CLINICAL RESEARCH

Unpredictability of Intravenous Busulfan Pharmacokinetics in Children Undergoing Hematopoietic Stem Cell Transplantation for Advanced Beta Thalassemia: Limited Toxicity with a Dose-Adjustment Policy

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β-thalassemia is a major health problem worldwide, and stem cell transplantation (SCT) is the only curative option. Oral Busulfan (Bu) based conditioning is widely used in this setting. Due to the variability of Bu systemic exposure, intravenous (i.v.) Bu has been proposed as a standard of care, with no need for drug monitoring and dose adjustment. Patients with β-thalassemia from countries with limited resources might be at higher risk of erratic Bu metabolism because of liver dysfunction, severe iron overload, and specific ethnic/genetic features. We studied Bu pharmacokinetics in 53 children with advanced β-thalassemia from Middle Eastern countries who underwent a total of 57 matched related donor SCTs. Forty-two percent of the children required dose adjustment because they did not achieve the therapeutic window after the first dose. With a Bu dose-adjustment policy, regimen-related toxicity was limited. At a median follow-up of 564 days, the probabilities of 2-year survival, current thalassemia-free survival, rejection, and treatment-related mortality were 96%, 88%, 21%, and 4%, respectively. Conditioning with i.v. Bu and dose adjustment is feasible and well tolerated, although recurrence of thalassemia remains an unsolved problem in children with advanced disease.

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KEY WORDS: Hemoglobinopathies, Conditioning regimen, Regimen-related toxicity

INTRODUCTION

β-thalassemia is the most frequent monogenic syndrome causing major health problems worldwide. The global estimated annual birth incidence is 40,000/year, with the highest rates in the Mediterranean, Middle East and South Asia [1]. The prognosis for β-thalassemia with conventional therapy has improved dramatically, and most children now reach adulthood with an open-ended survival. Nevertheless, endocrine, cardiac, and hepatic complications of iron overload still occur, affecting quality of life and representing the principal causes of death [2]. Children living in countries with limited resources may have impaired access to safe blood products and iron-chelating drugs, leading to a life expectancy of <20 years [1]. However, in the risk/benefit balance between stem cell transplantation (SCT) and medical care, severely ill patients with organ dysfunction also are at greater risk for regimen-related toxicity (RRT) [3].

Busulfan (Bu) is an ablative alkylating agent used in conditioning regimens for β-thalassemia, mainly in concert with cyclophosphamide (Cy) [4]. The use of oral Bu in the pediatric population is characterized by extensive absorption variations, with wide interpatient and intrapatient variability in systemic exposure,

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resulting in overexposure (eg, veno-occlusive disease [VOD], mucositis) or underexposure (eg, graft rejection) [5]. Intravenous (i.v.) Bu (Busilvex) has been introduced for clinical use with a new body weight–based dose calculation in children [6]. In a pediatric population affected mostly by malignancies, the new i.v. dosing regimen has preliminarily demonstrated a successful target of the therapeutic area under the plasma concentration versus time curve (AUC) range of 900-1500 μmol*min, resulting in efficient engraftment and mild to moderate nonhematologic toxicity [7]. Consequently, the fixed i.v. Bu dosing for pediatric patients has been proposed as standard practice before SCT, with no need for drug monitoring and dose adjustment. However, recent reports have described variable pharmacokinetics (PK) of i.v. Bu in heterogeneous groups of children undergoing SCT [8-10], suggesting that the PK of i.v. Bu might be influenced by age, comorbidities, genetic background, and underlying disease.

We postulated that children with advanced β-thalassemia could be at greater risk of erratic Bu metabolism with increased RRT because of organ dysfunction resulting from severe iron overload, infectious diseases, and ethnic/genetic specific features. Consequently, we conducted a prospective i.v. Bu PK study in a large, homogeneous group of children with β-thalassemia from Middle Eastern countries undergoing SCT from a matched family donor. The aim of this study was to evaluate the reliability of standard i.v Bu fixed dosing in achieving the therapeutic AUC. In addition, we analyzed the impact of patients' pretransplantation clinical and laboratory features on i.v. Bu PK and the association between Bu PK and transplantation outcomes, such as overall survival (OS), disease-free survival (DFS), engraftment and RRT.

