

Conditioning regimens

Randomized trial of two different conditioning regimens for bone marrow transplantation in thalassemia – the role of busulfan pharmacokinetics in determining outcome

M Chandy¹, P Balasubramanian¹, SV Ramachandran¹, V Mathews¹, B George¹, D Dennison², R Krishnamoorthy³ and A Srivastava¹

¹Department of Hematology, Christian Medical College, Vellore, India; ²Sultan Qaboos University Hospital, Muscat, Oman; and ³INSERM, U458, Hospital Robert Debre, Paris, France

Summary:

In total, 94 patients with homozygous beta thalassemia were randomized to two different conditioning regimens: busulfan 600 mg/m² + cyclophosphamide 200 mg/kg or busulfan 16 mg/kg + cyclophosphamide 200 mg/kg and antilymphocyte globulin (47 in each group), for bone marrow transplantation, to see whether increased myeloablation or increased immunosuppression would reduce rejection. Busulfan pharmacokinetics in determining outcome was evaluated. There was no significant difference in engraftment, graft-versus-host disease, rejection, and overall and disease-free survival in the two groups. Systemic exposure to busulfan was significantly higher in the 600 mg/m² group, but in both groups there was a wide interindividual variation in the busulfan kinetics. Six patients rejected the graft, two in the busulfan 600 mg group and four in busulfan 16 mg group ($P = 0.677$ CI –0.17, 0.07), but in five patients (pharmacokinetic data not available in one patient) who rejected the graft busulfan first dose trough level ($C_{\min-1}$) was below 150 ng/ml while it was above this level in the 66 of 68 patients with successful engraftment ($P \leq 0.001$). This randomized trial shows that rejection is influenced by busulfan levels and suggests that monitoring of busulfan levels and dose adjustment based on first-dose kinetics may reduce the risk of rejection.

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Bone marrow transplantation (BMT) is today an alternative to life-long chelation and transfusion for children with thalassemia major. Lucarelli *et al* have shown that for

children who have no hepatomegaly or fibrosis in the liver and have been adequately chelated (Class I), an event-free survival (EFS) of 92% can be achieved with transplantation, but for children who have been inadequately managed before transplant with all three risk factors (Class III), the transplant-related complications and rejection are high and the EFS is only 60%.^{1,2} Rejection is a major problem in BMT for thalassemia, with rates varying from 11 to 55% in different series of BMT for thalassemia.^{3,4}

Busulfan (Bu) and cyclophosphamide (Cy) are the two agents used most commonly for conditioning in BMT for thalassemia and of these two, Bu is considered to be the main agent for myeloablation. Systemic exposure to Bu may be a critical determinant of the degree of myeloablation achieved and we and others have reported a significant interindividual variation in busulfan kinetics in patients conditioned with Bu and Cy.^{5,6} It is also known that the clearance of busulfan in children is higher than that in adults and that for a given dose, the area under the curve is lower for children than in adults. Vassal *et al*⁷ have shown that children need to be given a dose of 600 mg/m² in order to achieve a systemic exposure to Bu similar to adults given 16 mg/kg. A child, whose body surface area is 0.5 m², may therefore receive as much as 28 mg/kg of Bu.

Storb *et al*⁸ have shown that it is possible to reduce rejection in allogeneic BMT for severe aplastic anemia by the addition of ALG to the conditioning regime of Cy 200 mg/m².

We therefore decided to evaluate in a randomized manner whether increased systemic exposure to Bu with 600 mg/m² and consequently more effective myeloablation or increased immunosuppression with antilymphocyte globulin (ALG) combined with the standard dose of 16 mg/kg of Bu would reduce rejection in patients receiving an allogeneic BMT for thalassemia. In both groups, Bu pharmacokinetics was determined to evaluate its effect on outcome.

