Biology of Blood and Marrow Transplantation 8:619-624 (2002)

A Limited Sampling Strategy for Pharmacokinetic Directed Therapy with Intravenous Busulfan

William P. Vaughan, Delicia Carey, Stephanie Perry, Andrew O. Westfall, Donna E. Salzman

Departments of Medicine (Division of Hematology/Oncology) and Pharmacology, Bone Marrow Transplantation Program, and Comprehensive Cancer Center, The University of Alabama at Birmingham, Birmingham, Alabama

Correspondence and reprint requests: William P. Vaughan, MD, MBA, University of Alabama at Birmingham, 541 Tinsley Harrison Tower, 1900 University Blvd, Birmingham, AL 35294-0006 (e-mail: WVaughan@uabmc.edu).

Received February 20, 2002; accepted August 9, 2002

ABSTRACT

High-dose busulfan is widely used in allogeneic and autologous marrow transplantation preparative regimens. Variation in the area under the concentration/time curve (AUC) for oral busulfan results in substantial risk of over or under treatment with excess risk of toxicity or relapse. Use of the IV formulation reduces this variability by eliminating variability in absorption. Variability due to drug metabolism remains, but simplified pharmacokinetic study may be employed to achieve a specific target AUC. In conventional sampling strategies for determining AUC after oral administration, 12 samples are used over 6 hours to assure accurate tracking of erratic absorption. With IV busulfan there is no necessity for measuring plasma levels during the infusion because busulfan pharmacokinetics are well described with a single-compartment, first-order elimination model. In theory, only peak and trough levels should be necessary, but for assurance of reliability in clinical decision making, it must be possible to identify outlier values. This process requires at least 4 samples. We studied a total of 59 adult patients receiving a 2-hour IV busulfan infusion to develop a limited sampling strategy (LSS). At the end of a 2-hour infusion, we collected 11 samples from 18 patients and compared the AUC obtained when all samples were used with the AUC obtained when samples were collected only hourly. The mean AUC calculation was 5% higher (1002 versus 956 μM-min) and the coefficient of variation (CV) was substantially better (4.6% versus 8.2%) when only the postinfusion samples were used. A follow-up study of 41 consecutive patients demonstrated that all patients were easily evaluable with a coefficient of variation (CV) for the AUC of 2.6%. To validate this approach, we analyzed pharmacokinetic data on 60 patients in the phase II clinical trial of the IV formulation described by Anderson et al. Data on an additional 36 patients from a companion study also were analyzed. The AUC based on all 11 samples from each patient were compared with the AUC based on the 5 postinfusion samples. The results of this analysis confirmed comparable reliability and possibly superior precision of the University of Alabama at Birmingham 5-sample LSS. These results validated that LSS for IV busulfan will make possible meaningful and accurate comparisons of busulfan versus TBI-based preparative regimens and comparison of dose intensity of busulfan-containing preparative regimens in trials of submyeloablative transplantation.

KEY WORDS

Busulfan • Limited sampling strategy • Pharmacokinetic directed therapy

W.P.V. served as a consultant to Orphan Medical Inc. in the preparation of the Busulfex New Drug Application and for the preparation and presentation of the pharmacokinetics data from the phase I and II Busulfex registration trials to the Oncology Drug Advisory Committee of the U.S. Food and Drug Administration. He has served intermittently since then as a consultant to Orphan Medical in pharmacokinetics and marketing.

This work was presented in part at the February 1999 meeting of the American Society of Blood and Bone Marrow Transplantation in Anaheim, CA, and at the February 2000 meeting of that society in Keystone, CO.

INTRODUCTION

In the 1970s Tutschka and Santos [1,2] developed busulfan as a substitute for total body irradiation (TBI) in bone marrow transplantation preparative regimens, first in a rat myelocytic leukemia model and then in clinical trials. Santos et al. [1] reported in a phase I trial that very high dose escalation was possible before nonmarrow toxicity was encountered and that there was no delay in engraftment, or even enhancement of engraftment. Busulfan was given in an every-6-hour

(q6h) schedule for 16 doses and was followed by cyclophosphamide 50 mg/kg daily for 4 consecutive days. The busulfan dose was escalated from 8 to 20 mg/kg (total dose) before a high incidence of pulmonary toxicity was encountered. The response rate in this group of 51 patients with mostly advanced acute myelocytic leukemia was excellent.