PATIENTS AND METHODS

Patient Characteristics

Fifty-four children with β-thalassemia major or transfusion-dependent thalassemia intermedia were referred to our institution from Mediterranean and Middle Eastern countries to undergo SCT. The cost of treatment was borne by a cooperative program funded by the Mediterranean Institute of Haematology (www.fondazioneime.org). In an intention-to-treat analysis, 1 patient with β-thalassemia intermedia was not enrolled to SCT because transfusion independency was achieved after splenectomy. Between December 2005, and September 2008, 53 consecutive children underwent 57 i.v. Bu-conditioned SCTs from a matched related donor and were included in this analysis. Patient characteristics are summarized in Table 1. All patients were assigned to 1 of 3 risk classes proposed by Lucarelli et al. [11] based on hepatomegaly, portal fibrosis in pre-SCT liver biopsy and quality of chelation. The 53 treated patients included 2 patients in risk class I, 26 patients in risk class II, and 25 patients in risk class III. Most patients had severe iron overload, as evidenced by a median serum ferritin level of 3.267 μg/L (range, 956-14.280 μg/L) and a median liver iron concentration of 24 mg/g dry weight (range, 4-95.7 mg/g). The median portal fibrosis score (Ishak score 1-6) was 3 (range, 1-5); 3 patients (5%) exhibited early signs of liver cirrhosis. Fifteen patients (28%) had positive antibodies for hepatitis C virus (HCV).

Before SCT, all patients received a comprehensive pretransplantation workup and underwent intensive iron chelation therapy with deferoxamine. Splenectomy was performed in 12 of the 53 patients (23%) because of splenomegaly below the umbilicus and the consequent risk of poor graft function posttransplantation.

Table 1. Patient Characteristics at Transplantation

	n	%
Number of patients	53	100
Sex, male/female	29/24	55/45
Age, years		
Median	8	
Range	2-17	
Country of origin		
Iraq	19	36
Lebanon	9	17
Syria	21	40
Palestinian territories	3	5
Other	1	2
Pesaro class at time of SCT		
Class I	2	4
Class II	26	49
Class III	25	47
Splenectomy, yes/no	12/41	23/77
Iron chelation, regular/irregular	9/44	17/83
Number of pretransplantation red blood cell units		
Median	76	
Range	15-230	
Serum ferritin, μg/L		
Median	3267	
Range	956-14.280	
Liver iron concentration, mg/g dry weight*		
Median	24	
Range	4-95.7	
Ishak grading (portal fibrosis, range 1-6)		
Median	3	
Range	1-5	
AST, U/L		
Median	58	
Range	18-371	
ALT, U/L		
Median	72	
Range	12-545	
HCV antibody–positive, yes/no	15/38	28/72
Anti HLA antibodies		
Pesaro class I-II	16	57
Pesaro class III	25	100

SCT indicates stem cell transplantation; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.
*Data available for only 40 patients.

Written informed consent was obtained from each patient's parent or legal guardian, with the collaboration of 2 mother language (Arabic and Kurdish) non-official translators employed full time to fulfill patients' and families' needs.