Patients and methods

Patients with an HLA six antigen serologically identical donor with transfusion-dependent beta thalassemia major were considered for allogeneic BMT at the Christian

Correspondence: Professor M Chandy, Department of Hematology, Christian Medical College, Vellore 632 004, India;
E-mail: mammen@cmcvellore.ac.in
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Medical College Hospital, Vellore and enrolled in the study, which was conducted from January 1994 to December 2002. Ethical clearance was obtained from the institutional review board and informed consent was obtained from the parents of all children entered on this study.

Data analysis was performed in December 2004.

Conditioning

Patients were randomized using a computer generated randomization list, to receive one of the following conditioning regimens. Assuming a 10% difference in rejection between the two groups the sample size would be 148 in each arm.

1. REGIMEN A [Bu600] – busulfan 600 mg/m² given as four divided doses over 4 days and cyclophosphamide 200 mg/kg given over 4 days (50 mg/kg/day i.v over 1 h).
2. REGIMEN B [Bu16] – busulfan 16 mg/kg as 1 mg/kg/dose four times daily \times 4 days, cyclophosphamide 200 mg/kg given over 4 days (50 mg/kg/day i.v over 1 h) and ALG (Pasteur Merieux) 30 mg/kg/day for 3 days.

Details regarding pre-transplant evaluation, prevention of hemorrhagic cystitis, infection control policies, growth factor use, graft-versus-host disease (GVHD) prophylaxis, bone marrow harvest procedures and documentation of chimerism have been reported earlier.⁹

The Glucksberg scale was used to grade acute GVHD¹⁰ and regimen-related toxicity was graded according to Bearman's¹¹ criteria. Hepatic veno-occlusive was diagnosed according to Baltimore criteria.¹²

Bu levels and pharmacokinetic analysis

The first dose of busulphan was given at 6am to all patients. All doses were given to patients on an empty stomach. Blood samples (3–5 ml) were taken from a central venous catheter into glass tubes containing 150 IU heparin. They were collected immediately before starting the drug and at 0.5, 1, 1.5, 2, 4 and 6 h after the first, second and 13th doses. Samples were immediately refrigerated and centrifuged within 1.5–3 h of collection and stored at 80°C until analysis. Plasma levels of Bu were measured after dose 1, 2, 6 and 13 using a newly developed HPLC method¹³ that was validated with the previously reported GC-MS method¹⁴ and showed significant correlation. Pharmacokinetic parameters were analyzed using the TOPFIT program.¹⁵ Parameters like elimination half-life ($T_{1/2}$), elimination rate constant (Kel), and AUC were estimated directly from the data using noncompartmental analysis. $T_{1/2}$ was determined from the linear portions of the log plasma AUC curves. All the other parameters were derived. The mean concentration at steady state (C_{ss}) was calculated from the following formulae:

Expected C_{ss} = AUC0- α of 1st dose/dosing interval, that is, 6 h.

Observed C_{ss} = AUC0-6 h of the 13th dose/dosing interval.

Clearance (Cl/F) was calculated as the ratio of dose to AUC. Vd/F (in l/kg) was calculated as the ratio of Cl/F to Kel. The minimum concentration (C_{min}) was the trough level of Bu before each dose. Mean residual value (MRV) was calculated as the mean of all trough levels from dose 2 to dose 16. Maximum concentration (C_{max}) was the peak value of Bu after the first and 13th doses.

Statistical analysis

The Mann–Whitney *U*-test was used to compare between the two groups using the statistical program SPSS version 11.0. Probabilities of disease-free survival (DFS) and overall survival (OS) following transplant were estimated by the Kaplan–Meier method and the significance was assessed by log-rank test. Since interim analysis showed no statistical difference in rejection between the two groups and a relationship with busulfan pharmacokinetics independent of the regimen, further recruitment was stopped.

