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Conditioning Therapy With Intravenous Busulfan and Cyclophosphamide (IV BuCy2) for Hematologic Malignancies Prior to Allogeneic Stem Cell Transplantation: A Phase II Study

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ABSTRACT

Busulfan (Bu) is commonly used as a component of conditioning regimens for hematopoietic stem cell transplantation. Precise delivery of the oral formulation is compromised by erratic gastrointestinal absorption. An IV Bu formulation was developed to provide dose assurance and complete bioavailability. In a phase I study, the plasma bioequivalence of IV Bu was established at approximately 80% of the oral dose. We now report the findings of the first phase II study, in which 61 adults with hematologic cancers were treated with a Bu-cyclophosphamide (BuCy) regimen consisting of IV Bu (0.8 mg/kg every 6 hours × 16) followed by Cy (60 mg/kg qd × 2) and transplantation of stem cells from an HLA-matched sibling donor. The median age of study participants was 37 years; 75% of patients had active disease; 48% were heavily pretreated, and 13% had undergone a prior transplantation. Median follow-up was 2.3 years; median time to engraftment (absolute neutrophil count, >0.5 × 10^9/L) was 13 days; 100% of patients with cytogenetic and/or molecular markers had documented chimerism; and there were no engraftment failures. Two-year overall and disease-free survival were 67% and 42%, respectively. There were no unexpected toxic reactions. Fatal veno-occlusive disease occurred in 2 patients, 1 of whom had undergone a prior transplantation. Treatment-related mortality at 100 days was 9.8% (6/61). Bu pharmacokinetics after IV drug administration demonstrated high inter- and intrapatient consistency; 86% of patients maintained an area under the curve between 800 and 1500 μMol-min. In conclusion, the IV Bu in this regimen was very well tolerated and demonstrated excellent antitumor efficacy, most likely because of dose assurance with predictable pharmacokinetics.

KEY WORDS

Allogeneic hematopoietic stem cell transplantation • Busulfan • Cyclophosphamide • Intravenous busulfan

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established treatment modality for patients with hematologic malignancies [1]. The most commonly used pretransplantation conditioning therapy is a combination of total body irradiation (TBI) and cyclophosphamide (Cy), possibly with the addition of other cytotoxic agents [2]. Although the delivery of TBI is very precise, it is fraught with late complications such as cataracts, secondary tumors, and retardation of physical and intellectual development in children [3-12]. In addition, TBI is often contraindicated for patients who have had previous therapeutic radiation

therapy. As an alternative, high-dose oral busulfan (Bu) in combination with Cy was introduced [13,14]. After modification of the Cy dose (BuCy2) [15], this regimen became widely accepted, and it is now the most commonly used non–TBI-based pretransplantation conditioning treatment [2].

Although the oral BuCy2 regimen is generally well tolerated, there has been concern as to whether oral BuCy2 is immunosuppressive enough to consistently allow engraftment, especially when the donor is partially mismatched or when an unrelated donor is used [16-19]. Hepatic and neurological toxicities are also worrisome, particularly in heavily pretreated patients. Several investigators have associated the serious side effects of Bu-based therapy with systemic drug exposure. Thus, a high area under the plasma concentration–versus–time curve (AUC) has been associated with an increased risk for hepatic veno-occlusive disease (VOD) [19-24]. In addition, the hepatic first-pass Bu exposure has been proposed to contribute to VOD [25]. Furthermore, high Bu levels and consequent drug penetration of the central nervous system (CNS) have produced seizures [26,27]. Ljungman et al. suggested that an increased risk for serious treatment-related toxicity/mortality is connected with high Bu blood concentrations [28]. Conversely, low Bu AUC levels have been correlated with an increased risk for graft rejection and leukemic relapse [17-19].

Unpredictable and erratic intestinal absorption of Bu contributes to wide interpatient variations in bioavailability and AUC measurements. The interpatient variability associated with oral Bu has been estimated to be as high as 10-fold or more [20-23,26,27]. To reduce this variability and improve the safety of oral Bu administration, a practice of individualized, targeted dosing evolved [20-22]. Although this approach is intellectually appealing, it appears to yield an AUC within the desired interval in only about half of the patients, making further modifications of subsequent doses necessary [19,20,29]. The requirement for dose modification stems from multiple problems inherent with the use of oral Bu. First, nausea and vomiting due to gastric irritation interferes with intestinal Bu absorption. Second, the interdose bioavailability may vary as much as 2- to 3-fold [27]. Third, delayed Bu absorption and elimination occurs in 10% to 25% of the patients, pushing Bu blood concentration to its maximum more than 6 hours after drug ingestion and thus greatly interfering with the reliability and reproducibility of the pharmacokinetic (PK) information [23,30,31]. These problems have called into question the value to a particular patient of an individualized dosing strategy [32].

