, 29% matched unrelated, 10% mismatched

unrelated and in 1% of the cases a mismatched relative donors. The GVHD prophylaxis consisted of CSA and MTX in 42% of transplants.

SOS incidence, risk factors and influence on outcomes

Cumulative incidence of hepatic SOS (using Seattle criteria) at 6 months was 7.8 ± 2% ($n = 20$ patients). All 20 patients had bilirubin ⩾2 mg/dL (or ⩾34 mmol/L), 11 had right upper quadrant pain, six had hepatomegaly confirmed by ultrasound, three patients had hepatomegaly without pain, and all patients had unexplained weight gain of (>2% basal weight).

Previously, mylotarg was not given to patients presenting SOS. Baltimore criteria were available in 17 of the 20 patients, and using these criteria, 12 patients had SOS. Cumulative incidence of SOS using the Baltimore criteria was 5%. As this was a retrospective assignment, all data pertaining to SOS are based on Seattle criteria. Median day of diagnosis of SOS was 11 days (range, 5–62). Severe SOS was observed in eight out of 20 patients (40%) and an overall SOS-associated mortality rate of 15%, and 38% within the group of patients with severe SOS. Interestingly, seven of the eight patients with severe VOD were treated with supportive care only, of whom five patients survived.

According to Richardson's criteria of severity, three patients had severe SOS and died of multi organ failure. However using Seattle criteria, eight patients had severe SOS, and only one patient was alive after treatment with defibrotide.

Interestingly, six patients developed SOS after day 21, four patients received MAC regimen and two patients received RIC (both used fludarabine associated to i.v. BU). In univariate analysis (Table 2), SOS was more frequently diagnosed in patients who received a transplant from donors other than HLA-matched siblings ($P = 0.004$). It was also associated with advanced disease status at the time of transplantation: it was 4.4% in patients transplanted in remission compared with 16.3% in those transplanted with primary refractory disease or relapse ($P = 0.001$). The incidence of SOS following MAC was not statistically higher after receiving RIC ($P = 0.42$). In multivariate analyses, factors associated with SOS were: HLA-mismatched donors (Hazard ratio (HR): 6.25; 95% confidence interval (CI): 1.99–16.6; $P = 0.002$) (Table 3) and patients transplanted in non remission (HR: 4; 95% CI: 1.69–10; $P = 0.002$). The occurrence of SOS as a time-dependent covariate in a multivariate analysis was associated with higher NRM (HR: 3.3; 95% CI: 1.16–9.44; $P = 0.03$) but not with OS ($P = 0.94$)

Other outcomes

Engraftment and graft-vs host disease. The median days for neutrophil recovery $>0.5 \times 10^9$/L and platelet recovery $>20 \times 10^9$/L was 14 days[4–45] and 15 days (8–383), respectively. Full donor chimerism was observed in 70% of the transplanted patients and 28.3% had mixed chimerism.

Overall, 119 patients suffered of acute GVHD. Seventy one patients (28%) had Grade II–IV GVHD without significant differences between the donor graft characteristics. Out of 221 patients who were alive at day 100, 98 patients (44%) had chronic GVHD. Altogether, the 2-year cumulative incidence of chronic GVHD was 37 ± 3%.

Relapse incidence and LFS. LFS for patients transplanted at CR1 and CR2 was 55 ± 4% and 58 ± 7%, respectively and it was 20 ± 5% for patients in advanced disease. Relapse incidence for patients transplanted at CR1 and CR2 was 28 ± 4% and 22 ± 6%, respectively and it was 68 ± 6% for patients in advanced disease. There were no differences in outcomes according to conditioning regimen (Table 4).