Results

Table 1 (patient characteristics) shows that there was no significant difference between the two groups in relation to sex, age and Lucarelli class. The liver size, degree of hepatic fibrosis, splenectomy status, serum ferritin levels and positivity for viral markers was also not different in the two groups. The donor age ranged from 2 to 42 years with 67% of the donors being heterozygous for beta thalassemia. There was no significant difference in donor characteristics between the two groups. In total, 26% of the transplants were major group mis-matched. There was no difference between the two groups in relation to engraftment: time to ANC > 500/mm³ was 14.5 days in the Bu 600 group and 16 days in the Bu 16 group and time to reach an unsupported platelet count of 20 000/mm³ was 24.2 days in the Bu 600 group and 26.3 days in the Bu 16 group. There was no significant difference in the number of days in hospital and in the incidence or severity of acute and chronic GVHD in the two groups (acute GVHD 50%, with 20% being grade III/IV in both groups).

Table 1 Clinical details of patients

	Bu 600/Cy 200 (n = 47)		Bu 16/Cy 200/ALG (n = 47)		P-value
	n	(%)	n	(%)	
Age (years)					
Mean \pm s.d.	6.74	3.31	7.55	2.97	0.516
Sex					
Males	31	(66)	30	(64)	1.000
Females	16	(34)	17	(36)	
Risk classification					
Class II	21	(45)	22	(47)	1.000
Class III	25	(53)	25	(53)	
Class II or III	1	(2)	—	—	
Serum ferritin (ng/ml)					
Mean \pm s.d.	3881.55	2042.24	3600.96	1659.10	0.680

Table 2 Data on regimen-related toxicity

	<i>Bu</i> 600/ <i>Cy</i> 200 (<i>n</i> = 47)		<i>Bu</i> 16/ <i>Cy</i> 200/ <i>ALG</i> (<i>n</i> = 47)		<i>P</i> -value
	<i>n</i>	(%)	<i>n</i>	(%)	
<i>Stomatitis – evaluable</i>	45/46	(96)	41/47	(87)	0.053
Grade I and II	16	(36)	26	(63)	
Grade III–IV	29	(64)	15	(37)	
<i>Gastrointestinal</i>					0.526
Grade I–II	4	(9)	6	(13)	
GI Hemorrhage	31	(66)	20	(43)	0.038
<i>Bladder (hemorrhagic cystitis)</i>					0.035
Grade I and II	4	(8)	11	(23)	
Grade III–IV	3	(6)	3	(6)	
<i>Hepatic</i>					0.662
Grade I and II	31	(66)	27	(57)	
Grade III	2	(4)	3	(6)	
<i>Central nervous system</i>					1.000
Grade III	1	(2)	1	(2)	
<i>Pulmonary</i>					0.617
Grade III	1	(2)	3	(6)	
<i>Veno-occlusive disease of liver</i>					1.000
Seattle criteria	27	(57)	27	(57)	
Baltimore criteria	8	(17)	12	(26)	0.450

The differences in regimen-related toxicity in the two groups is illustrated in Table 2. There was an increase in the incidence of hepatic veno-occlusive disease in the 16 mg group when the Baltimore criteria were used, but this was not statistically significant ($P = 0.45$). There was a statistically significant increase in the incidence of GI hemorrhage in the group receiving 600 mg/m² of busulfan ($P = 0.038$). Independent of the two groups there was a higher incidence of bleeding in patients with higher busulfan levels (MRV mean + s.d. (ng/ml) 322 ± 142 in patients with bleeding vs 255 ± 91 in those with no bleeding ($P = 0.006$). In Table 3, the overall outcome and outcome in relation to Lucarelli class is illustrated; again, there was no significant difference between the two groups. In nine patients the mortality was due to infections: cytomegalovirus – 3, viral pneumonia – 2, bacterial sepsis – 3 and fungal pneumonia – 1. The miscellaneous causes included intracranial hemorrhage – 2, cardiac tamponade – 1, hemorrhagic cystitis – 2, myocarditis – 1 and graft failure – 2. Analysis of engraftment in relation to growth factor use was not different in the two groups.