Precise, predictable Bu dosing is important in pretransplantation conditioning therapy, and the unpredictable bioavailability of oral Bu prompted us to develop an alternative. We designed a pharmaceutically acceptable intravenous (IV) Bu formation to address the erratic intestinal absorption and any hepatic first-pass effect [33,34], then determined that 0.8 mg/kg IV should yield a median AUC of 1100 to 1200 μMol-min [35]. This IV Bu dose plus Cy in a modified BuCy2 regimen should be cytoreductive and immunosuppressive enough to consistently ensure engraftment, yet stay short of the 1500–μMol-min level associated with an increased risk for serious VOD [23]. A 2-hour infusion was chosen to mimic a typical Bu elimination pattern following oral dosing.

This IV BuCy2 regimen has now been used without PK-guided dose adjustment as pretransplantation conditioning therapy for patients undergoing allogeneic HSCT for hematologic cancers. We now report the clinical outcome of these patients and the PK data obtained.

PATIENTS AND METHODS

Eligibility Criteria

Patients with the following hematologic malignancies were eligible for this study, provided that they did not qualify for a protocol of higher institutional priority: (1) acute leukemia failing induction chemotherapy, in first remission with a high risk for relapse or past first remission; (2) chronic myelogenous leukemia (CML); (3) myelodysplastic syndrome (MDS); and (4) primary refractory or resistant relapsed Hodgkin's disease (HD) or non-Hodgkin's lymphoma (NHL). Patients were to have a physiologic age between 15 and 55 years and a Zubrod performance status of <2. The eligibility criteria also included normal (or with minor deviations, judged not to be of clinical significance) renal and hepatic function (serum creatinine ≤1.5 mg/100 mL, bilirubin ≤1.0 mg/100 mL, alanine aminotransferase ≤3× the upper normal limit), a cardiac left ventricular ejection fraction ≥50%, pulmonary function tests including forced expiratory volume in 1 second ($FEV_{1.0}$) and diffusion capacity of the lung for carbon monoxide (DLCO) ≥50% of predicted, negative serology for hepatitis B and human immunodeficiency virus (HIV), and a life expectancy of at least 12 weeks. Patients were also required to have either marrow or granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood progenitor cells (PBPC) available from an HLA-matched related donor (without T-cell depletion). All patients voluntarily signed a written informed consent in accordance with institutional guidelines.

Preparatory Regimen

The treatment was modified from Tutschka et al. [15]: Bu was administered at 0.8 mg/kg body weight IV over 2 hours every 6 hours for 16 doses followed by Cy at 60 mg/kg IV over 1 hour for 2 doses on 2 consecutive days. After a day of rest, the HSCT was performed. The Bu formulation used in this trial, Busulfex (busulfan) injection, (Orphan Medical, Minnetonka, MN), consisted of Bu (6 mg/mL) dissolved in dimethylacetamide (DMA, 33%, vol/vol) and polyethylene glycol-400 (PEG400, 67%, vol/vol) [33,34]. The IV Bu dose was diluted in normal saline or 5% dextrose to approximately 0.5 mg/mL and infused via a controlled-rate infusion pump through a central venous catheter. The final Bu administration solution was stable for 8 hours at room temperature and for 12 hours if refrigerated [36]. A fixed-dose regimen for Bu was used and was calculated based on the lower of actual or ideal body weight or based on adjusted ideal body weight, the choice depending on the participating institution's practice.

Supportive Care

Phenytoin was administered before and during Bu treatment. MESNA, antiemetics, blood components, and other supportive measures, such as the use of recombinant G-CSF, were used according to the guidelines of the participating

institutions. Prophylaxis against graft-versus-host disease (GVHD) consisted of tacrolimus or cyclosporin A combined with low-dose methotrexate (MTX) and/or steroids.

Evaluation of Therapy

Clinical. The clinical end points of the study consisted of the regimen-related toxicity pattern, engraftment, overall survival (OS), disease-free survival (DFS), and relapse. Engraftment was defined as the first day the absolute neutrophil count (ANC) exceeded 0.5×10^9 cells/L. Failure to reach an ANC of 0.5×10^9 cells/L by day 100 after HSCT was defined as no engraftment. Late graft failure was defined as initial engraftment with documented donor-derived hematopoiesis followed by loss of graft function. Informative cytogenetic restriction fragment length polymorphism (RFLP) and/or fluorescence in situ hybridization (FISH) data were collected to support the clinical impression of engraftment, but these assays were not a mandatory part of the protocol, and the data were not available for all patients. Clinical remission was defined for patients with leukemia as the absence of malignant cells in the marrow with normalization of marrow morphology and peripheral blood counts; for patients with lymphoma, remission was defined as the resolution of mass disease on physical examination, computed tomography (CT) scans, and/or gallium scan, as appropriate. Time to relapse and progressive disease were calculated from transplantation to the day of detection. Length of survival was defined as time from HSCT to the day of death, with the cause of death noted. Kaplan-Meier estimates were calculated for OS and DFS [37]. The latter was defined as survival in continuous clinical remission (CCR): relapse and death were events, and patients surviving in CCR were censored at last follow-up. Descriptive statistics were computed for time to myeloablation, duration of neutropenia, time to engraftment, time to disease progression, and survival.

During hospitalization, all patients were monitored daily for adverse events and hematologic parameters. Clinical chemistry parameters were evaluated at least twice weekly. After discharge and up to day 100 post-HSCT, all patients were followed for treatment-related toxicity (weekly), for the quality of engraftment, and for relapse. After HSCT day 100, disease status and survival were followed at least quarterly.