Transplantation Procedures

Fifty-one patients received a transplant from an HLA-identical sibling, and 2 patients received a transplant from a phenotypically identical mother. Thirty-two donors (60%) were heterozygous for β-thalassemia. Four patients with graft failure underwent a second SCT from the same donor. Forty patient–donor pairs were ABO-compatible, 6 had major ABO incompatibility, 6 had minor ABO incompatibility, and 1 had bidirectional ABO incompatibility. Fresh bone marrow (BM) provided the stem cell source for all patients. The median number of infused BM nucleated cells was 5×10^8/kg (range 1.4-11.5 $\times 10^8$/kg), that of $CD34^+$ cells was 7.7×10^6/kg (range 3.3-21.7 $\times 10^6$/kg), and that of $CD3^+$ cells was 73×10^6/kg (range, 23-126 $\times 10^6$/kg). The conditioning regimen for patients in risk class I-II consisted of i.v. Bu (detailed below) and Cy 200 mg/kg total dose (on days −5 to −2) in patients aged ≥4 years, with thiotepa (TT) 10 mg/kg (on day −6) added for patients aged <4 years. Pretransplantation chemotherapy for risk class III patients consisted of a "preconditioning" regimen [12] with hydroxyurea 30 mg/kg daily and azathioprine 3 mg/kg daily (on days −45 to −11), followed by fludarabine (Flu) 100 mg/m^2 total dose (on days −17 to −13), i.v. Bu, and Cy 160 mg/kg total dose (on days −5 to −2). Because of the occurrence of 3 graft failures in the first 6 patients in class III, the immunosuppression in risk class III patients was strengthened with a higher dose of Flu (150 mg/m^2) and the addition of rabbit antithymocyte globulin (ATG; Thymoglobulin) 7.5-10 mg/kg total dose (on days −7 to −4). Patients undergoing a second SCT received a standard preconditioning regimen, followed by i.v. Bu, Cy 200 mg/kg, TT 10 mg/kg, and ATG 8-12.5 mg/kg [13]. Graft-versus-host disease (GVHD) prophylaxis consisted of oral cyclosporine (CsA) adjusted to a trough plasma level of 150-250 ng/mL. In the absence of GVHD, CsA was tapered from day 60 until discontinuation at 1 year. All patients received a short course of i.v. methotrexate (MTX) 10 mg/m^2 on days 1, 3, and 6. Additional GVHD prophylaxis consisted of methylprednisolone 0.5 mg/kg from day -1 to day 25 in 33 patients, ATG in 19 patients, and methylprednisolone plus ATG in 5 patients. Supportive therapy consisted of ceftriaxone during neutropenia, acyclovir during neutropenia and immunosuppression and prophylactic liposomal amphotericin B 1 mg/kg daily from day 8 until the lymphocyte count exceeded 1×10^9/L. A preemptive approach with twice-weekly polymerase chain reaction (PCR) monitoring was adopted for cytomegalovirus (CMV) treatment, and no prophylaxis was given. Seizure prophylaxis consisted of oral clonazepam starting 24 hours before the first i.v. Bu dose and continuing up to 24 hours after the last dose. VOD prophylaxis consisted of defibrotide from day −11 to day 30.

Engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count (ANC) of $>0.5 \times 10^9$/L and an unsupported platelet count of $>20 \times 10^9$/L. Engraftment was monitored by short tandem repeat variability testing on peripheral blood (PB) and BM. Mixed chimerism was defined as the presence of recipient DNA >5%. GVHD was graded according to the criteria of the Seattle group. RRT was evaluated using the National Cancer Institute's common toxicity criteria (NCI CTC); the overall score was assigned on signs and symptoms recorded during the first 100 days after SCT.

Intravenous Bu Administration, Pharmacokinetics Studies and Bu Dose-Adjustment Policy

According to the standard [6], 5 different i.v. Bu doses were given based on weight: 1.0 mg/kg for <9 kg, 1.2 mg/kg for 9-16 kg, 1.1 mg/kg for 16-23 kg, 0.95 mg/kg for 23-34 kg, and 0.8 mg/kg for >34 kg. Bu was administered as a 2-hour i.v. infusion every 6 hours for a total of 16 doses from day −9 to day −6. Bu concentrations in plasma were measured by a validated high-performance liquid chromatography (HPLC) assay involving protein precipitation, precolumn derivatization, liquid/liquid extraction, and ultraviolet detection [14]. Calculation of the total $AUC_{0-\infty}$ was based on PB samples at 0, 2, 3, 4, and 6 hours of the first dose (AUC_{0-6} by trapezoidal rule and $AUC_{6-\infty}$ by extrapolation to infinity). The prediction of AUC_{0-6} during the dosing interval at steady state was based on the equality of the total AUC after the first dose and the AUC during the dosing interval at steady state after multiple doses. The reliability of a national reference pharmacology laboratory allowed us to apply a limited sampling strategy as preferred by others [15]. Bu AUC values >900 μmol*min and <1500 μmol/min were considered to lie within the therapeutic window. The Bu dose was adjusted from dose 4 using the following equation: adjusted dose = actual dose × target AUC/actual AUC (target AUC = 1250 μmol*min). This dose was used for the remaining course of therapy.