Data on Bu pharmacokinetics are given in Table 4. There were problems with sample collection and processing in 11 patients in the Bu 600 group and in one patient in the Bu 16 group. There were significantly higher MRV, C_{\max} , C_{\min} , T_{\max} , C_{ss} and AUC in the Bu 600 mg group than in the 16 mg group. There was a marginal increase in clearance in the Bu16 group ($P = 0.05$). However, there was a significant inter-individual variation in both the groups with the AUC ranging from 2796 to 8899 ng/h/ml in the Bu 600 group and 1876–5757 ng/h/ml in the Bu 16 group. This wide range was

Table 3 Analysis of outcome in relation to busulfan dose

	<i>Bu</i> 600/ <i>Cy</i> 200		<i>Bu</i> 16/ <i>Cy</i> 200/ <i>ALG</i>		<i>P</i> -value
	<i>n</i>	(%)	<i>n</i>	(%)	
Overall survival	32	(68)	34	(47)	0.822
Disease-free survival	32	(68)	30	(64)	0.828
Follow-up (months) median	63	1–124	52	1–124	0.376
Rejection	2	(4)	4	(9)	0.677**
Mortality	15	(32)	13	(28)	0.652*
<i>Outcome by class</i>					
Class II	<i>n</i> = 21		<i>n</i> = 22		0.698
Overall survival	17	(81)	19	(86)	
Disease-free survival	17	(81)	19	(86)	
Rejection	—	—	—	—	
Mortality	4	(19)	3	(14)	
Class III	<i>n</i> = 26		<i>n</i> = 25		1.000
Overall survival	15	(58)	15	(60)	
Disease-free survival	15	(58)	11	(44)	
Rejection	2	(10)	4	(22)	
Mortality	9	(35)	10	(40)	

95% CI: *0.08, 0.16; **–0.17, 0.07.

also seen in C_{\max} , C_{\min} , MRV and C_{ss} , confirming the variability between individuals with regard to all indicators of Bu metabolism. In Table 5, the risk of VOD in relation to Bu levels in both groups is shown. Patients with higher clearance and lower C_{\max} , C_{ss} , C_{\min} and MRV had a significantly higher incidence of VOD.

Four patients rejected the graft in the Bu 16 mg group while two patients rejected it in the Bu 600 mg group, but this was not statistically significant. However, as illustrated in Table 6, there was a significant correlation between the risk of rejection and the Bu pharmacokinetics. In five of the six patients (for whom pharmacokinetic data is available) who rejected the graft, the C_{\min} was below 150 ng/ml, while all except two of the 68 evaluable patients with a C_{\min} greater than 150 ng/ml did not reject the graft ($P < 0.001$) and this is illustrated in Figure 1. The C_{ss} , which is a reflection of the AUC, was also lower in the group that rejected the graft (476 vs 651.9 ng/ml), but this did not reach statistical significance and the same is true for the C_{\max} (844 vs 1108 ng/ml).

Discussion

In the first allogeneic transplant carried out for thalassemia, Thomas *et al* in Seattle used a single dose of Bu 5 mg/kg i.v. and 200 mg of Cy for conditioning and the patient still has a stable graft.¹⁶ Subsequently, Lucarelli *et al*¹⁷ have evaluated different doses of Bu (14–16 mg/kg) and Cy 120–200 mg/kg as conditioning for BMT in thalassemia. In children with Class I between 1983 and 1994, conditioning consisted of Bu orally three times daily for 4 days (total dose, 14 mg/kg body weight), followed by intravenous Cy once daily for 4 days (total dose, 200 mg/kg), with most patients receiving cyclosporine alone as GVHD