Hepatic VOD was diagnosed by each site's principal investigator based on clinical examination and laboratory findings. This primary assessment was verified by an independent reviewer, who did not participate as a site investigator. The reviewer retrospectively applied the Jones criteria [38] to all reported VOD cases. Additionally, he searched the clinical database for subjects with a bilirubin >2 mg/dL who also fulfilled at least 1 of the Jones criteria to try to identify any additional patients with VOD. All other toxic events were defined by the modified National Cancer Institute criteria. Data were collected across all participating centers on standardized case report forms using prospectively established data collection guidelines. All data were 100% monitored by an independent clinical research associate. Database entry was double-entry verified and set up with established edit checks.

Pharmacokinetics. The objective was to describe the PK characteristics of IV Bu when administered in the prescribed regimen. Blood samples for assay of Bu concentrations were drawn in conjunction with the first and ninth (steady-state) infusions: immediately before drug infusion (trough) and at 15, 30, and 45 minutes after the start of infusion, at 5 minutes before the end of infusion (peak; end of infusion), and at 15, 30, 60, 120, 180, and 240 minutes after the end of the infusion. In addition, a sample was taken immediately before the 13th infusion (trough) and 5 minutes before its completion (peak). All blood samples were collected from a peripheral IV line. The samples were placed on ice and carried to the laboratory. After centrifugation in a refrigerated centrifuge, the plasma was cryopreserved at –70°C until assayed with a gas chromatographic–mass selective detection assay (GC-MSD) [17, modified from 39].

The PK parameter calculations were performed with a noncompartmental subroutine [40]. The peak Bu concentrations (C_{max}) and the corresponding peak time (T_{max}) were observed values. The AUC was calculated by the linear trapezoidal rule; the AUC at dose 1 (AUC_{inf}) included an extrapolated area to time infinity after the last measurable plasma concentration, and the AUC at steady state (AUC_{ss}) was calculated for dose 9. The terminal half-life ($T_{1/2}$) was obtained by log-linear regression analysis of the terminal phase of the concentration-versus-time curves. Plasma clearances (CL for the initial intravenous infusion and CL/F for the steady-state dose) were determined using the dose-area relationship and were normalized to actual body weight. Volume of distribution was determined from the ratio of the apparent clearance to the elimination rate constant (λZ). Descriptive statistics were computed for pertinent PK parameters for doses 1 and 9, and comparisons of peak and trough concentrations were done for doses 1, 9, and 13. Projected dose 9 C_{max} was compared with the actual dose 9 C_{max}. The projected dose 9 C_{max} was based on dose 1 C_{max} multiplied by the accumulation factor ($1/1 - e^{-\lambda\tau}$), where λ is the elimination rate constant and τ is the dosing interval [41]. The Bu plasma analyses were performed at a centralized laboratory, and all PK analyses were performed by an independent contractor.

RESULTS

Patient and Disease Characteristics

Sixty-one patients were treated between June 1996 and December 1997. The demographics of the patients are summarized in Table 1. The median age was 37 years (range, 20-63 years), with 11 patients (18%) aged between 50 to 63 years. There were slightly more men than women enrolled (36/25). Twenty-six patients (43%) had acute myelogenous leukemia (AML), 9 (15%) had MDS, 17 (27%) had CML, 5 (8%) had NHL, and 4 (7%) had HD. Most patients (56/61; 92%) were considered to be at high risk for treatment-related toxicity and recurrent disease, based on any combination of the following criteria: active disease at the time of transplantation, ≥3 prior chemotherapy regimens, prior radiation therapy, or prior HSCT. Seven of the 11 patients ≥50 years of age (64%) met the above high-risk criteria.

Most of the patients entered the program at an advanced stage of disease (Table 1). Four of the AML patients were refractory to induction chemotherapy, and 8 patients were in first complete remission (CR). Of the

Table 1. *Patient Demographics and Disease Characteristics**

Demographics	n (%)
Age, median (range), y	37 (20-63)
18-29	11 (18)
30-39	21 (34)
40-49	18 (30)
50-64	11 (18)
Sex	
Male	36 (59)
Female	25 (41)
Active disease	42 (69)†
Pretreatment	29 (48)
≥3 Chemo regimens	9 (15)
Previous XRT	7 (11)
≥3 Chemo regimens + previous XRT	5 (8)
Previous BMT	8 (13)
Stem cell source	
Bone marrow	27 (44)
Peripheral blood	34 (56)
Disease	
AML	26 (43)
Induction failure	4 (7)
CR1	8 (13)
>CR1	14 (23)
CML	17 (27)
Chronic phase	4 (7)
Acute phase	11 (18)
Blast crisis	2 (3)
Lymphoma	9 (15)
HD	4 (7)
NHL	5 (8)
MDS	9 (15)
De novo	4 (7)
Secondary	5 (8)
Total	61

*XRT indicates radiation therapy; CR1 indicates patient was in first complete remission; >CR1, patient was beyond CR1.

†Excluding the 4 chronic phase CML patients, who were regarded as good-risk patients for treatment-related complications.

