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Safety and Efficacy of Targeted-Dose Busulfan and Bortezomib as a Conditioning Regimen for Patients with Relapsed Multiple Myeloma Undergoing a Second Autologous Blood Progenitor Cell Transplantation

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A B S T R A C T

Patients with multiple myeloma (MM) who relapse after autologous transplantation have limited therapeutic options. We conducted a prospective, multicenter, phase IIa study to investigate the safety and efficacy of i.v. busulfan (Bu) in combination with bortezomib as a conditioning regimen for a second autotransplantation. Because a safe Bu exposure was unknown in patients receiving this combination, Bu was initially targeted to a total area under the concentration−time curve (AUC) of 20,000 μM × minute. As no concentration-limiting toxicity was observed in 6 patients, this Bu exposure was utilized in the following treatment cohort (n = 24). Individualized Bu dose, based on test dose .8 mg/kg pharmacokinetics (PK), was administered daily for 4 consecutive days starting 5 days before transplantation, followed by a single dose of bortezomib (1.3 mg/m^2) 1 day before transplantation. The total mean dose of i.v. Bu (including the test dose and 4-day administration) was 14.2 mg/kg (standard deviation = 2.48; range, 8.7 to 19.2). Confirmatory PK demonstrated that only 2 of 30 patients who underwent transplantation were dosed outside the Bu AUC target and dose adjustments were made for the last 2 doses of i.v. Bu. The median age was 59 years (range, 48 to 73). Median time from first to second transplantation was 28.0 months (range, 12 to 119). Of 26 evaluable patients, 10 patients attained a partial response (PR) or better at 3 months after transplantation, with 2 patients attaining a complete response. At 6 months after transplantation, 5 of 12 evaluable patients had maintained or improved their disease status. Median progression-free survival was 191 days, whereas median overall survival was not reached during the study period. The most common grade 3 and 4 toxicities were febrile neutropenia (50.0%) and stomatitis (43.3%). One transplantation-related death was observed. A combination of dose-targeted i.v. Bu and bortezomib induced PR or better in one third of patients with MM who underwent a second autotransplantation, with acceptable toxicity.

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INTRODUCTION

There are limited therapeutic options for patients with multiple myeloma (MM) who relapse after autologous blood progenitor cell transplantation. Immune modulators and proteosome inhibitors are frequently used after autotransplantation, but patients eventually relapse or develop toxicities that preclude therapy with these agents. A second autotransplantation can induce durable remissions in selected patients with MM [1-7].

The addition of oral busulfan (Bu) to melphalan as a conditioning regimen for autotransplantation resulted in better disease control of MM compared with melphalan monotherapy [8,9]. Unfortunately, sinusoidal obstructive syndrome (SOS), a frequent complication of oral Bu, hindered its use as part of the conditioning regimen [10]. Because Bu improves the response to melphalan, we elected to investigate the safety and efficacy of a higher dose of i.v. Bu in patients who relapsed after autologous transplantation, as these patients are at least partially resistant to melphalan. Intravenous Bu eliminates the unpredictable bioavailability of the oral formulation, which results in decreased incidence of SOS [11,12]. However, differences in Bu metabolism can cause suboptimal tumor exposure in approximately one third of patients when i.v. Bu dose is calculated by body weight [13]. Recent studies have demonstrated that the optimal Bu exposure is correlated with good clinical outcomes in other hematologic malignancies [14]. When doses are adjusted based on pharmacokinetic (PK) results, interindividual variability of the Bu metabolism can be taken into account, resulting in optimal therapeutic exposure [15-18]. Thus, therapeutic dose monitoring is useful for safety and efficacy.

Preclinical and clinical studies suggest that bortezomib potentiates the cytotoxicity of alkylating agents and other chemotherapy agents [19,20]. Bortezomib has been added to high-dose melphalan as part of the conditioning regimen for multiple myeloma [21,22]. This combination is safe and effective in patients with MM and correlative studies suggest that bortezomib administered after the melphalan induces more apoptosis of myeloma cells that when administered before the melphalan [21]. The combination of Bu and bortezomib has not been investigated. Here, we report the results from a prospective multicenter phase IIa study to examine the safety and efficacy of dose-targeted i.v. Bu in combination with bortezomib as a novel conditioning regimen for a second autotransplantation in patients with MM who relapsed after a previous autotransplantation. We also wanted to ascertain if PK analysis after a test dose of i.v. Bu could be used to determine the individual dose that is necessary to reach the target total area under the concentration–time curve (AUC) of Bu used in this conditioning regimen.