Table 4 Busulfan pharmacokinetics

	<i>Bu 600/Cy 200 (n = 36)</i>		<i>Bu 16/Cy 200/ALG (n = 46)</i>		<i>P-value</i>
	<i>Mean</i>	<i>s.d.</i>	<i>Mean</i>	<i>s.d.</i>	
Mean residual value (ng/ml)	343.18	126.87	234.4	87.36	<0.001
C_{max} (ng/ml)	1318	416	902	325	<0.001
C_{min} (ng/ml)	282.44	127.75	198.96	65.6	0.002
T_{max} (h)	1.53	1.03	1.33	0.78	0.321
AUC (ng h/ml)	5074.94	1639.07	3119.15	837.43	<0.001
$T_{1/2}$ (/h)	2.31	0.88	2.46	0.87	0.486
Cl (l/h/kg)	0.31	0.07	0.35	0.08	0.029
C_{ss} (ng/ml)	845.83	273.12	529.41	148.41	<0.001

C_{max} , C_{min} , T_{max} , AUC, $T_{1/2}$, Cl, C_{ss} are for dose 1.

Pharmacokinetic data were not available in 11 patients in Regimen A and one patient in Regimen B.

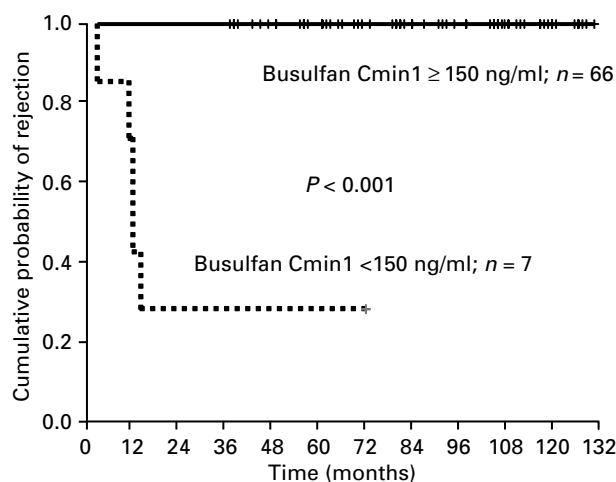
Table 5 HVOD (Baltimore Criteria) in relation to busulfan pharmacokinetics (both groups combined)

	<i>With HVOD</i>			<i>No HVOD</i>			<i>P-value</i>
	<i>n</i>	<i>Mean</i>	<i>s.d.</i>	<i>n</i>	<i>Mean</i>	<i>s.d.</i>	
C_{ss} -1 (ng/ml)	20	577.0	181.53	62	697.79	279.85	0.030
C_{min} -1 (ng/ml)	20	204.7	64.81	62	245.58	114.55	0.051
Cl/F-1 (l/h/kg)	20	0.35	0.06	62	0.33	0.08	0.001
C_{max} -1 (ng/ml)	20	911.4	289.02	63	1143.43	442.40	0.009
AUC-1 (ng h/ml)	20	3461.9	1089.7	62	4144.2	1685.2	0.040
MRV (ng/ml)	20	247.9	79.55	66	294.59	27.77	0.055

Table 6 Graft rejection in relation to busulfan pharmacokinetics (both groups combined)

	<i>Rejection (n = 5)</i>		<i>No rejection (n = 68)</i>		<i>P-value</i>
	<i>Mean</i>	<i>s.d.</i>	<i>Mean</i>	<i>s.d.</i>	
C_{min} -1 (ng/ml)	113.8	20.3	240.4	103.1	<0.001
C_{ss} -1 (ng/ml)	475.6	180.5	678.6	251.9	0.049
Cl/F-1 (l/h/kg)	0.41	0.09	0.33	0.07	0.081
C_{max} -1 (ng/ml)	844.0	301.1	1138.6	420.1	0.066
AUC-1 (ng h/ml)	2854.4	1082.1	4032.7	1514.9	0.052

Complete pharmacokinetics data were evaluable in 73 cases that were evaluable for rejection.