NRM, OS and causes of death. Overall, 2-year cumulative incidence of NRM was 17 ± 3% and 20 ± 6% for patients

Table 2. Risk factors and CI at 1 year of hepatic SOS after HSCT using i.v. BU in the conditioning regimen

	n *(%)*	*1-year CI (%) ± s.d.*	P-*value*
1-year CI	8.4 ± 2%		
By conditioning			
MAC	18/208 (9%)	8.4 ± 2	0.42
RIC	2/49 (4%)	5 ± 3	
By transplant			
HLA-Sib	6/153 (4%)	3.9 ± 1	0.004
HLA-MUD	8/75 (11%)	10.8 ± 4	
HLA-MMUD	6/29 (21%)	21.4 ± 8	
By status			
CR1	4/146 (3%)	CR: 4.4 ± 1	0.001
CR > 2	4/48 (8%)		
Relapse	7/48 (15%)	No CR: 16.3 ± 4	
Primary refractory	5/26 (19%)		

Abbreviations: CI = cumulative incidence; Id Sib = Identical siblings; MAC = myeloablative chemotherapy; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; RIC = reduced-intensity conditioning.

Table 3. Multivariate analysis for SOS

	HR	*95% CI*	P-*value*
MUD vs MSD	2.49	0.87–7.16	0.9
MUD vs MMUD	6.25	1.99–19.6	0.002
No CR vs CR	4	0.69–10	0.002

Abbreviations: CI = confidential interval; HR = hazard ratio; MAC = myeloablative; MMUD = mismatched unrelated donor; MUD = matched unrelated donor; RIC = reduced-intensity conditioning.

Table 4. Overall results: 2-year cumulative incidence of NRM, RI and probability of LFS in 269 patients with AML according to disease status, type of conditioning regimen and donor type

	100-day NRM ± s.d. (%)	*2-year NRM ± s.d. (%)*	*2-year RI ± s.d. (%)*	*2-year LFS ± s.d. (%)*
All patients	5 ± 1	16 ± 2	38 ± 3	46 ± 3
Status at allo-HSCT				
CR1	4 ± 2	17 ± 3	28 ± 4	54 ± 4
CR2	9 ± 1	20 ± 6	22 ± 6	58 ± 7
Advanced	4 ± 2	12 ± 4	68 ± 6	20 ± 5
By conditioning				
MAC	6 ± 2	16 ± 3	38 ± 3	46 ± 3
RIC	0	15 ± 6	43 ± 8	42 ± 8
By donor				
Id Sib	3 ± 2	16 ± 4	34 ± 4	49 ± 4
HLA-MUD	7 ± 2	11 ± 4	49 ± 8	40 ± 6
HLA-MM	7 ± 5	26 ± 9	34 ± 10	39 ± 10

Abbreviations: Allo-HSCT = Allogeneic hematopoietic SCT; Id Sib = Identical siblings; MAC = myeloablative; MM = Mismatch (either related or unrelated); MUD = matched unrelated donor; NRM = non relapse mortality; RI = relapse incidence; RIC = reduced-intensity conditioning.

transplanted at CR1 and CR2, respectively, and it was 12 ± 4% for patients in advanced disease. With median follow-up of 26 months (range, 3–70), 51% of the patients were alive with 45% of the patients in CR, whereas 49% (125 patients) had died. Out of the patients who died, 72 patients (59%) had disease relapse, 21 patients (17%) died of GVHD and 14% died of posttreatment infections. Death related to organ toxicity was observed in 13 patients (10%) including SOS in three patients, cardiac toxicity and interstitial pneumonitis.

DISCUSSION

In this study we aimed to assess the incidence and risk factors for SOS and to make a survey on the outcomes of 257 AML patients who underwent allo-HSCT using i.v. BU-based conditioning.

Our results demonstrate a low incidence of hepatic SOS compared with previous reports pertaining to oral BU.[27–29] One could argue that we have used the Seattle classification of SOS instead of the Baltimore classification that is more appropriate for adults. However, our study is a multicenter-based registry analysis and Seattle criteria were previously established in the questionnaire forms. We have revised the Baltimore criteria in the 17 out of 20 patients who had SOS and in fact SOS was observed in 12 patients, representing a cumulative incidence of 5%. The difference in the incidence of SOS between Seattle and Baltimore criteria was recently described by Carreras E *et al.*,[19] who have found a cumulative incidence for SOS of 13.8% and 8.8% using the Seattle and the Baltimore diagnostic criteria, respectively in 845 allo-HSCTs over a period of 24 years. The limitations of this study are the use of Seattle criteria and performing a multicenter retrospective analysis. However, our findings that HLA-mismatched donor HSCT and patients transplanted in non-remission are similar to factors associated with SOS after allo-HSCT with other pre-HSCT conditioning regimen.