MATERIALS AND METHODS
Study Design

This was a phase IIa, single-arm, open-label, exploratory study. The primary objective was to evaluate the safety and efficacy of the novel combination of i.v. Bu and bortezomib. The study consisted of 2 segments: selection of the target Bu exposure, and the safety and efficacy component. A secondary objective was to examine whether PK analysis after a test dose of i.v. Bu allowed for accurate dose targeting of Bu as part of the conditioning regimen.

Study Eligibility

Patients ages 18 to 75 years with an Eastern Cooperative Oncology Group performance status of 0 to 2 were enrolled. All patients had relapsed MM after a first autotransplantation and were eligible for a second autotransplantation as salvage therapy. The first autotransplantation had to be performed at least 1 year before the second autotransplantation.

It was required that patients had adequate pretransplantation organ function, defined as left ventricular ejection fraction $\geq$ 45% without uncontrolled arrhythmias or symptomatic cardiac disease; forced expiratory volume in 1 second, forced vital capacity, and carbon monoxide diffusion capacity of at least 50% of predicted; liver transaminases $\leq$ 3 times the upper limit of normal; and serum creatinine $<$ 2 mg/dL. Patients must have a minimum peripheral blood stem cell dose of 2.0×10^6 CD34$^+$ cells/kg.

Patients who had t(4;14) or p53 gene deletion at any time during the disease were ineligible, as were patients with systemic amyloidosis [23]. We also excluded patients who previously underwent allogeneic transplantation and those with a history of having a total serum bilirubin >2 mg/dL after chemotherapy or at study screening. Patients with grade 1 neuropathy with pain or > grade 2 neuropathy without pain were also excluded. All patients provided written informed consent to participate in this study in accordance with the Declaration of Helsinki ethical principles. The trial was registered at www.clinicaltrials.gov as NCT01009840.

Determination of Target Total AUC

Because a safe Bu exposure was unknown in patients receiving this combination, Bu exposure was initially targeted to an AUC of 20,000 μM × minute, as this dose was well tolerated in people with other hematologic malignancies [24,25]. Six to 12 patients were to be enrolled in a 3-patient cohort schedule to determine the safety of the Bu exposure. If concentration-limiting toxicity (CLT)—defined as treatment-related mortality (TRM) or SOS—occurred, it was planned to de-escalate the target AUC to 16,000 μM × minute. If no CLT occurred during a period of observation of $\geq$30 days, 3 additional patients were to be treated at the same target. If no CLT were observed in the second cohort, the target dose of 20,000 μM × minute were to be used in the next study segment to determine the safety and efficacy of this combination in 24 additional patients. No dose escalation above 20,000 μM × minute was planned as it is known that SOS risk is higher when the total Bu AUC exceeds 24,000 μM × minute [26-28].

Test Dose, PK Analysis, and Dose Recommendations

A test dose, .8 mg/kg, of i.v. Bu based on actual body weight (BW) or adjusted ideal BW (AIBW) was administered over 2 hours once between days −12 to −9 (Figure 1). The dosing algorithm for the test dose was as follows: first, the ideal BW (IBW) was calculated using the formulas: IBW (kg) = 50 + .91 × (height in cm−152) for men; IBW (kg) = 45 + .91 × (height in cm−152) for women. The actual BW was used when the actual BW was less than or equal to the IBW; the AIBW was used when the actual BW was greater than the IBW. The AIBW was calculated as the IBW plus 25% of the difference between the actual BW and the IBW.