**Figure 1** Probability of graft rejection depending on busulfan C_{min} -1.

prophylaxis. The OS, EFS and mortality were 96, 90 and 4%, respectively. Using a similar protocol in Class II patients, the OS, EFS and mortality were 86, 83 and 14%,

with 3% rejection. However, in 55 patients with Class III treated with the same protocol, the mortality was 42%; hence, from 1989, 165 patients were treated with 14–16 mg of Bu and 120–160 mg of Cy with OS, EFS, rejection and nonrejection mortality of 79, 58, 30 and 18%. The reduction in mortality was at the price of increased rejection.¹⁷ Ball *et al*⁴ from the Netherlands report a rejection rate of 55% in 21 transplants for thalassemia using Bu/Cy conditioning with or without ATG. In Taiwan, DFS was only 44% with graft rejection being the major cause for failure.¹⁸ However, data from Hong Kong show results similar to those achieved in Pesaro,⁵ while Lawson *et al*¹⁹ from UK, using Bu14 and Cy200 in 55 consecutive transplants for thalassemia, had an 81% thalassemia-free survival and 13.2% rejection. In this study, we have been able to achieve an overall and DFS of 84% in 43 patients who were in Lucarelli class II with no rejection, and a mortality of 16.3% that is similar to data from Pesaro. The DFS of 51% in the Class III patients in this study is also close to the 58% figure of Lucarelli, but the mortality of 37 and 30% in the two studies remains unacceptably high. Alternative approaches to reduce

toxicity and rejection by using a nonmyeloablative regime have been tried, but in one study using fludarabine and TBI all six patients with sickle cell anemia and one with thalassemia rejected, suggesting that this approach would not work in children who have been multiply transfused.²⁰ The more recent approach that the Pesaro group has taken using hydroxyurea and azathioprine appears to be promising and the report of improvement in outcome of sickle cell anemia patients who were on hydroxyurea prior to transplant supports this concept for conditioning.^{21,22}

There are no randomized trials on different conditioning approaches for BMT in thalassemia published in the English language literature. The basic conditioning regimen that we decided to use was Bu at 16 mg/kg; we did not want to reduce the Bu dose to 14 mg/kg since these were multiply transfused children with an increased risk of rejection, and we kept the dose of Cy at 200 mg/kg and added ALG. The addition of ALG was based on the data from Seattle, which showed that the addition of ALG to Cy 200 mg/m² for multiply transfused patients with aplastic anemia reduced the rejection rate.²³ With this regimen (B), no patient in Lucarelli Class II rejected the graft and the mortality was low (16.3%).

The first hypothesis that was tested in this study was to see whether optimizing the systemic exposure to Bu by dosing at 600 mg/m² would reduce the rejection rate without increasing toxicity. Vassal and co-workers^{8,24} first studied the disposition of Bu in children below 3 years and noted a higher clearance, particularly in children with lysosomal storage disorders, and suggest that 600 mg/m² may be a more appropriate dose for children. Hassan *et al*²⁵ demonstrated that the difference in clearance of Bu between children and adults was not significant when normalized to body surface areas, which is in agreement with the data of Vassal and co-workers.^{8,24} In two clinical trials of BMT in children with 600 or 650 mg/m² of Bu^{26,27} using the Bu/Cy regimen, there was no significant increase in HVOD.

In this study we have shown for the first time, in children with thalassemia, that giving Bu at 600 mg/m² is feasible with only two rejections (4.25%) and the only toxicity observed was an increase in GI hemorrhage. This study also shows that Bu at 600 mg/m² is equivalent to the standard conditioning of Bu16/Cy200 with ALG. The design of this study does not allow us to determine the independent contribution of ALG in reducing rejection since we did not have a group that received 16 mg/kg Bu + 200 mg/kg Cy without ALG.