Statistical Analyses

Numerical values are expressed as median and range, and categorical values are expressed as proportion and percentage. The χ^2 test (for categorical variables) and logistic regression (for continuous variables) were used to explore for any correlation

between pretransplantation patient characteristics (ie, Pesaro class, serum ferritin, Ishak staging, serum alanine aminotransferase [ALT] and aspartate aminotransferase [AST], HCV infection, age, and sex) and Bu AUC range (in range vs out of range). Comparisons of Bu AUC between SCTs with NCI CTC grade III-IV toxicity and uncomplicated SCTs were done using the Mann-Whitney *U* test. OS was defined as the probability of survival regardless of disease status, and DFS was defined as the probability of being alive and disease-free. Both OS and DFS were estimated by the Kaplan-Meier method, and comparisons among groups were made using the log-rank test. Current DFS also was evaluated, including patients who were in subsequent remission after treatment for graft rejection. Treatment-related mortality (TRM) was defined as death from a complication of transplantation. The incidences of TRM and both acute and chronic GVHD (aGVHD, cGVHD) were calculated for evaluable SCTs by estimating cumulative incidence. All outcomes were evaluated from the day of stem cell infusion, and actuarial estimates (with 95% confidence intervals [CIs]) were calculated at 2 years after SCT. Patients who underwent a second SCT were censored at the day of documented graft failure. A P value $<.05$ was considered statistically significant.

RESULTS

Bu Pharmacokinetic Studies

All patients received the full 16-dose course of i.v. Bu. The median Bu AUC of the whole population (53 SCTs) after the first dose was 1083 μmol*min (range, 669-2698 μmol*min). PK studies following the first i.v. Bu dose showed that 31 patients (58%) achieved an AUC within the therapeutic window (900-1500 μmol*min), 13 patients (25%) had an AUC below the lower target limit, and 9 patients (17%) had an AUC above the upper target limit. No difference in AUC was found between class I-II patients and class III patients ($P = .96$). Although the numbers are small, intrapatient Bu AUC variability was high. In the 4 patients receiving i.v. Bu–based conditioning following rejection of the first SCT, Bu AUCs following the first and second exposures to Bu were 1461/1391, 1237/1106, 1959/1085, and 1798/1019 μmol*min, respectively.

Bu Pharmacokinetics and Pretransplantation Characterstics

No significant correlation was found between a Bu AUC outside the therapeutic window and specific pretransplantation clinical features. This indicates that Pesaro risk stratification, serum ferritin, liver fibrosis, ALT and AST, HCV infection, age, and sex are not associated with an unfavorable Bu systemic exposure if a dose-adjustment policy is adopted.

Table 2. Severe RRT (NCI CTC Grade III-IV) in Patients with Class I-II and Class III Thalassemia

	Class I-II (n = 28)	Class III (n = 29)	Overall (n = 57)
Infections	17 (60%)	21 (72%)	38 (66%)
Bladder	1 (3%)	4 (14%)	5 (9%)
Pulmonary	1 (3%)	3 (10%)	4 (7%)
Neurologic	1 (3%)	2 (7%)	3 (5%)
Stomatitis	0	2 (7%)	2 (3%)
Renal failure	0	2 (7%)	2 (3%)
Liver (VOD)	0	1 (3%)	1 (2%)
Multiorgan failure	0	1 (3%)*	1 (2%)
Diarrhea	0	0	0

RRT indicates regimen related toxicity; NCI CTC, National Cancer Institute's common toxicity criteria; VOD, veno-occlusive disease.
*This patient died due to multiorgan failure after primary graft failure without autologous reconstitution.