Six serial blood samples were drawn as follows: at the end of infusion (EOI), immediately after a 2-hour infusion, EOI + 15 minutes, EOI + 30 minutes, and 240, 300, and 360 minutes after the start of the infusion of i.v. Bu. The Seattle Cancer Care Alliance measured Bu concentrations, determined Bu exposure as AUC using WinNonlin software version 5.2 (Pharsight Corporation, Mountain View, CA), and recommended individualized PK-directed dosing for the conditioning regimen [29,30]. Target daily AUC during the conditioning regimen was calculated as: (20,000 μM × minute−test PK AUC)/4. The individualized daily dose for the conditioning regimen was calculated as: (test PK dose/test PK AUC) × target daily AUC. The individualized daily dose was calculated to achieve 20,000 μM × minute as a total AUC, including the AUC exposure from the test PK.

Conditioning Regimen and Confirmatory PK Analysis

Individually dosed i.v. Bu was administered over 3 hours once daily from day −5 through day −2. Confirmatory PK was performed on day −5 (Figure 2). Samples were collected immediately at EOI, EOI + 15 minutes, EOI + 30 minutes, and 270, 360, and 480 minutes after start of infusion. If confirmatory PK analysis demonstrated that the Bu exposure would be outside the target range (20,000 μM × minute ± 20%, or 16,000 to 24,000 μM × minute), the dose of i.v. Bu on days −3 and −2 was adjusted. On day −1, bortezomib 1.3 mg/m^2 was administered as a 3 to 5-second bolus i.v. injection. Seizure prophylaxis with lorazepam and/or levetiracetam started 1 day before the initiation of Bu and continued until the day after the last Bu dose [31].

Concomitant Medications

Concomitant medications were accounted for during the study period. The following drugs known to have drug interactions with busulfan were prohibited 72 hours before i.v. Bu treatment through 48 hours after treatment: acetaminophen, voriconazole, metronidazole, digoxin, other alkylating agents, vaccines, herbal supplements, filgrastim, or sargramostin. The following medications were discouraged during the trial: nonsteroidal anti-
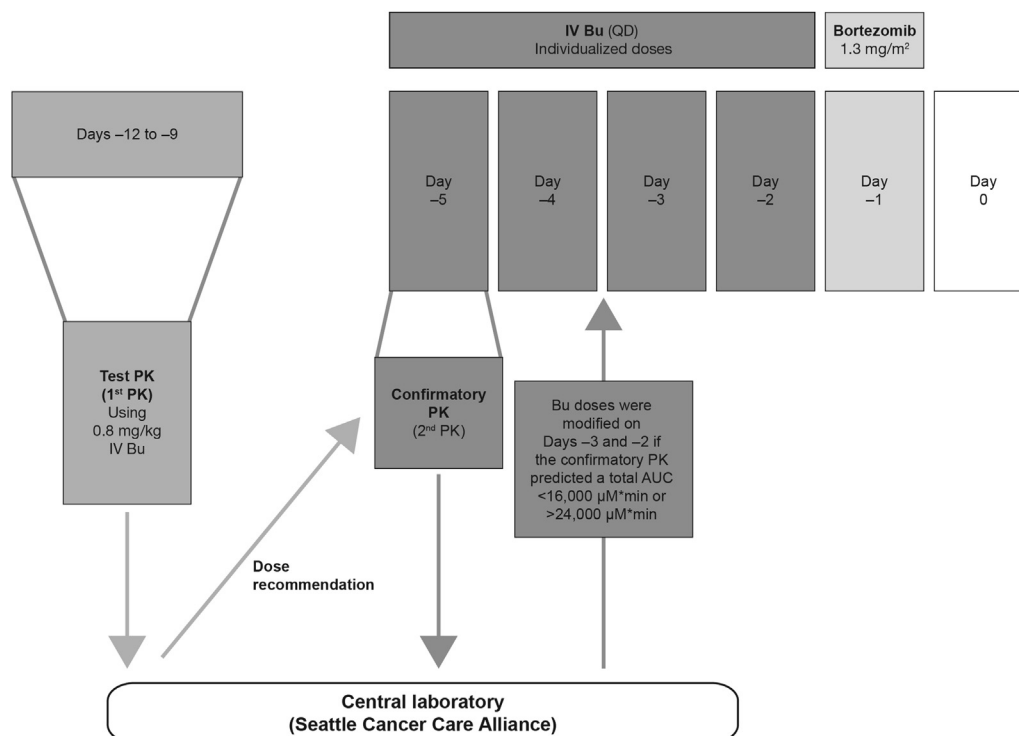
Figure 1. Preconditioning test pharmacokinetics (PK) (first PK) and conditioning regimen. A test dose, .8 mg/kg, of i.v. busulfan (Bu) based on actual body weight (BW) or adjusted ideal BW was administered over 2 hours between days −12 and −9. Plasma samples were used to calculate Bu concentrations at the laboratory of Seattle Cancer Care Alliance. Bu exposure was measured as area under the curve (AUC) using WinNonlin software and the individualized daily dose for the conditioning regimen was calculated as: (test PK dose/test PK AUC) × target daily AUC, to achieve 20,000 μM × minute as a total AUC, including the AUC exposure from the test PK. The individualized dose of i.v. Bu was administered over 3 hours once daily from day −5 through day −2. The second PK (confirmatory PK) was performed on day −5. Only when confirmatory PK analysis indicated that Bu exposure would be outside the target range were doses on days −3 and −2 adjusted. Bortezomib (1.3 mg/m^2 once daily) was administered as a 3 to 5-second bolus i.v. injection on day −1.