Tutchska and colleagues [2] reported results of a phase II study of a reduced dose regimen containing busulfan at 16 mg/kg and cyclophosphamide at 120 mg/kg (BuCy2). The preparative regimen–related mortality in this trial was much lower than in the previous study, and there was demonstration of antileukemia benefit in acute and chronic myeloid leukemia as well as acute lymphocytic leukemia. High-dose etoposide (VP16) (60 mg/kg over 4 h) was added to BuCy2 in a clinical trial at the University of Nebraska with the intent of further strengthening the activity in lymphocytic malignant disease, especially malignant lymphoma [3]. No significant increase in regimen-related mortality was seen with this addition, and the number and duration of responses were encouraging. The BuCy2 regimen and such derivatives have become the "standard" for comparison against TBI-based regimens. More than 500 trials involving more than 15,000 patients receiving allogeneic or autologous transplants have been described in the literature (G. Bream, personal communication, February 2000). Complete response rates for busulfan-based regimens exceed 50% in the aggregate for acute monocytic leukemia (AML), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), and non-Hodgkin's lymphoma [1-10].

Until recently a major drawback to the use of high-dose busulfan was that it was available only in an oral formulation. The erratic and unpredictable absorption of this formulation from the gastrointestinal tract can result in wide interpatient and intrapatient variations in the busulfan plasma concentrations achieved [11-15]. Several investigators have reported hepatic venoocclusive disease (VOD) of the liver, which leads to fatal liver failure, as the most serious side effect [11-13]. The literature reports that VOD occurs with a frequency of approximately 20% in patients who receive oral busulfan–based preparative regimens (administered at the standard dose of 1.0 mg/kg q6h for 16 doses) prior to hematopoietic progenitor cell transplantation [16]. Grochow et al. [11] and Dix et al. [13] have demonstrated an association between the AUC for busulfan plasma concentration and the risk of this serious and often fatal complication. Dix et al. further demonstrated that VOD is seldom observed when the AUC of first-dose busulfan in a 16-dose q6h oral administration regimen is less than 1500 μM-min [13]. Thus busulfan dose reduction can reduce the risk of VOD, but Bolinger et al. [17] have reported that simple dose reduction may not improve overall survival owing to a correlation between low busulfan AUC and increased relapse risk, at least in ALL.

In a phase I clinical trial conducted at MD Anderson Cancer Center, Stanford University, and the University of Alabama at Birmingham (UAB) [18], dose escalation of an IV busulfan formulation was administered as the first dose of an otherwise all-oral 16-dose busulfan regimen in the BuCy2 preparative regimen. The results of this trial demonstrated that an IV busulfan dose of 0.8 mg/kg was approximately equivalent to a 1-mg/kg oral dose with respect to AUC achieved. This formulation was then taken into multicenter phase II clinical trials in autologous and allogeneic transplantation for hematologic malignant disease. The results of the allogeneic transplantation trial were reported by Andersson and colleagues [19]. With the 0.8-mg/kg dose in an otherwise standard BuCy2 regimen, excellent response rates and duration were achieved in myelodysplasia syndrome, AML, CML, and malignant lymphoma.

A subgroup of 12 patients in this clinical trial were given oral busulfan for dose 1 at 1 mg/kg and the remaining 15 doses of the IV formulation at 0.8 mg/kg. Pharmacokinetic analysis of doses 1 and 9 in these trials demonstrated equivalent AUC and half-life ($T_{1/2}$) for these doses of the oral and IV preparation, respectively, but a very much more predictable shape and timing of the AUC curve for the IV preparation. Pharmacokinetic study of IV busulfan in 96 patients in these 2 trials demonstrated remarkable intrapatient consistency in AUC achieved between doses 1, 9, and 13 but a still substantial persistent interpatient variability ranging from 600 to more than 1600 μM-min AUC for dose 1.