Bu PK and RRT

Table 2 summarizes grade III-IV RRT in the study population. Notably, 37 patients (66%) developed 41 episodes of severe infection, represented by culture negative febrile neutropenia (n = 24), *Staphylococcus* non-*aureus* sepsis (n = 6), *Pseudomonas aeruginosa* sepsis (n = 3), vancomycin-resistant *Enterococcus faecium* (n = 1), *Klebsiella* spp sepsis (n = 1), BK virus hemorrhagic cystitis (n = 5), and severe *Pseudomonas aeruginosa* vulvovaginitis (n = 1). None of these infections was fatal. Five patients (9%) experienced moderate to severe hemorrhagic cystitis, and 2 patients (3%) developed secondary obstructive renal failure that resolved after surgery. Four patients (7%) presented with grade III-IV pulmonary complications with acute respiratory distress at 15-52 days, which was treated successfully with noninvasive ventilation. Three patients (5%) experienced CsA-related posterior reversible encephalopathy syndrome. The incidences of stomatitis (3%) and VOD (2%) were low. Seven patients developed grade II-IV aGVHD, with a cumulative incidence of 13% (95% CI = 4%-22%). All were treated successfully, except for 1 patient who died at day 56 of grade IV aGVHD not responsive to several lines of immunosuppression (ie steroids, ATG, etanercept, and infusion of third-party mesenchymal stem cells). Four patients developed limited cGVHD, with a cumulative incidence of 8% (95% CI = 1%-15%). Three patients evolved from aGVHD, and 1 patient developed vitiligo as a sole de novo expression of cGVHD. Only 1 of the patients with cGVHD was receiving immunosuppressive treatment at the last follow-up. We found no correlation between the severity of adverse events after transplantation and Bu AUC after the first dose ($P = .57$). With the exception of the patient who experienced hepatic VOD 11 days after SCT, none of the other patients with grade III-IV RRT had a Bu AUC >1500 μmol*min (Figure 1).

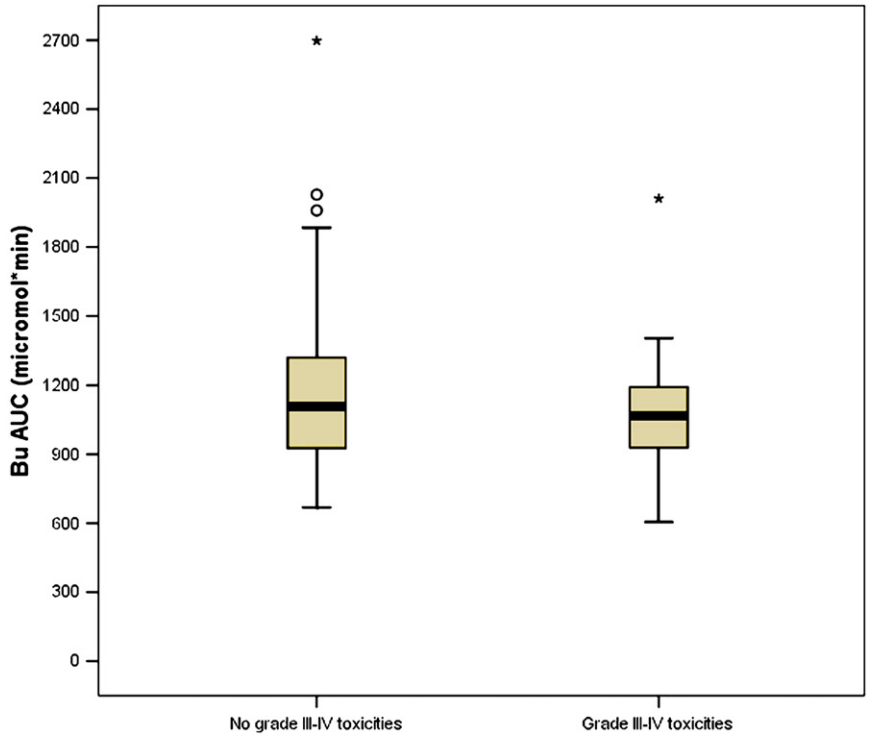

Figure 1. Boxplot of Bu AUC values in 53 thalassemic children with or without grade III-IV toxicity. Outliers are represented as circles (<3 box lengths) or asterisks (>3 box lengths).

Engraftment, OS and DFS

Fifty patients (94%) engrafted, and 3 patients (6%) experienced primary graft failure. Of these 3 patients, 2 had autologous reconstitution and 1 died in aplasia at day 67. Among the patients who engrafted, the median time to neutrophil engraftment was 19 days (range, 12-39 days), and the median time to platelet engraftment was 18 days (range, 12-73 days). Time to red cell transfusion independence differed in recipients of first SCT (median, 22 days; range, 0-336 days) and recipients of a second SCT (median, 90 days; range, 0-216 days).