inflammatory drugs, salicylates, anticoagulants, ethotoin, phosphenytoin, thioguanine, or immunosuppressive agents.

Maintenance Therapy After Second Transplantation

There was no restriction for maintenance therapy after the second autotransplantation because its standard use had not been established at the time of the study.

Safety Assessment

The Common Terminology Criteria for Adverse Events version 3 was utilized to define adverse events (AEs). A 12-lead electrocardiogram (ECG) was obtained on screening and 3 consecutive ECGs were obtained on day −1 to rule out QT prolongation. A data safety monitoring board, chaired by an external transplantation physician, reviewed the safety data at the end of the AUC selection and after one half of the study subjects were enrolled.
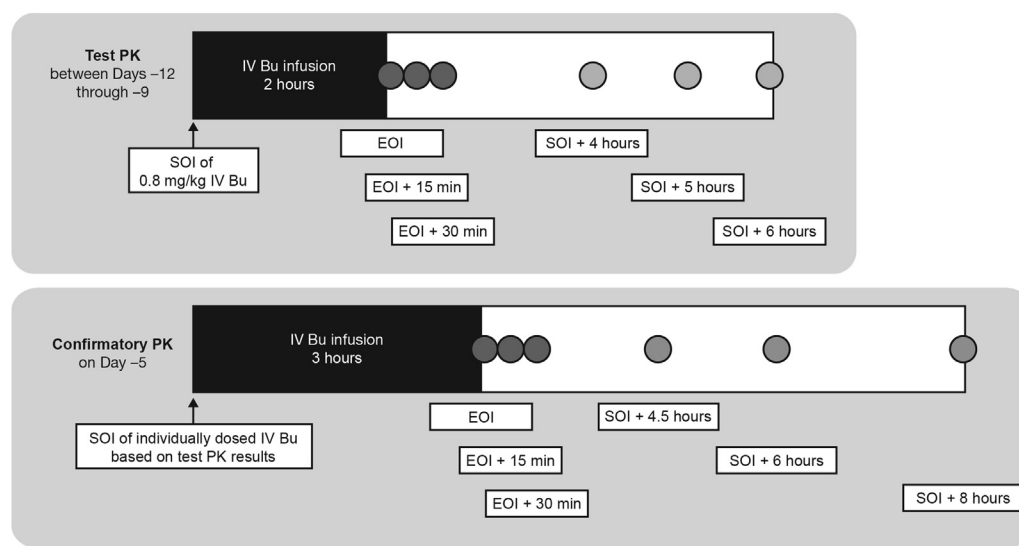

Figure 2. Pharmacokinetics (PK) sampling times for preconditioning test PK (first PK) and confirmatory PK (second PK). For test PK, 6 serial blood samples were drawn at the end of infusion (EOI) after a 2-hour infusion of i.v. Bu, EOI + 15 minutes, EOI + 30 minutes, and 4, 5, and 6 hours after the start of the infusion of intravenous (i.v.) Bu. For the confirmatory PK, plasma samples were collected at the EOI after a 3-hour infusion of i.v. Bu, EOI + 15 minutes, EOI + 30 minutes, 4.5, 6, and 8 hours after start of the infusion of i.v. Bu.