There was no pharmacokinetic dose adjustment in either of these trials. The mean AUC for first-dose IV busulfan in the allogeneic trial reported by Andersson was 1106 μM-min, and the range was 556 to 1673 μM-min. The incidence of VOD was 5 of 61 patients (8.2%), and 2 cases of VOD were fatal (3.3%). Two of the 5 cases of VOD occurred among the 4 patients with a first-dose AUC >1500, and 1 of these cases was fatal [20].

The predictability of the shape of the AUC for IV busulfan suggests that a limited sampling strategy (LSS) could be developed that would allow pharmacokinetically determined precise busulfan dosing. Using the large Orphan Medical data set from these 2 pivotal trials, including the one reported by Andersson et al. [19], we describe the development of such an LSS and its validation.

PATIENTS AND METHODS

Development of the LSS

Between March 1999 and September 2000 at UAB, the pharmacokinetics of busulfan were analyzed for the first dose of the IV busulfan component of the preparative regimens of 59 patients. The IV busulfan was given first in all of these regimens and was followed by either fludarabine or cyclophosphamide with or without etoposide. The initial busulfan dose schedule in each case was 27.5 mg/m^2 infused over 2 hours q6h for 16 doses. The dose and schedule were adjusted on the basis of dose 1 pharmacokinetics to achieve a protocol specified or a patient-specific target AUC beginning with dose 8.

Busulfan was infused with a fresh infusion set whenever pharmacokinetic assessments were to be performed. The tubing was primed with the busulfan, and the entire volume plus a volume of normal saline flush equal to the priming volume was infused at a constant rate into a central venous catheter over the 2-hour infusion time. The samples were drawn through the same line after additional flushing. The actual start and stop times were recorded, as were the exact times when the blood levels were drawn. Busulfan levels were obtained with a properly validated technique in the Emory University Hospital special chemistry laboratory. In

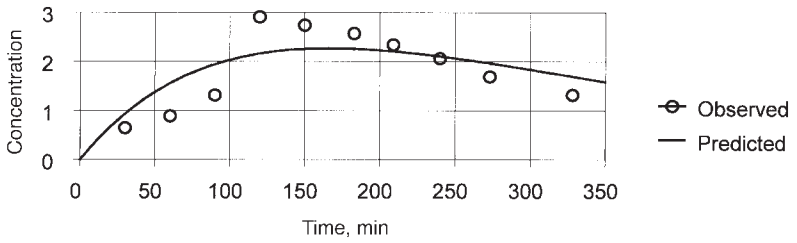

Figure 1. Delayed and variable absorption of oral busulfan prohibits the use of modeling to develop an LSS.

a few exceptions, busulfan levels were measured at Quest Laboratories (4 sets) or in the laboratory of Dr. John Slattery (1 set) at the Fred Hutchinson Cancer Research Center, Seattle, when the Emory laboratory was unavailable. The data were analyzed with the WinNonlin program version 2.5 or 3.0 (Pharsight Corporation, Mountain View, CA).

The first cohort studied (cohort A) consisted of 18 patients who had the historical standard 12 samples drawn for busulfan levels. On the basis of results of analysis of the pharmacokinetic data obtained for these patients, a LSS with only 5 postinfusion samples was studied for the subsequent 41 patients (cohort B). Several patients had only 4 valid samples analyzed owing to errors in obtaining samples, labeling samples, documenting sampling time, or problems at the laboratory. No patient had fewer than 4 valid samples for analysis.