Two-year OS at a median follow-up of 564 days (range, 123-1144 days) was 96% (51 of 53 patients). One patient died of grade IV aGVHD on day 56, and 1 patient died of primary graft failure, as discussed earlier. Secondary graft failure occurred in 5 patients (9%) and was always followed by autologous reconstitution. We found no correlation between the incidence of graft failure and low Bu systemic exposure following the institution of the dose-adjustment policy; in fact, none of the 8 patients who experienced primary or secondary graft failure had a Bu AUC below the lower target limit of 900 μmol*min. Graft failure (primary or secondary) occurred mainly in class III patients, with only 1 occurring (in the phenoidentical recipient) in class I-II patients and 7 occurring in class III patients ($P = .02$, χ^2 test). Four of the 8 patients who experienced graft failure underwent retransplantation from the same donor, with a median interval between the 2 procedures of 12 months (range, 7-16 months). After the second SCT, all patients engrafted with sustained full donor chimerism. Considering all patients, chimerism at the last follow-up was 100% donor in 46 patients (87%), 0% donor in 4 patients (7%), and mixed in 3 patients (6%). Outcomes estimated by Kaplan-Meier probability were OS, 96% (95% CI = 91%-100%); DFS, 79% (95% CI = 67%-91%); current DFS accounting for second SCT, 88% (95% CI = 78%-98%); rejection, 21% (95% CI = 9%-33%); and TRM, 4% (95% CI = 0-9%). Transplantation outcome was strongly influenced by the Pesaro risk class stratification; the class III patients had an estimated OS of 96% (95% CI = 88%-100%), an estimated DFS of 66% (95% CI = 45%-87%), and a current DFS of 85% (95% CI = 69%-100%) (Figure 2), whereas the class I-II patients had an OS of 96% (95% CI = 89%-100%) and a DFS of 91% (95% CI = 80%-100%; $P = .04$ for DFS) (Figure 3).

DISCUSSION

Our results demonstrate that standard i.v. Bu fixed dosing in children with advanced β-thalassemia results in unpredictable systemic exposure requiring a dose-adjustment policy. In fact, only 58% of our patients

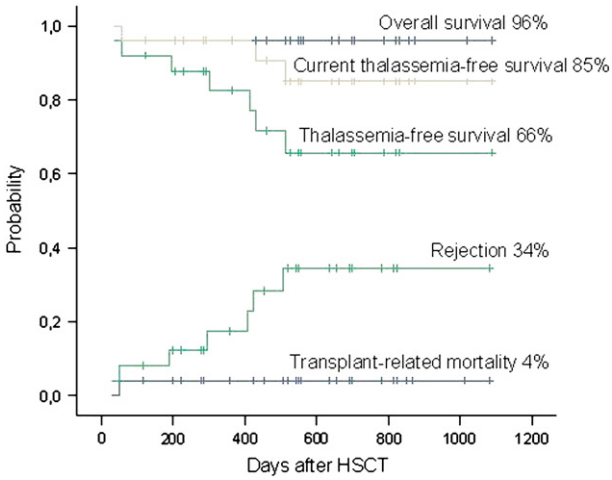

Figure 2. Kaplan-Meier probabilities of OS, DFS, current thalassemia-free survival, rejection, and TRM in 25 patients with class III thalassemia.

achieved the targeted AUC range after the first dose, which differs significantly from the results of Vassal et al. [7], in which 91% of children undergoing SCT achieved the Bu therapeutic window. Our findings are more in line with the large interindividual variation of AUC reported by other authors, in which up to 66% of children being treated for an heterogeneous group of diseases required Bu dose adjustment [8-10]. Our study is unique in terms of the homogeneity of disease, the severity of pretransplantation organ dysfunction, and the specific ethnic origin of the patients. All of these characteristics may have influenced i.v. Bu PK.

As has been noted previously [16,17], a marked increase in glutathione S-transferase (GST) concentration in thalassemic patients could be responsible for the unpredictable Bu PK. GST is the enzyme largely

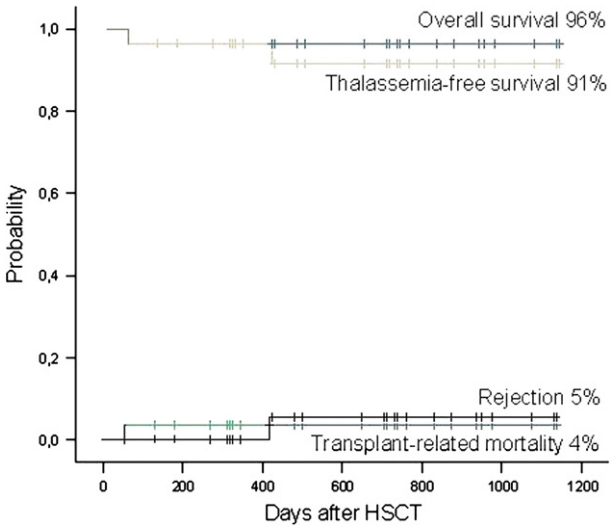

Figure 3. Kaplan-Meier probabilities of OS, DFS, rejection, and TRM in 28 patients with class I-II thalassemia.