Table 1
Subject Demographics

Variable	Value
Age, median (range), yr	59 (48-73)
Gender	
Male	25 (83.3)
Female	5 (16.7)
Race	
Caucasian	26 (86.7)
African American	4 (13.3)
ECOG performance status	
Grade 0	11 (36.6)
Grade 1	18 (60.0)
Grade 2	1 (3.3)
Body weight, median (range), kg	89.9 (51.5-131.6)
Body mass index, median (range), kg/m^2	31.2 (18.9-41.0)
Body surface area, median (range), m^2	2.08 (1.52-2.58)
Ig subtype*	
IgG	18 (60.0)
IgA	4 (13.3)
Light chain	9 (30.0)
Cytogenetic abnormality at initial diagnosis	
Yes	11 (36.7)
No	17 (56.7)
Unknown/not evaluable	2 (6.7)
Residual neuropathy without pain at enrollment	
Grade 1	18 (60.0)
Grade 2	2 (6.7)
Prior chemotherapy history	
Bortezomib	26 (86.7)
Thalidomide	14 (46.7)
Lenalidomide	20 (66.7)
Salvage or reinduction therapy for relapsed myeloma before the second autotransplantation	
Bortezomib-based regimens	11 (36.7)
Lenalidomide-based regimens	5 (16.7)
Lenalidomide, bortezomib, and dexamethasone	4 (13.3)
Dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, and etoposide	2 (6.7)
Bendamustine-based regimen	1 (3.3)
Time from first to second transplantation, median (range), mo	28.0 (12-119)
Time from initial diagnosis to second transplantation, median (range), mo	38.5 (19-125)
Disease response at second autotransplantation	
VGPR	7 (23.3)
PR	12 (40.0)
SD	2 (6.7)
PD	9 (30.0)

ECOG indicates Eastern Cooperative Oncology Group; VGPR, very good partial response; SD, stable disease.
Data presented are n (%) unless otherwise indicated.
  * One case was reported as biclonal gammopathy, which is primarily IgA-kappa with a smaller IgG-kappa band.

Statistical Analyses

The primary endpoint was to evaluate the 6-month response by International Myeloma Working Group uniform response criteria [32]. The secondary efficacy endpoints included overall survival (OS) and progression-free survival (PFS). These endpoints were analyzed as time-to-event variables, which were defined as the time from transplantation to death for OS and the time from transplantation to disease progression or death, whichever occurred first, for PFS. The event-free probabilities for these endpoints were estimated using the Kaplan-Meier method. Patients without events were censored at the last follow-up for OS and at the last disease evaluation for PFS. Safety evaluation included TRM, which was defined as death after transplantation due to any cause other than disease progression and as SOS as defined by the Baltimore criteria [33].

RESULTS
Patient Demographics

Thirty patients were enrolled at 11 institutions from the United States and Canada between June 2010 and July 2011.

All enrolled patients completed the protocol regimen and received a second salvage autotransplantation. Of the patients, 83.3% were male and 86.7% were Caucasian (Table 1). Median time from initial diagnosis to second autologous transplantation was 38.5 months (range, 19 to 125). Median time from first to second transplantation was 28.0 months (range, 12 to 119). Median age at second transplantation was 59 years (range, 48 to 73). Extramedullary disease was present in 4 patients (13.3%) at study screening.

At initial diagnosis, 17 patients (56.7%) had normal cytogenetics, 11 patients (36.7%) had cytogenetic abnormalities, and 1 patient had no evaluable metaphases (3.3%). Cytogenetic abnormalities were reported in 6 patients (20.0%) at study screening. Cytogenetic 13q deletion was recorded in 3 patients at initial diagnosis and in 1 patient at second transplantation.

Before the second transplantation, all patients were treated with at least 1 of 3 drugs—bortezomib, thalidomide, or lenalidomide—and 13 patients (43.3%) received radiotherapy for myeloma. Twenty-six patients (86.7%) received prior therapy with bortezomib and 23 patients (77.0%) received prior therapy with thalidomide and/or lenalidomide. Single-agent melphalan was used as the conditioning regimen for the first autotransplantation in all patients. One patient had a tandem transplantation before enrolling in the study; this patient was excluded from the survival analysis. The salvage or reinduction therapy for relapsed myeloma before the second autotransplantation is illustrated in Table 1. Twenty patients (66.7%) had residual sensory neuropathy without pain (18 with grade 1 and 2 with grade 2) at enrollment. Eastern Cooperative Oncology Group performance status was grade 0 in 11 patients (36.6%), grade 1 in 18 patients (60.0%), and grade 2 in 1 patient (3.3%).