However, we did find significant differences in rejection in relation to Bu kinetic parameters irrespective of whether the patient received 16 mg/kg or 600 mg/m² of Bu. This suggests that systemic exposure to Bu is an important determinant of the efficacy of myeloablation/immunosuppression and therefore transplant outcome in relation to rejection. Since there was no reduction of rejection in the 600 mg/m² group despite the much greater dose in younger children, the effect of interindividual differences in pharmacokinetics is extremely significant. Slattery *et al*²⁸ also showed the wide variability in plasma concentrations in patients receiving oral Bu, and that low concentrations predispose to graft rejection while high levels increase the

risk of toxicity. They subsequently showed in a study of BMT for CML that the Bu C_{ss} median 917 ng/ml was the only statistically significant determinant of relapse in univariate or multivariate analysis.²⁹ This group has also reported that busulfan C_{ss} was the only statistically significant predictor of rejection on univariate logistic regression analysis, with the risk of rejection decreasing with an increase in busulfan C_{ss} in a multivariate Cox time-to-rejection analysis in children undergoing BMT for diseases other than thalassemia.³⁰ They found that an average concentration of Bu at steady state of at least 200 ng/ml was needed to avoid rejection of a matched-sibling graft.²⁸

In this study, in 66 of 68 patients with successful engraftment, the C_{min-1} was greater than 150 ng/ml while in five of the six patients who rejected the graft, the C_{min-1} was below 150 ng/ml. These data are not in accordance with the previously published data by Pawlowska *et al*³¹ from the Pesaro group who did not find any association with Bu kinetics and rejection or mortality. However in their study, which is the only other large series other than this report, that has looked at the pharmacokinetics of Bu in patients with thalassemia, the dose of Bu was 14–16 mg/kg and the Cy dose varied from 120 to 200 mg/kg.

Pawlowska *et al*³¹ also did not find any correlation between Bu levels and toxicity. Grochow *et al* noted the wide inter-individual variation in Bu kinetics and the relationship to VOD, with all six patients who developed VOD in their series having an AUC that was greater than the mean.³² Others have also subsequently noted this association between VOD and Bu kinetics.^{33,34} Ljungman *et al*³⁵ reported a correlation between the minimum level of BU and the severity of alopecia following transplantation, but do not mention whether the AUC or C_{max} was related. Grochow *et al*³² found that in 27 patients with high AUCs (>1500 μ mol/min/l) after the first dose, when the fifth through 16th doses of Bu were decreased, the incidence of VOD was only 18% while those patients with high AUCs who did not receive dose adjustments had a 75% incidence of VOD. The data from this study, however, differ from that published so far in that we found a significantly higher clearance in patients who developed VOD with lower AUC, C_{max} , C_{min} and MRV.⁶ Subsequently, we have found an association between GSTM1 and clearance and speculate that accelerated clearance produces VOD either by depletion of glutathione or by increased production of a toxic metabolite.³⁶ There was an association between gastrointestinal bleeding and high Bu levels. Studies showing inter-patient variability and relationship of toxicity, relapse and rejection to Bu levels have led to monitoring of drug levels and dose adjustment based on the kinetics of the first dose administered. Bleyzac *et al* showed that this strategy resulted in a dose reduction in 69%, increase in 27.6% and no change in only 0.03%. They also showed that this resulted in a reduction in graft failure, VOD and an improvement in survival when compared to a control group where no drug monitoring or dose adjustment was made.³⁷ Demirer,³⁸ however, reports that dose escalation of BU based on targeted plasma levels did not improve the outcome in patients with CML undergoing BMT. Dupuis *et al* from Toronto found that a change from the initial Bu

dose was required to achieve the target AUC in 34 courses (87%). Most children >1 to 5 years required dose increments, while most children >5 years required dose reductions.³⁹ Current data suggest that therapeutic drug monitoring may improve outcome by ensuring adequate systemic exposure and therefore reduced relapse, rejection and regimen-related toxicity.⁴⁰ However, the level of different pharmacokinetic parameters of Bu to achieve this result may depend on the disease for which transplantation is being done and specific host and donor characteristics. Intravenous busulfan may reduce the variability due to differences in gastrointestinal absorption and first-pass metabolism and have less VOD, but this needs to be documented in a randomized trial for thalassemia.

This study shows that the systemic exposure to Bu is important in reducing rejection and the best way to ensure this would be by individualized dosing based on first dose pharmacokinetics.

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