Toxicity of the preparative regimen is a major limiting factor in HSCT. Regimens using only chemotherapy, instead of irradiation-based regimens, have been developed to minimize these toxicities. Nevertheless, early toxicities are an important problem with BU-containing regimens in particular, in SOS of the liver.[4,7–9] Therefore, the benefit of allo-HSCT therapy for treating AML is offset by high rates of organ toxicities and patients' morbidity. In 1991, Morgan *et al.*[30] analyzed the pretransplant conditioning-related toxicity in 233 patients transplanted for acute or chronic leukemia with an HLA-identical sibling donor comparing the toxicity of 67 patients that received the BU–CY preparation vs 166 patients that received TBI–CY conditioning. VOD appeared to be higher in the BU–CY group (19% vs 13%, $P<0.0005$). There was a trend toward a higher mortality from a nonleukemic cause in the BU group.[30]

In contrast, i.v. BU has shown a more predictable PK and favorable toxicity profile. Anderson *et al.*[15] compared incidence of VOD and VOD-related mortality with P.O. BU/CY ($n=61$) vs i.v. BU/CY ($n=30$) conditioning regimens in heavily pretreated patients. In multivariate analysis, it was demonstrated that the use i.v. compared with oral BU was the strongest predictor for VOD (33% vs 8%) and VOD-related mortality (20% vs 3.3%), respectively (HR − 7.5%, 95% CI (2.1–27.2%); $P<0.002$). Day 100 OS was also significantly higher in the i.v. BU group vs the PO BU group 13% vs 33%, respectively ($P<0.02$). We have to be aware that some differences in the incidence of SOS observed in the oral BU era could be in part due to the lack of knowledge on the high-resolution matching between donors and recipients. In our analysis, 11% of the transplants were HLA mismatched and this group of patients had a higher incidence of SOS, showing that HLA-mismatched transplant recipients are at a risk of developing SOS.

Similar results were observed in a Center for International Blood and Marrow Transplant Research study comparing i.v. BU/CY with PO BU/CY conditioning regimens. Logistic regression analyses showed that only the mode of BU administration was a significant factor for the risk of VOD. I.v. BU was associated with a greatly reduced risk ($P<0.004$).VOD incidence was 4.6% vs 20.3% ($P<0.001$) and day 100 mortality was 8.7% vs 22.5%, in patients treated with i.v. BU vs oral BU, respectively ($P<0.015$).[34]

This retrospective-based analysis revealed a SOS incidence of 7.8 ± 2% (using Seattle criteria) and 5% (using Baltimore criteria) with an incidence of severe SOS in eight out of 20 patients (40%) and an overall SOS-associated mortality rate of 15%, and 38% within the group of patients with severe SOS. Interestingly, seven of eight patients with a severe VOD were treated with supportive care only, of whom five survived. This is unusual as the overall mortality in the pre-defibrotide era is over 80%. The survey data is comparable with recent results despite unfavorable risk factors, and SOS incidence was lower than what was reported with oral BU, which is in line with previous publications. Advanced disease status and mismatched transplants at HSCT were the main risk factors for SOS ($P<0.05$). However, in spite of the low incidence of SOS, its occurrence is still associated with higher mortality. It was interesting to note that in spite of median days of onset of SOS at 11 days, there were six patients presenting late SOS (after day + 21), as it has been described recently with i.v. BU.[25]

Hasegawa *et al.*[26] reviewed 140 children with hematologic malignancies that underwent allogeneic BMT to clarify the incidence, onset time and risk factors for VOD of the liver. Multivariate analysis showed that low serum albumin levels ($\leqslant 3.7$ g/dL) before the start of pretransplant conditioning and donor mismatch (other than HLA-matched relatives) were most significantly associated with the development of SOS.