Validation of the LSS

Extensive pharmacokinetic data (supplied by Orphan Medical, Minnetonka, MN) were obtained in 2 simultaneous phase II trials of IV busulfan in autologous and allogeneic hematopoietic stem cell transplantation between 1996 and 1998. Busulfan, 0.8 mg/kg, was administered as a 2-hour IV infusion q6h in the BuCy2 regimen [2]. Ideal, actual, or adjusted ideal body weight was used according to institutional preference. Eleven serum samples were obtained for busulfan levels over the 6-hour dosing interval of the first dose. Complete dose 1 data for 96 of the 102 patients in these 2 trials were analyzable. The larger of the 2 trials described by Andersson et al. [19] was conducted with 60 patients fully evaluable for pharmacokinetics. Consequently we reanalyzed this data set for the validation of our LSS. All 96 patients' entire data were then analyzed to demonstrate accuracy of fit to model.

The data from these trials were originally analyzed with NONMEM software (GloboMax, Hanover, MD) and a noncompartmental assumption to allow comparison with oral busulfan pharmacokinetics, which cannot be modeled owing to variable absorption from the gastrointestinal tract. To calculate pharmacokinetic parameter estimates, we reanalyzed these data using all 11 samples or only the 4 or 5 samples drawn after the end of the infusion. This analysis was performed with the same WinNonlin software and assumptions used for the UAB data, including the single-compartment, first-order elimination model.

Statistical Methods

This study was designed to validate the LSS for pharmacokinetically directed dosing of high-dose IV busulfan in bone marrow transplantation preparative regimens. The analyses were based on 3 groups, 11 samples per patient, 5 postinfusion samples per patient, and the peak-trough samples per patient. Descriptive statistics (mean, median, range, standard deviation, and CV were calculated on patient pharmacokinetic parameter estimates. Statistical analyses were performed with the SAS statistical package version 8.0 [21]. All variables passed the Kolmogorov-Smirnov test for normality of distribution either with or without transformation. Thus presentation of the data as mean and standard deviation and results of parametric statistical analysis was most appropriate. An unpaired *t* test was used to compare the mean AUC, $T_{1/2}$, and CV for the 3 possible pairwise group combinations. All results of statistical analysis were similar when nonparametric statistical tests were used.

RESULTS

Unlike data on oral busulfan administration, the serial drug level data on IV administration in our studies was found to fit very well to a single-compartment, first-order elimination pharmacokinetic model available in the WinNonlin program and many other standard statistical packages (Figures 1 and 2). On the basis of this observation, we used this pharmacokinetic model to determine dose 1 pharmacokinetics in 18 patients in cohort A. Pharmacokinetic parameters, including AUC and $T_{1/2}$, were computed with all 10 to 12 samples drawn and compared with the same parameters computed with only the 4 or 5 postinfusion samples (Table 1). The calculated AUC was slightly higher when only the postinfusion samples were used for this analysis, but this difference was not statistically significant. However, this analysis demonstrated greater precision (fit to model) when only the postinfusion specimens were used for the calculation of AUC. The mean CV for the dose 1 AUC for this group of 18 patients was 4.6% with the postinfusion drug levels versus 8.2% with all levels obtained. Results for an additional group of 41 patients (cohort B) studied only with 4 or 5 postinfusion samples confirmed this excellent fit to model.

The original pharmacokinetic analyses in the Orphan Medical–sponsored phase II trails were performed separately for the 2 trials with noncompartmental NONMEM statistics. For comparison, we analyzed the larger trial data set (59 patients) with the WinNonlin software and the single-compartment, first-order elimination assumption. All 11 samples obtained were used in both computations. Table 2 demonstrates that the results are essentially identical.

All combined first-dose data from both phase II trials were reanalyzed with this model and software. We compared