14 patients beyond first CR, 8 patients were in relapse and 6 patients were in remission. Three of the 8 AML patients in first CR and 5 of the 9 MDS patients had a history of a preceding malignant disease. Eleven of the 17 CML patients had accelerated-phase disease, and 2 patients were in the blastic phase. Four of the 5 NHL patients had relapsed active disease (3 of the 4 patients were refractory to conventional chemotherapy). One patient had both NHL and MDS but, for the purpose of this analysis, was counted as having lymphoma. The fifth NHL patient was refractory to induction chemotherapy. The 4 HD patients had primary refractory or resistant relapsed disease; 1 of these patients had progressive disease after a previous autologous HSCT. Twenty-seven patients (44%) received bone marrow, and 34 patients (56%) had received a PBPC graft.

Toxicity

All patients received the entire IV BuCy2 regimen as prescribed. In no case was the IV Bu treatment interrupted because of side effects. Table 2 provides a summary of primary causes of death for all patients. Up to and including day 28 after HSCT, 2 patients died (3.3%), 1 of Aspergillus pneumonia on HSCT day 20, before engraftment could be evaluated, and the other of cytomegalovirus pneumonia complicated by diffuse alveolar hemorrhage (DAH) and acute respiratory distress syndrome (ARDS) on HSCT day 27. Between HSCT days 29 and 100, 6 other patients died, 2 of whom had VOD (HSCT days 30 and 31): 1 was an AML patient in second CR, and the other was an MDS patient who underwent a second HSCT (Table 3). One patient died of pneumonia complicated by DAH (day 62), and 1 patient died of interstitial pneumonitis (IP)/ARDS (day 98). The latter patient had previously received 40 Gy mantle radiation for HD. Two patients died of recurrent leukemia (days 42 and 80). The treatment-related and overall mortality rates in the first 100 days post-HSCT were 9.8% (6/61) and 13.1% (8/61), respectively.

The CNS, pulmonary, gastrointestinal tract, and hepatic toxicities are described below.

Central Nervous System. No seizures were reported during the defined 36-day study period (HSCT day –7 through day 28). Three patients experienced hallucinatory events, all grade 1; 1 event occurred during IV Bu administration and the other 2 events occurred approximately 3 weeks after the last IV Bu infusion. Isolated incidents of mild CNS disturbances were encountered and resolved. The only exceptions were seen in the 2 patients with lethal VOD, who became confused shortly before they died.

Lungs. There were no additional pulmonary adverse events aside from the above incidents of pneumonia, IP, and DAH.

Gastrointestinal Tract. There were no grade 4 toxic events aside from self-limited anorexia in 1 patient (2%). Four patients (7%) experienced grade 3 nausea; 1 patient (2%) had grade 3 vomiting. The overall incidence of vomiting during Busulfex administration (days –7 through –4) was 43%; all occurrences were considered mild, of grades 1 or 2. Twenty-seven patients (44%) developed grade 2 mucositis, and 16 patients (26%) had grade 3 mucositis lasting a median of 6 days (range, 2-11 days).

Hepatic. The site investigators recognized 5 incidents of VOD (5/61; 8.2%). No additional patients were identified through a search of the database. VOD resolved in 3 patients and was fatal in 2 patients (3.3%). The overall incidence of VOD in patients undergoing their first transplantation was 5.7% (3/53), and 1 of them died (1.9%). The independent reviewer concluded that only 3 of the 5 reported patients (3/61; 4.9%) fulfilled the Jones criteria for VOD [38] (see

Table 2. *Primary Causes of Death by Study Period*

	HSCT Study Day Period	
	Days –7 to 28, n	Days 29 to 100, n
Infection, including pneumonia	1	
Pneumonia with secondary DAH	1	1
VOD		2
IP/ARDS		1
GVHD		
Disease progression		2
Total	2	6

Table 3. *Characteristics and Outcome of Patients Developing VOD**

Patient Disease	VOD Site†	Jones‡	Prior Therapy	Dose 1 AUC, μMol-min	Dose 9 AUC, μMol-min	Outcome
HD	Yes	No	R,C,T	1256	1170	Resolved
AML	Yes	Yes	C	1106	1194	Died
MDS	Yes	Yes	R	1225	978	Resolved
MDS	Yes	Yes	R,C,T	1644	1617	Died
CML	Yes	No	R	1567	1604	Resolved

*R indicates prior radiation; C, ≥3 prior chemotherapy regimens; T, prior transplantation.

†Diagnosis of VOD by the site principal investigator based on clinical examination and laboratory findings.

‡Diagnosis of VOD by bilirubin >2 mg/dL with at least 2 of the following 3 findings: painful hepatomegaly, weight gain ≥5% from baseline, ascites [38].

Table 3 for details). In addition to the patients who suffered VOD, increased serum bilirubin (>1.0 mg/dL) was recorded in 5 patients who had GVHD. Mild, reversible, and self-limiting increases in serum bilirubin (median, 2.9 mg/dL) were also recorded in 9 patients during the early posttransplantation period, approximately at the time that low-dose MTX was given.