Obviously, the occurrence of SOS in this population can also be attributed to other concomitant hepatotoxic drugs like CY and others.[35,36] Similarly, genetic predisposition likely has some role in the occurrence of SOS owing to the presence of pharmacogenetic polymorphisms that will be associated with lower or increased metabolism of chemotherapeutic drugs.[37–39]

In this retrospective-based registry analysis, we have also reported the overall outcomes of 257 AML patients given a HSCT with a conditioning regimen containing i.v. BU. The rate of severe GVHD in our survey, considering the relatively large number of unrelated and mismatched transplants (40%), seems reduced. This was also suggested in other studies.[21–23,40–42] Acute GVHD is related in part to tissue injury and cytokine release,[43,44] and limitation of tissue injury with this regimen may have contributed to this observation. However, severe acute GVHD remains a major obstacle to transplant success and better methods for GVHD prevention with preservation of antileukemic effects need to be explored.

The global 100-day NRM was remarkably low (5%), despite the presence of patients transplanted with a graft from a mismatched unrelated donor. These favorable results are confirmed at 2 years with a NRM incidence of only 16%.

Disease recurrence remains the major cause of treatment failure. The status of disease at the time of transplantation was the major predictor for relapse. The 2-year projected relapse risk ranged from 28% in early leukemia to 68% in refractory advanced AML.

Altogether, the 2-year OS and LFS rates in this study were 53% and 46%, respectively. These results despite the heterogeneity of the patient group, including different type of conditioning regime, type of donor and disease status the overall results using i.v. BU are very encouraging. The improved outcome is probably related to the more predictable PK and favorable toxicity profile related to i.v. BU. Retrospective and prospective studies comparing outcomes of conditioning regimens containing i.v. BU with TBI in patients with acute leukemia are ongoing and will probably provide sufficient data, showing decreased NRM using i.v. BU.

Another limitation of our study is the lack of PK of i.v. BU[16,22,42] as there are many centers in Europe where BU levels are not

measured. The lack of measurement could impact the outcomes. However, because of the more predictable absorption and less variability with i.v. BU[14,15,18] the PK of i.v. BU and monitoring of plasma levels are less crucial using i.v. BU vs oral BU. Recently, Veal *et al.*[45] reported that, in a pediatric population study in which PK analyses on BU were performed, 87% of the i.v. BU patients achieved values within the target of 900–1500 μM/min vs only 56% of patients following oral BU. Nevertheless, it is remarkable that the quoted literature, although mostly in very small uncontrolled trials, showed a three–fivefold reduction of the incidence of SOS. Considering the difference of 87% vs 56% incidence in reaching target values, differences in the incidence of SOS need to be viewed critically.

Finally, in view of our results, despite the fact that the use of i.v. BU seems to reduce the SOS incidence, clinicians should be aware of the importance of some risk factors with the aim to diagnose promptly and use drugs like defibrotide in patients with a high risk of developing SOS.[34,46]

In conclusion, in this registry-based study of AML patients conditioned with i.v. BU-based regimens in a rather large cohort, we were able to demonstrate low hepatic SOS incidence, while risk factors being HLA-mismatched allogeneic transplantations and not being in remission. However, relatively reduced rate of hepatic SOS is still correlated with high mortality rates. Therefore, preventive measures and prophylaxis are of major importance regardless of the conditioning used.

CONFLICT OF INTEREST

M. Mohty, A. Nagler and V. Rocha received lecture honoraria from Pierre-Fabre Medicament whose product is discussed in this paper.

ACKNOWLEDGEMENTS

This study was supported by an unrestricted educational grant from Pierre-Fabre Medicament. Vanderson Rocha was supported, in part, by the National Institute for Health Research (NIHR), Oxford Biomedical Research Center at Oxford University Hospitals NHS Trust and University of Oxford. We thank all European Blood and Marrow Transplant centers and national registries for contributing patients to the study (Supplement) and Data Managers for their superb work.