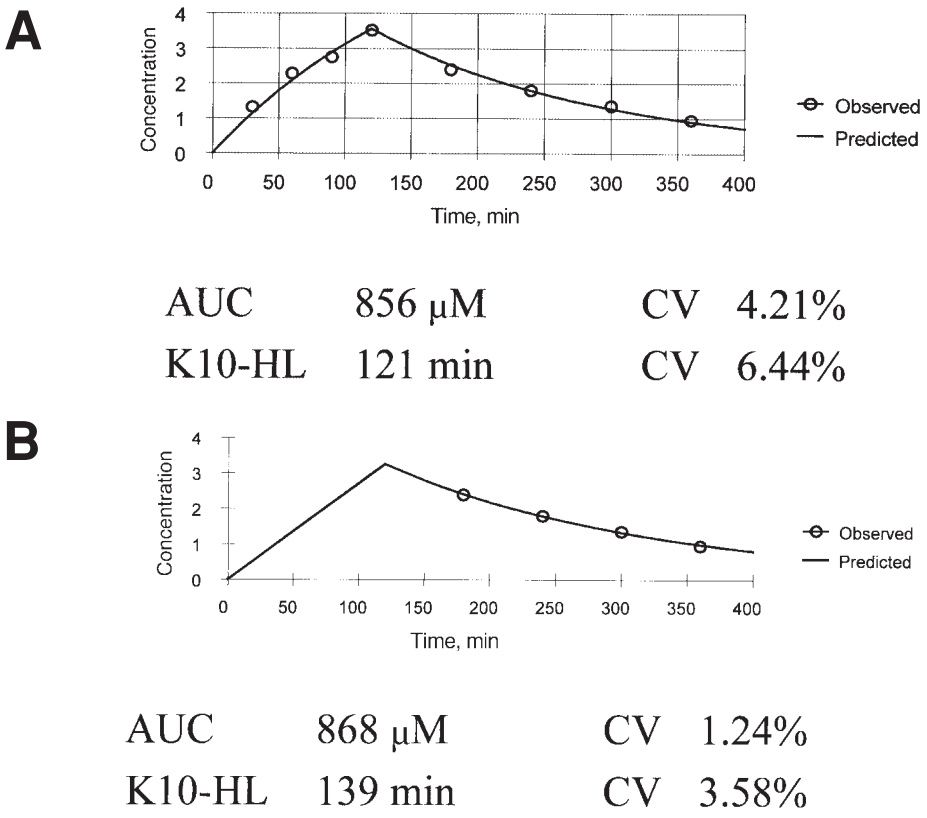

Figure 2. A, Fit to single-compartment, first-order elimination model for IV busulfan. B, Improved fit to single-compartment, first-order elimination model for IV busulfan with an LSS.

the data obtained when all 11 samples were used with the data obtained when only the postinfusion samples were used. Tables 3 and 4 illustrate the accuracy, precision, and validity of the UAB LSS. Table 3 demonstrates the accuracy of the UAB LSS and the only slightly less accurate results obtained when only peak and trough measurements were used. The means and ranges for the AUC and $T_{1/2}$ calculations with the 3 different sample sets were essentially identical. Table 4 demonstrates the superior precision of the UAB LSS. The mean CV for AUC and $T_{1/2}$ determination with the UAB LSS is less than half that for the 11-sample whole-data set, and this difference is highly significant. Examination of the graphic outputs demonstrated that this better fit is the result of greater variation from fit to model for the levels obtained during the infusion (Figure 2).

Table 1. *Comparison of Mean AUC and CV of AUC Fit to Model for IV Busulfan with Single-Compartment, First-Order Elimination Assumption in Pharmacokinetic Analysis**

Group	n	Busulfan Level		Mean AUC, μM-min	Mean CV
		Drawn	Used		
A	18	10-12	All	956	8.2%
A	18	10-12	4-5	1002	4.6%
B	41	4-5	4-5	1069	2.6%

*All $P > .5$.

DISCUSSION

The difficulty in assessing dose delivered with oral administration of high-dose busulfan in preparative regimens for hematopoietic stem cell transplantation results in significant risk of lethal toxicity due to inadvertent overdosing and the potential for recurrent or persistent malignant disease after transplantation owing to inadvertent underdosing. Pharmacokinetic studies of oral busulfan have demonstrated wide variation in time to peak concentration and AUC, primarily because of wide variation in rate of intestinal absorption and uncertainty of dosing owing to losses from vomiting. Pharmacokinetic analysis requires sophisticated modeling and interpretation not widely available, and

Table 2. *Comparison of AUC and $T_{1/2}$ Calculation with a Noncompartmental Compiled Analysis versus the Single-Compartment, First-Order Assumption in the WinNonlin Software for 60 Patients Treated in a Single Phase II Clinical Trial**

	AUC, μM-min		$T_{1/2}$, h	
	Mean	SD	Mean	SD
Noncompartmental				
11-Sample	1135	345	2.9	0.93
Compartmental				
11-Sample	1105	346	2.7	0.97

*All $P > .5$.