Engraftment and Chimerism

All 60 evaluable patients achieved engraftment at a median of 13 days after transplantation (range, 9-29 days); 1 patient died prior to engraftment. Patients who received marrow (n = 27) achieved engraftment in an average of 16 days (range, 11-29 days), whereas those who received PBPC (n = 34) achieved engraftment in 13 days (range, 9-22 days) ($P = .029$). Chemotherapy-induced pancytopenia developed slowly, with the median time to reach an ANC of $<0.5 \times 10^9$ cells/L being HSCT day 4 (range, day –7 to day +5). The median duration of neutropenia was therefore only a brief 11 days (range, 6-27 days). The time to engraftment was considerably shorter for the 47 patients who received G-CSF (12 days; range, 9-22 days) than for the 13 patients who did not receive G-CSF (18 days; range, 13-29 days). Donor-derived hematopoiesis was further documented by cytogenetic markers and/or FISH and/or RFLP analysis in 43 of the 60 evaluable patients at 1 and/or 3 months posttransplantation. Eleven patients had indeterminate results because of the lack of an appropriate marker, and 6 patients did not have any test performed. All 43 patients (100%) had documented donor-cell engraftment; 38 patients (88%) had complete chimerism, and the remaining 5 patients (12%) had mixed chimerism (RFLP) with host-type DNA contributed by persistent or recurrent leukemic marrow cells. We did not observe autologous recovery or late graft failure posttransplantation in any patient who had cytogenetic, FISH, and/or RFLP data available.

Fifty-three patients (87%) required packed red blood cell (PRBC) transfusion support, and 60 patients (95%) received platelet transfusions. The median number of PRBC and platelet transfusions was 4 (range, 1-12) and 6 (range, 1-27), respectively.

Graft-Versus-Host Disease

Acute GVHD was documented in 13 patients (22%), with 6 patients (10%) having grades III to IV GVHD. No patient died of GVHD prior to HSCT day 100, but beyond HSCT day 100, 3 patients (4.9%) died of GVHD or its secondary complications.

Response, Relapse, and Survival

The median follow-up time for patients alive, in CCR, and still available for follow-up (n = 25) was 28 months (range, 12-39 months). Of the 25 patients, 20 patients had a DFS of more than 2 years, and 3 patients had a DFS ≥3 years (2 patients were censored in CCR as they were lost to follow-up at 12 and 17 months). The OS and DFS rates at 2 years were 67% and 42%, respectively (Figure 1).

Pharmacokinetics

Complete PK profiles were assessed at Busulfex doses 1 and 9. Additionally, peak and trough levels were obtained at dose 13. The analyses were performed on blood samples obtained from all 61 patients. Ninety-seven percent of patients (59/61) were evaluable for all PK parameters for both dose 1 and 9 (61/61 for dose 1 and 59/61 for dose 9). The resulting parameters are listed in Table 4. Following the initial dose, the mean AUC_{inf} was 1106 μMol-min (range, 413-2511 μMol-min), the mean $T_{1/2}$ was 2.83 hours (range, 1.69-6.81 hours), and the mean CL normalized to actual body weight (CL/ABW) was 2.74 mL/min per kg (range, 1.28-6.00 mL/min per kg). Following dose 9, the mean AUC_{ss} was 1167 μMol-min (range, 556-1673 μMol-min), the mean $T_{1/2}$ was 2.99 hours (range, 2.11-5.05 hours), and the mean CL/ABW was 2.52 mL/min per kg (range, 1.49-4.31 mL/min per kg). The variability around these 3 parameters at steady state was small: the coefficient of variation (CV) ranged from 19% to 24%.

Without dose adjustment, 55 (90%) of 61 patients had a dose 1 AUC_{inf} <1500 μMol-min, whereas 55 (93%) of 59 maintained an AUC <1500 μMol-min at dose 9 (steady state). Eighty-six percent (51/59) of the patients maintained an AUC between 800 and 1500 μMol-min (the 4 patients with AUC below 800 μMol-min had AUCs of 556, 741, 771, and 794 μMol-min; the 4 patients with AUC >1500 μMol-min had levels of 1586, 1604, 1617, and 1673 μMol-min). The AUC comparisons between dose 1 and dose 9 are presented in Figure 2. This graph shows tightly clustered, predominantly horizontal lines demonstrating both intra- and interpatient predictability and consistency; the dose 1 outlier (AUC = 2511 μMol-min) had an unexplained 6-hour trough value that yielded a very high residual AUC, but this value normalized at the dose 9 assessment (Figure 2).