REFERENCES

1 Thomas E, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE *et al.* Bone-marrow transplantation (first of two parts). *N Engl J Med* 1975; **292**: 832–843.
2 Armitage JO. Bone marrow transplantation. *N Engl J Med* 1994; **330**: 827–838.
3 Bolinger AM, Zangwill AB, Slattery JT, Glidden D, DeSantes K, Heyn L *et al.* An evaluation of engraftment, toxicity and busulfan concentration in children receiving bone marrow transplantation for leukemia or genetic disease. *Bone Marrow Transplant* 2000; **25**: 925–930.
4 Slattery JT, Sanders JE, Buckner CD, Schaffer RL, Lambert KW, Langer FP *et al.* Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. *Bone Marrow Transplant* 1995; **16**: 31–42.
5 Tran HT, Madden T, Petropoulos D, Worth LL, Felix EA, Sprigg-Saenz HA *et al.* Individualizing high-dose oral busulfan: prospective dose adjustment in a pediatric population undergoing allogeneic stem cell transplantation for advanced hematologic malignancies. *Bone Marrow Transplant* 2000; **26**: 463–470.
6 Hassan M. The role of busulfan in bone marrow transplantation. *Med Oncol* 1999; **16**: 166–176.
7 Dix SP, Wingard JR, Mullins RE, Jerkunica I, Davidson TG, Gilmore CE *et al.* Association of busulfan area under the curve with veno-occlusive disease following BMT. *Bone Marrow Transplant* 1996; **17**: 225–230.
8 Grochow LB, Jones RJ, Brundrett RB, Braine HG, Chen TL, Saral R *et al.* Pharmacokinetics of busulfan: correlation with veno-occlusive disease in patients undergoing bone marrow transplantation. *Cancer Chemother Pharmacol* 1989; **25**: 55–61.
9 Ljungman P, Hassan M, Bekassy AN, Ringden O, Oberg G. High busulfan concentrations are associated with increased transplant-related mortality in allogeneic bone marrow transplant patients. *Bone Marrow Transplant* 1997; **20**: 909–913.
10 McCune JS, Gooley T, Gibbs JP, Sanders JE, Petersdorf EW, Appelbaum FR *et al.* Busulfan concentration and graft rejection in pediatric patients undergoing hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2002; **30**: 167–173.
11 Slattery JT, Clift RA, Buckner CD, Radich J, Storer B, Bensinger WI *et al.* Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. *Blood* 1997; **89**: 3055–3060.
12 Bolinger AM, Zangwill AB, Slattery JT, Risler LJ, Glidden DV, Sultan DH *et al.* Target dose adjustment of busulfan in pediatric patients undergoing bone marrow transplantation. *Bone Marrow Transplant* 2001; **28**: 1013–1018.
13 Deeg HJ, Storer B, Slattery JT, Anasetti C, Doney KC, Hansen JA *et al.* Conditioning with targeted busulfan and cyclophosphamide for hemopoietic stem cell transplantation from related and unrelated donors in patients with myelodysplastic syndrome. *Blood* 2002; **100**: 1201–1207.
14 Andersson BS, Gajewski J, Donato M, Giralt S, Gian V, Wingard J *et al.* Allogeneic stem cell transplantation (BMT) for AML and MDS following i.v. busulfan and cyclophosphamide (i.v. BuCy). *Bone Marrow Transplant* 2000; **25**: S35–S38.
15 Andersson BS, Kashyap A, Gian V, Wingard JR, Fernandez H, Cagnoni PJ *et al.* Conditioning therapy with intravenous busulfan and cyclophosphamide (IV BuCy2) for hematologic malignancies prior to allogeneic stem cell transplantation: a phase II study. *Biol Blood Marrow Transplant* 2002; **8**: 145–154.