Table 3. *Comparison of 11-Sample versus UAB LSS for Busulfan Pharmacokinetic Parameters for 96 Patients from the Combined Phase II IV Busulfan Clinical Trials Data in a Single-Compartment, First-Order Elimination Model*

	AUC, μM-min		$T_{1/2}$, h	
	Mean	SD	Mean	SD
11-Sample	1134	329	2.9	1.03
UAB LSS	1138	308	2.9	0.75
Peak and trough	1192	378	3.1	1.02

accurate determination of AUC cannot be reliably established in as many as 35% of patients [13]. Studies with IV busulfan have demonstrated that almost all patients are evaluable and that there is excellent intrapatient consistency in level achieved as a function of dose given.

In this study we developed an LSS and used a single-compartment, first-order elimination model (WinNonlin 3.0) for reliable analysis of busulfan AUC and $T_{1/2.}$ We further showed by applying this LSS and the compartmental model to the data on 96 patients in phase II trials sponsored by Orphan Medical that there is no difference in the calculated AUC or $T_{1/2}$ from the values originally calculated with all 11 samples and a noncompartmental analysis. The precision of the LSS was higher than for the traditional 11-sample strategy, as demonstrated by the statistically significantly lower average CV for both AUC and $T_{1/2}$. This result is to be expected because clinical errors such as specimen contamination with drug, variation in infusion rate, or inaccurate sample time recording would be expected to result in larger variation in the levels obtained during infusion of the drug rather than after infusion.

Theoretically only peak and trough concentrations should be required for accurate determination of AUC and $T_{1/2}$ when the drug used has a pharmacokinetic profile that can be described with a single-compartment, first-order elimination model. However, the need for rapid turnaround and the inability to repeat questionable results in a timely manner argue for the 4- or 5-sample strategy. That this simple and easy to use method cannot be applied to oral busulfan is illustrated by Figure 1.

This LSS combined with test dosing should allow a precisely targeted AUC of busulfan for clinical use to specify drug exposure rather than dose administered. This will improve the risk profile for busulfan in clinical practice, be useful for comparative trials between busulfan and other preparative regimens, especially TBI-based regimens, and allow safer study of new combinations of preparative regimens. Use of the LSS should also reduce cost, because the number of samples is fewer and the analysis can be performed without highly sophisticated consultative expertise.

The ability to acquire busulfan dosing precision will allow better studies of the trade-off between regimen-related toxicity and antimalignancy effect in the setting in which graft-versus-malignancy benefit may be the primary treatment goal (submyeloablative allogeneic hematopoietic stem cell transplantation) [10,22,23].

Table 4. *Comparison of 11-Sample versus UAB LSS for Fit to a Single-Compartment, First-Order Elimination Model for 96 Patients with the Combined Phase II Trials Busulfan Pharmacokinetic Data*

	CV of AUC		CV of $T_{1/2}$	
	Mean	SD	Mean	SD
11 Sample	12.5	8.7	19.3	12.66
UAB LSS	5.8	9.0	9.8	13.95
P	<.0001		<.0001	

ACKNOWLEDGMENT

This work was sponsored through grants FD-R-001112-02 and FD-R-001650-02-01 from the US Food and Drug Administration to Orphan Medical, Inc. (OMI).