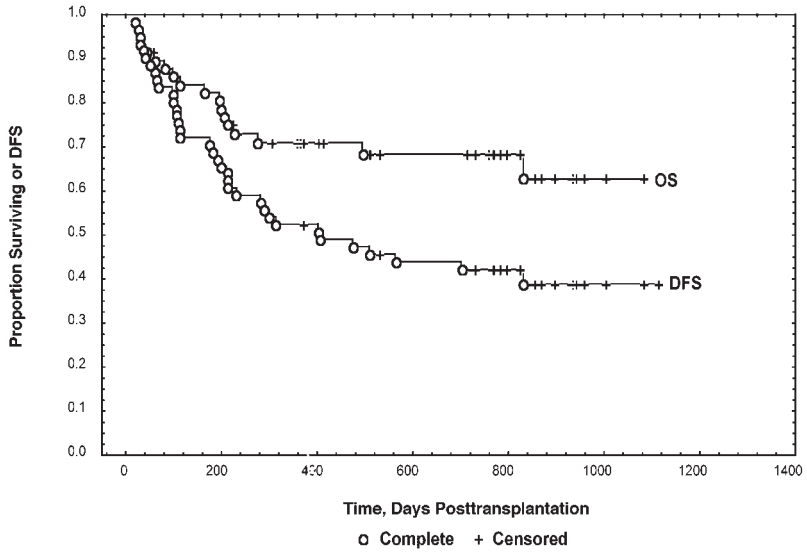

Figure 1. The probability of OS and DFS after HSCT using HLA-matched family donors after the IV BuCy2 regimen in patients with advanced hematologic malignancies.

Additionally, a comparison of the mean AUC estimates between doses 1 and 9, and the peak and trough plasma Bu concentrations of doses 1, 9, and 13 displayed an excellent interdose reproducibility (Figure 3). Using a standard formula for calculation of a predicted concentration (see "Patients and Methods"), the dose 1 C_{max} values predicted the actual dose 9 C_{max} values; there was no significant difference between the predicted result (1251.7 ± 399.8 ng/mL) and the actual result (1221.8 ± 216.1 ng/mL) (P = .615).

A comparison of dose 1 and dose 9 AUC (n = 59) showed a difference of 5.5% (Figure 3), which was significant (P = .042). This difference is expected, because dose 9 is at steady state. When we applied the 2-sided t test to determine the 90% confidence interval for the ratio between dose 1 AUC_{inf} and the dose 9 AUC_{ss}, we found a 90% confidence interval of 103.4% to 111.5%. This interval falls within the defined pharmaceutical definition of equivalence interval for bioequivalence (80%-120%). Thus, dose 1 AUC_{inf} predicted dose 9 AUC_{ss}.

DISCUSSION

Our phase II trial of an IV Bu formulation in preparation for HSCT yielded encouraging results. Two-year OS and DFS rates were 67% and 42%, respectively. Fatal VOD occurred in only 2 patients (3.3%), and treatment-related mortality at 100 days was <10%. The PK data were consistent between dose 1 and steady state (dose 9).

The development of an IV Bu formulation was a logical step to overcome the bioavailability and dose assurance

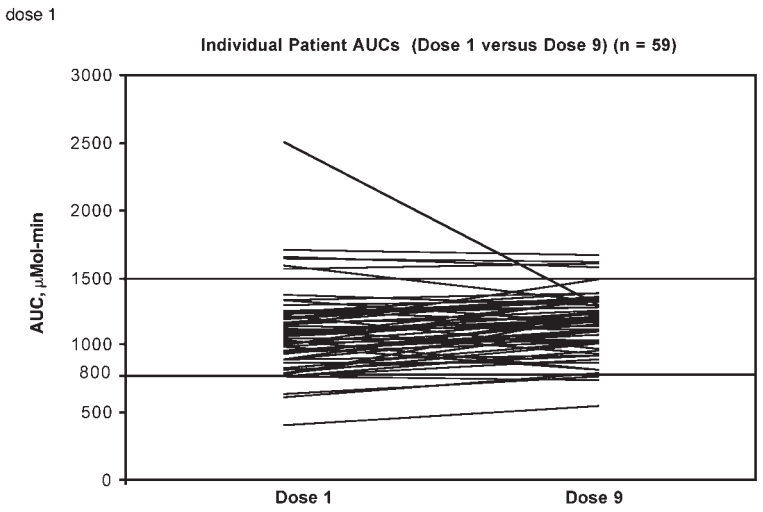

Figure 2. Individual patient AUC values for dose 1 and dose 9 (59 patients). Interpatient results for the entire group (n = 59) for both dose 1 and dose 9 are represented vertically and show consistency and predictability across all patients. Results for each patient are represented horizontally where each line joins a single patient's dose 1 and dose 9 AUC value (intrapatient results). For details, see text.