16 Andersson BS, Madden T, Tran HT, Hu WW, Blume KG, Chow DS *et al.* Acute safety and pharmacokinetics of intravenous busulfan when used with oral busulfan and cyclophosphamide as pretransplantation conditioning therapy: a phase I study. *Biol Blood Marrow Transplant* 2000; **6**: 548–554.
17 Schuler US, Renner UD, Kroschinsky F, Johne C, Jenke A, Naumann R *et al.* Intravenous busulphan for conditioning before autologous or allogeneic human blood stem cell transplantation. *Br J Haematol* 2001; **114**: 944–950.
18 Shimoni A, Bielorai B, Toren A, Hardan I, Avigdor A, Yeshurun M *et al.* Intravenous busulfan-based conditioning prior to allogeneic hematopoietic stem cell transplantation: myeloablation with reduced toxicity. *Exp Hematol* 2003; **31**: 428–434.
19 Carreras E, Díaz-Beyá M, Rosiñol L, Martínez C, Fernández-Avilés F, Rovira M. The incidence of veno-occlusive disease following allogeneic hematopoietic stem cell transplantation has diminished and the outcome improved over the last decade. *Biol Blood Marrow Transplant* 2011; **17**: 1713–1720.
20 Thall PF, Champlin RE, Andersson BS. Comparison of 100-day mortality rates associated with i.v. busulfan and cyclophosphamide vs other preparative regimens in allogeneic bone marrow transplantation for chronic myelogenous leukemia: Bayesian sensitivity analyses of confounded treatment and center effects. *Bone Marrow Transplant* 2004; **33**: 1191–1199.
21 Andersson BS, de Lima M, Thall PF, Wang X, Couriel D, Korbling M *et al.* Once daily i.v. busulfan and fludarabine (i.v. Bu-Flu) compares favorably with i.v. busulfan and cyclophosphamide (i.v. BuCy2) as pretransplant conditioning therapy in AML/MDS. *Biol Blood Marrow Transplant* 2008; **14**: 672–684.
22 de Lima M, Couriel D, Thall PF, Wang X, Madden T, Jones R *et al.* Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. *Blood* 2004; **104**: 857–864.
23 Perkins JB, Kim J, Anasetti C, Fernandez HF, Perez LE, Ayala E *et al.* Maximally tolerated busulfan systemic exposure in combination with fludarabine as conditioning before allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2012; **18**: 1099–1107.
24 McDonald GB, Hinds MS, Fisher LD, Schoch HG, Wolford JL, Banaji M *et al.* Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. *Ann Intern Med* 1993; **118**: 255–267.
25 Pai RK, van Besien K, Hart J, Artz AS, O'Donnell PH. Clinicopathologic features of late-onset veno-occlusive disease/sinusoidal obstruction syndrome after high dose intravenous busulfan and hematopoietic cell transplant. *Leuk Lymphoma* 2012; **53**: 1552–1557.
26 Hasegawa S, Horibe K, Kawabe T, Kato K, Kojima S, Matsuyama T *et al.* Veno-occlusive disease of the liver after allogeneic bone marrow transplantation in children with hematologic malignancies: incidence, onset time and risk factors. *Bone Marrow Transplant* 1998; **22**: 1191–1197.
27 Blaise D, Maraninchi D, Archimbaud E, Reiffers J, Devergie A, Jouet JP *et al.* Allogeneic bone marrow transplantation for acute myeloid leukemia in first remission: a randomized trial of a busulfan-Cytoxan versus Cytoxan-total body irradiation as preparative regimen: a report from the Group d'Etudes de la Greffe de Moelle Osseuse. *Blood* 1992; **79**: 2578–2582.
28 Ringden O, Ruutu T, Remberger M, Nikoskelainen J, Volin L, Vindelov L *et al.* A randomized trial comparing busulfan with total body irradiation as conditioning in allogeneic marrow transplant recipients with leukemia: a report from the Nordic Bone Marrow Transplantation Group. *Blood* 1994; **83**: 2723–2730.