REFERENCES

1. Santos GW, Tutschka PJ, Brookmeyer R, et al. Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. *N Engl J Med.* 1983;309:1347-1353.
2. Tutschka PJ, Copelan EA, Klein JP. Bone marrow transplantation for leukemia following a new busulfan and cyclophosphamide regimen. *Blood.* 1987;70:1382-1388.
3. Vaughan WP, Dennison JD, Reed EC, et al. Improved results of allogeneic bone marrow transplantation for advanced hematologic malignancy using busulfan, cyclophosphamide and etoposide as cytoreductive and immunosuppressive therapy. *Bone Marrow Transplant.* 1991;4:489-495.
4. Deeg HJ, Shulman H, Anderson J, et al. Allogeneic and syngeneic marrow transplantation for myelodysplastic syndrome in patients 55 to 66 years of age. *Blood.* 2000;95:1188-1194.
5. Toze CL, Shepherd JD, Connors JM, et al. Allogeneic bone marrow transplantation for low-grade lymphoma and chronic lymphocytic leukemia. *Bone Marrow Transplant.* 2000;25:605-612.
6. van Besien K, Sobocinski KA, Rowlings PA, et al. Allogeneic bone marrow transplantation for low-grade lymphoma. *Blood.* 1998;92:1832-1836.
7. Salzman DE, Briggs AD, Vaughan WP. Bone marrow transplantation for non-Hodgkin's lymphoma: a review. *Am J Med Sci.* 1997;313:228-235.
8. Przepiorka D, Anderlini P, Ippoliti C, et al. Allogeneic blood stem cell transplantation in advanced hematologic cancers. *Bone Marrow Transplant.* 1997;19:455-460.
9. Boussiotis VA, Freedman AS, Nadler LM. Bone marrow transplantation for low-grade lymphoma and chronic lymphocytic leukemia [review]. *Semin Hematol.* 1999;36:209-216.
10. Sahebi F, Copelan E, Crilley P, et al. Unrelated allogeneic bone marrow transplantation using high-dose busulfan and cyclophosphamide (BU-CY) for the preparative regimen. *Bone Marrow Transplant.* 1996;17:685-689.
11. Grochow LB, Jones RJ, Brundrett RB, et al. Pharmacokinetics of busulfan: correlation with veno-occlusive disease in patients undergoing bone marrow transplantation. *Cancer Chemother Pharmacol.* 1989;25:55-61.
12. Vassal G, Hartmann O, Benhamou E. Busulfan and veno-occlusive disease of the liver. *Ann Intern Med.* 1990;112:881.
13. Dix SP, Wingard JR, Mullins RE, et al. Association of busulfan area under the curve with veno-occlusive disease following BMT. *Bone Marrow Transplant.* 1996;17:225-230.

14. Slattery JT, Sanders JE, Buckner CD, et al. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. *Bone Marrow Transplant.* 1995;16:31-42.
15. Buggia I, Locatelli F, Regazzi MB, Zecca M. Busulfan. *Ann Pharmacother.* 1994;28:1055-1062.
16. McDonald GB, Hinds MS, Fisher LD, et al. Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. *Ann Intern Med.* 1992;118:255-267.
17. Bolinger AM, Zangwill AB, Slattery JT, 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.
18. Andersson BS, Madden T, Tran HT, 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.
19. Andersson BS, Kashyap A, Gian V, et al. Conditioning therapy and 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.
20. Vaughan WP, Cagnoni P, Fernandez H, et al. Decreased incidence of and risk factors for hepatic veno-occlusive disease with an intravenous busulfan (BU) containing preparative regimen for hematopoietic stem cell transplantation (HSCT). Paper presented at: 40th Annual Meeting of the American Society of Hematology; December 4-8, 1998; Miami Beach, FL.
21. *SAS/STAT User's Guide.* Version 8, Volumes 1 and 2. 4th ed. Cary, NC: SAS Institute, Inc.; 1999-2001.
22. Weiden PL, Sullivan KM, Flournoy N, et al. Anti-leukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. *N Engl J Med.* 1981;304:1529-1533.
23. Champlin R, Khouri I, Komblau S, Molidrem J, Giralt S. Reinventing bone marrow transplantation: non-myeloablative preparative regimens and induction of graft-vs-malignancy effect. *Oncology (Huntingt).* 1999;13:621-628.