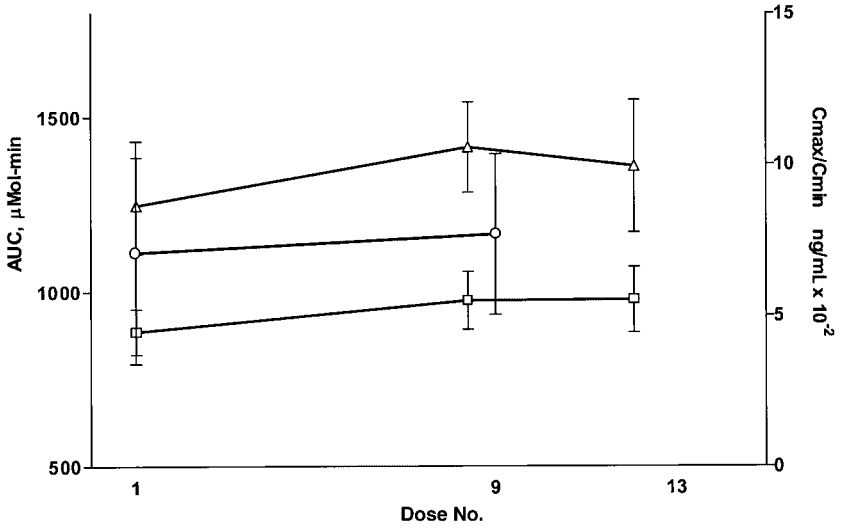

Figure 3. Graphic illustration of pharmacokinetic results of IV Bu for dose 1 (AUC, C_{min}, C_{max}), dose 9 (AUC, C_{min}, C_{max}), and dose 13 (C_{min}, C_{max}). △ indicates C_{max}; ○, area under the plasma concentration versus time curve; □, C_{min}.

problems associated with oral Bu. It was important to assure dose equivalency when the change was made from oral to IV Bu to enable a comparison of the clinical toxicity profile of the new formulation with that previously experienced with the oral drug. Our phase I study demonstrated a median bioavailability of 68% of the oral drug [35]. This result was virtually identical to the 70% bioavailability of oral high-dose Bu (1 mg/kg) reported by Schuler et al., using a dimethyl sulfoxide (DMSO)-based Bu formulation as the IV reference solution [42]. Similar estimates of oral drug bioavailability were made by Hassan et al. (68% in children and 80% in adults) [27]. Furthermore, the median AUC of oral Bu at 1 mg/kg of approximately 1106 µMol-min in the phase I study was similar to that reported by Somlo et al. (1050 µMol-min), who also used an oral dose of 1 mg/kg [43]. The similarities between the bioavailability estimates of different investigators indicated that the 0.8 mg/kg IV dose should be appropriate to use in a modified BuCy2 regimen for the first large-scale phase II study of this new formulation.

It is encouraging that the new IV Bu formulation was very well tolerated, in view of the high proportion of the patients who had been heavily pretreated. All 61 patients received all of their scheduled IV Bu doses, and all 60 evaluable patients achieved engraftment as assessed with white blood cell count recovery, and, if samples were available, confirmed with cytogenetics and/or RFLP studies. Also, there was no incident of secondary graft failure. All reported adverse effects were previously described problems, commonly encountered following myeloablative conditioning

Table 4. *Pharmacokinetics of Busulfex**

	Dose, mg†	C_{max}, ng/mL	T_{max}, h	$T_{1/2}$, h	AUC, µMol-min‡	CL/ABW, mL/min/kg§	Vz/ABW, L/kg§
Dose 1							
Mean	53.53	947	2.16	2.83	1106	2.74	0.64
Median	53.00	937	1.97	2.71	1084	2.63	0.63
SD	9.79	239	0.50	0.76	318	0.82	0.10
CV, %	18.30	25	23	27	29	30	16
Maximum	76.00	1768	4.20	6.81	2511	6.00	1.02
Minimum	33.00	415	0.25	1.69	413	1.28	0.38
Dose 9							
Mean	53.53	1222	2.07	2.99	1167	2.52	0.64
Median	53.00	1249	1.95	2.98	1180	2.40	0.61
SD	9.79	216	0.25	0.56	228	0.62	0.20
CV, %	18	18	12	19	20	24	30
Maximum	76.00	1684	3.00	5.05	1673	4.31	1.70
Minimum	33.00	496	1.75	2.11	556	1.49	0.37

*n = 59. Vz/ABW indicates volume of distribution normalized to actual body weight.

†Total busulfan dose (mg) per administration.

‡For dose 1, AUC_{inf} is presented; for dose 9, AUC_{ss} for the 6-hour dosing interval is presented.

§All patients were normalized to ABW for analysis.

therapy, whether based on TBI, on oral Bu, or on alternative chemotherapy combinations.

The Bu infusion through a central venous catheter avoided any hepatic first-pass effect but brought the possibility that an increased incidence of pulmonary complications might be encountered. This concern was not validated, and we did not detect any new side effects that could be associated with the Bu solvents DMA and PEG400.

The safety of this combination was further evidenced by the low treatment-related mortality, 3.3% at 28 days and 9.8% at 100 days posttransplantation. The causes of death—infections, VOD, DAH, IP, and GVHD—have been described after a variety of conditioning regimens. The incidence of serious but nonlethal toxicity, in particular VOD, was also very low. The incidence of fatal VOD was 3.3%, and only 1 of the first-transplantation patients died of VOD (1.9%). These results compare favorably with previously published reports on the use of high-dose oral Bu-based pretransplantation therapy in high-risk patients [44,45], and also with a group of patients treated (in parallel) with oral BuCy2 at one of the centers participating in the current study [46].

VOD is considered a most serious side effect of high-dose Bu-based conditioning therapy. Although several factors can predispose a patient to develop VOD, it appears to be more prevalent in patients whose Bu AUC is excessive (≥1500 μMol-min) [21-23], but not all investigators agree [32]. We did not find a predictive correlation between high AUC values and VOD in our patients [47]. Such an association may exist but be obscured by the low incidence of VOD in the present study, and the tight range of AUC values with the new drug formulation may make the association difficult to confirm.