29 Ferry C, Socie G. Busulfan-cyclophosphamide versus total body irradiation-cyclophosphamide as preparative regimen before allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia: what have we learned? *Exp Hematol* 2003; **31**: 1182–1186.
30 Morgan M, Dodds A, Atkinson K, Szer J, Downs K, Biggs J. The toxicity of busulphan and cyclophosphamide as the preparative regimen for bone marrow transplantation. *Br J Haematol* 1991; **77**: 529–534.
31 Richardson PG, Soiffer RJ, Antin JH, Uno H, Jin Z, Kurtzberg J *et al.* Defibrotide for the treatment of severe hepatic veno-occlusive disease and multiorgan failure after stem cell transplantation: a multicenter, randomized, dose-finding trial. *Biol Blood Marrow Transplant* 2010; **16**: 1005–1017.
32 Gooley TA, Leisenring W, Cullis JO, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med* 1999; **18**: 695–706.
33 Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assos* 1958; **53**: 457–481.
34 Sobocinski K, Thall P, Bekele B, Klein J, Lennon S, Horwitz M *et al.* Matched pairs analysis of IV vs PO Busulfan as a conditioning agent prior to transplantion. Blood. *104 Abs* 2004; **349.**
35 Johnson DB, Savani BN. How can we reduce hepatic veno-occlusive disease-related deaths after allogeneic stem cell transplantation? *Exp Hematol* **40**: 513–517 2012.
36 Suyanı E, Altındal Ş, Akı Ş, Sucak GT. Does fludarabine increase the incidence of sinusoidal obstruction syndrome when combined with Bu/Cy during conditioning? *Clin Transplant* **26**: E85–E86 2012.
37 Rocha V, Porcher R, Fernandes JF, Filion A, Bittencourt H, Silva Jr W *et al.* Association of drug metabolism gene polymorphisms with toxicities, graft-versus-host disease and survival after HLA-identical sibling hematopoietic stem cell transplantation for patients with leukemia. *Leukemia* 2009; **23**: 545–556.
38 Ritter CA, Sperker B, Grube M, Dressel D, Kunert-Keil C, Kroemer HK. Overexpression of glutathione S-transferase A1-1 in ECV 304 cells protects against busulfan mediated G2-arrest and induces tissue factor expression. *Br J Pharmacol* 2002; **137**: 1100–1106.
39 Bredschneider M, Klein K, Mürdter TE, Marx C, Eichelbaum M, Nüssler AK *et al.* Genetic polymorphisms of glutathione S-transferase A1, the major glutathione S-transferase in human liver: consequences for enzyme expression and busulfan conjugation. *Clin Pharmacol Ther* 2002; **71**: 479–487.
40 Alatrash G, de Lima M, Hamerschlak N, Pelosini M, Wang X, Xiao L *et al.* Myeloablative reduced-toxicity i.v. busulfan-fludarabine and allogeneic hematopoietic stem cell transplant for patients with acute myeloid leukemia or myelodysplastic syndrome in the sixth through eighth decades of life. *Biol Blood Marrow Transplant* 2011; **17**: 1490–1496.
41 Andersson BS, de Lima M, Thall PF, Madden T, Russell JA, Champlin RE. Reduced-toxicity conditioning therapy with allogeneic stem cell transplantation for acute leukemia. *Curr Opin Oncol* 2009; **21**: S11–S15.
42 Almog S, Kurnik D, Shimoni A, Loebstein R, Hassoun E, Gopher A *et al.* Linearity and stability of intravenous busulfan pharmacokinetics and the role of glutathione in busulfan elimination. *Biol Blood Marrow Transplant* 2011; **17**: 117–123.
43 Ferrara JL. Pathogenesis of acute graft-versus-host disease: cytokines and cellular effectors. *J Hematother Stem Cell Res* 2000; **9**: 299–306Review.
44 Harris AC, Ferrara JL, Braun TM, Holler E, Teshima T, Levine JE *et al.* Plasma biomarkers of lower gastrointestinal and liver acute GVHD. *Blood* 2012; **22**: 2960–2963.
45 Veal GJ, Nguyen L, Paci A, Riggi M, Amiel M, Valteau-Couanet D *et al.* Busulfan pharmacokinetics following intravenous and oral dosing regimens in children receiving high-dose myeloablative chemotherapy for high-risk neuroblastoma as part of the HR-NBL-1/SIOPEN trial. *Eur J Cancer* 2012; **48**: 3063–3072.
46 Corbacioglu S, Cesaro S, Faraci M, Valteau-Couanet D, Gruhn B, Rovelli A *et al.* ValtDefibrotide for prophylaxis of hepatic veno-occlusive disease in paediatric haemopoietic stem-cell transplantation: an open-label, phase 3, randomised controlled trial. *Lancet* 2012; **379**: 1301–1309.