responsible for Bu metabolism, and its activity is inversely correlated to Bu plasma concentration. Iron overload might induce GST via activation of the Nrvf2/keap1 pathway [17], thereby facilitating the conjugation of Bu to glutathione. In addition, abnormal liver function per se might affect Bu clearance and RRT, as suggested by studies finding an association between elevated serum ALT level and reduced Bu clearance [18]. Finally, GST-A1 isoforms are known to be distributed evenly in Caucasians, African Americans, and Hispanics [19,20]. As has been suggested previously [20], GST polymorphisms in different ethnic groups might affect Bu PK unpredictability. As has been reported for oral Bu [21], we found no clear correlation between the severity of specific clinical features (ie, Pesaro risk stratification, degree of iron overload or portal fibrosis, transaminase level, age, or presence of viral hepatitis) and Bu underexposure or overexposure. However, we speculate that for the aforementioned reasons, our series of patients with advanced β-thalassemia and severe liver disease demonstrated erratic and unpredictable Bu clearance. Lack of efficacy or RRT was not correlated with Bu AUC, most likely because of the Bu dose-adjustment policy.

In this series, the ablative conditioning based on targeted i.v. Bu translated into a high engraftment rate with low TRM and RRT despite the presence of clinical features of advanced disease. The mortality rate (4%) compared favorably to rates reported with oral Bu in children affected by hemoglobinopathies, including 12% in a recent update by Lucarelli and Gaziev [4], 8.7% by Di Bartolomeo et al. [22], and 5.4% by Lawson et al. [23]. TRM also was comparable to that seen in children with β-thalassemia treated with a treosulfan-based regimen with the aim of minimizing Bu-related toxicity [24]. The incidences of grade II-IV aGVHD and cGVHD were low (13% and 8%, respectively). Only 2 patients experienced grade III stomatitis, and only 1 patient (2%) developed reversible VOD. Targeted i.v. Bu and prophylactic defibrotide likely served as protective cofactors in this high-risk population. Defibrotide also may have contributed to low RRT and GVHD, as suggested by a recent multicenter trial [25]. Eight children presented with primary or secondary engraftment failure, resulting in an overall thalassemia-free survival of 79%. Four children underwent a second SCT and achieved complete and sustained donor engraftment, increasing the current thalassemia-free survival to 88%. A second SCT with another round of i.v. Bu conditioning was feasible and well tolerated without major organ toxicity [13]. Unfortunately, the application of a Bu dose-adjustment policy and intensive immunosuppression with steroids, CsA, and ATG did not resolve the problem of rejection in patients with advanced thalassemia. The rejection rate in patients with advanced disease

was high and in accord with that reported by Lucarelli and Gaziev [4] and by others in related [22,23] and unrelated donor SCTs [26]. We speculate that this population's poor socioeconomic background contributed to the increased risk of graft failure. Indirect evidence of strong alloimmunity was provided by the detection of high numbers of anti-HLA antibodies in all class III patients, as a result of chronic transfusions with non–leuko-depleted blood products. The addition of ATG to the regimen for class III patients after 3 graft failures in the first 6 patients treated was thus intended to facilitate a state of mutual tolerance between donor and recipient; however, this strategy failed to resolve the problem of rejection, with graft failure occurring in 4 of the 18 patients who subsequently underwent SCT.

In summary, our results indicate that an i.v. Bu-based conditioning regimen can be applied to a high-risk group of thalassemic patients with poor pretransplantation clinical conditions, but, because of unpredictable PK, therapeutic drug monitoring is strongly recommended to minimize the risk of RRT or lack of efficacy. These results may be taken into consideration for health care policy discussions in Middle Eastern countries in which implementation of transplantation programs is ongoing.

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