The fixed Bu dose of 0.8 mg/kg in this combination was confirmed as clinically appropriate; all evaluable patients achieved engraftment, and the DFS at 2 years was 42% (median follow-up, 2.3 years). Furthermore, this dose was well tolerated, and we did not find any increase in serious complications in older patients [48]. Based on our results, we suggest that an age above 50 years should not disqualify a patient with otherwise good performance status from allogeneic HSCT using a myeloablative IV Bu–based conditioning regimen.

In a review of oral Bu, Vassal [30] concluded that systemic exposure may vary by a factor as high as 20 from one patient to another with a fixed-dose oral Bu regimen, greatly influencing toxic and therapeutic effects. PK-guided dose adjustment has been advocated to reduce interpatient variability in systemic Bu exposure, thereby controlling toxicity and retaining or increasing therapeutic efficacy [20-22]. Slattery et al. defined a lower limit of acceptable Bu concentration at steady state (C_{ss}) to decrease the risk for graft rejection and/or leukemic relapse. This concentration was dependent on the degree of compatibility of the allogeneic graft. They suggested that a C_{ss} of at least 200 ng/mL (in the current study, corresponding to an AUC of approximately 300 μMol-min) is necessary to avoid rejection of a matched sibling graft, and that a C_{ss} of 600 ng/mL (AUC of approximately 900 μMol-min) is needed to insure engraftment when HLA–partially mismatched related or HLA-matched unrelated donors were used [17]. This group also reported a correlation between a high C_{ss} (>917 ng/mL; AUC of approximately 1350 μMol-min) and a low risk of leukemia recurrence after HSCT for CML [18].

Furthermore, Deeg et al. [49] reported that the use of oral BuCy2 with PK-guided dose adjustment to achieve C_{ss} plasma levels of 600 to 900 ng/mL (AUC of approximately 900-1350 μMol-min) yielded longer DFS than did oral BuCy2 without PK monitoring or with the use of Cy-TBI as conditioning therapy in older MDS patients (>55 years) undergoing allogeneic HSCT. In our study with the fixed-dose regimen, the median AUC_{ss} was 1167 μMol-min (range, 566-1673 μMol-min), and 81% of the patients had an AUC_{ss} in the range of 900 to 1350 μMol-min ± 5%. This result actually represents a tighter AUC interval than would be expected if oral Bu were used in conjunction with PK-guided individualized dosing [19,23,29]. IV Bu had consistent inter- and intrapatient PK, as demonstrated by the similarity both in the median AUCs of the first and ninth (steady state) doses and in peak and trough levels obtained at the first, ninth, and 13th doses. There was no statistically significant difference between the actual dose 9 C_{max} and the predicted dose 9 C_{max} value based on dose 1 PK. The AUC showed similar high reproducibility from dose 1 to dose 9. These data support the utility of a fixed-dose regimen to achieve acceptable PK parameters in the vast majority of patients. However, it must be recognized that no patient in our study received a partially matched or matched unrelated-donor graft.

The PK analysis showed similar values for AUC, clearance, and $T_{1/2}$ of the new IV Bu compared to published data for Bu obtained with the oral drug [21-23,27-32], and there were no significant changes in clearance, $T_{1/2}$, or AUC after doses 1 and 9. The findings suggest that the solvent vehicle does not significantly influence human Bu metabolism. The high interdose reproducibility of all PK parameters was documented by the available mean values of AUC, clearance, $T_{1/2}$, C_{max}, and C_{min} after doses 1 and 9, and C_{max} and C_{min} at doses 1, 9, and 13. This reproducibility is in contrast to the findings of high interdose variability between consecutive trough values reported with the use of oral Bu [31], probably because of elimination of the erratic intestinal oral drug absorption.

Improved investigator-controlled Bu dosing and predictable drug exposure with this pharmaceutically acceptable IV formulation offers an opportunity to explore other dosing regimens and compare outcomes with the confidence that drug delivery is well standardized. Investigations using PK-directed dosing regimens become especially appealing if sampling strategies using a limited number of samples can be applied. Hassan et al. showed a high correlation ($r = 0.998$, $P < .0001$, $n = 40$) between the estimated and the determined AUC from a sampling strategy based on 3 concentrations compared with that using a "complete set" of 10 to 13 blood samples [50]. Similarly, Perry et al. reported a high degree of reproducibility with a strategy based on 5 samples in patients treated with the new IV Bu formulation [51].

In summary, the good clinical tolerance paired with a modest incidence of serious toxicity, a highly reproducible PK profile (both intra- and interpatient), and a DFS rate of 42% at 2 years posttransplantation in patients with mostly advanced, heavily pretreated hematological cancers demonstrate that the new IV Bu formulation may have advantages over the standard Bu tablets in high-dose chemotherapy. The parenteral administration of Bu allows for predictable

systemic exposure without PK monitoring. However, if one elects to use PK monitoring and targeted dosing, this formulation is a tool to insure standardized drug delivery with the ability to use dose 1 or test dose PK data to predict steady-state levels and perform individualized dose adjustments with high precision.

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