At second transplantation, seven patients (23.3%) had a very good partial response, 12 patients (40.0%) had a partial response (PR), 2 patients (6.7%) had stable disease, and 9 patients (30.0%) had progressive disease (PD).

Seizure Prophylaxis

No specific drug or drug combination for seizure prophylaxis was required in the study. Twenty-one patients used lorazepam, 9 patients used levetiracetam, and 6 patients used both drugs.

Selection of Bu Exposure

No CLT was reported from the first 2 cohorts of 3 patients each, whose i.v. Bu was targeted to 20,000 μM × minute as a total AUC. Therefore, de-escalation of Bu was not necessary. After the data safety monitoring board reviewed safety data and verified that 20,000 μM × minute was a tolerable target total AUC, 24 additional patients were enrolled using this target AUC.

### Test Dose and Confirmatory PK (Supplemental Table 1)

Mean Bu clearance (CL) for the Bu test dose of .8 mg/kg was 3.03 mL/minute/kg. After the test dose, 40% of patients had an AUC outside of the expected range ($<$1000 or $>$1500 μM × minute) (Figure 3A). No clinical or laboratory parameter was capable of identifying patients whose AUC fell outside the target range. Based on this PK analysis, the dose of Bu for the conditioning regimen was determined for each patient.

Confirmatory PK results on day −5 of the conditioning regimen revealed that mean Bu CL was 2.93 mL/minute/kg, which was similar to the CL of the test dose. Accordingly,
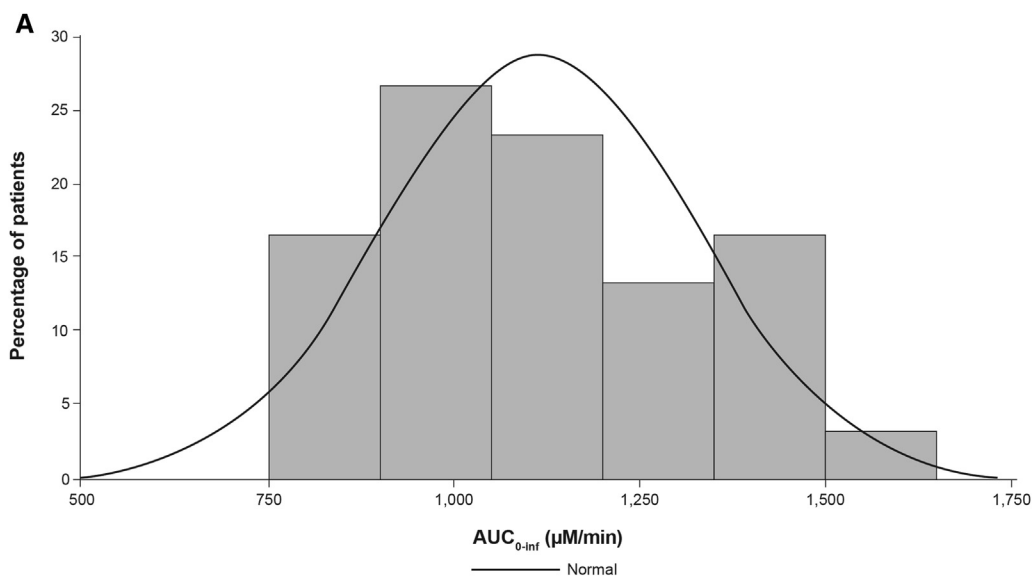
Bu clearance: median 2.95 ml/min/kg (range: 2.08–4.00)

AUC (µM*min)	<1,000	1,000–1,500	>1,500
Patient number (%, 95% CI)	n=11 (36.67%, 21.88–54.49)	n=18 (64.46%, 58.86–73.29)	n=1 (3.33%, 0.59–16.67)
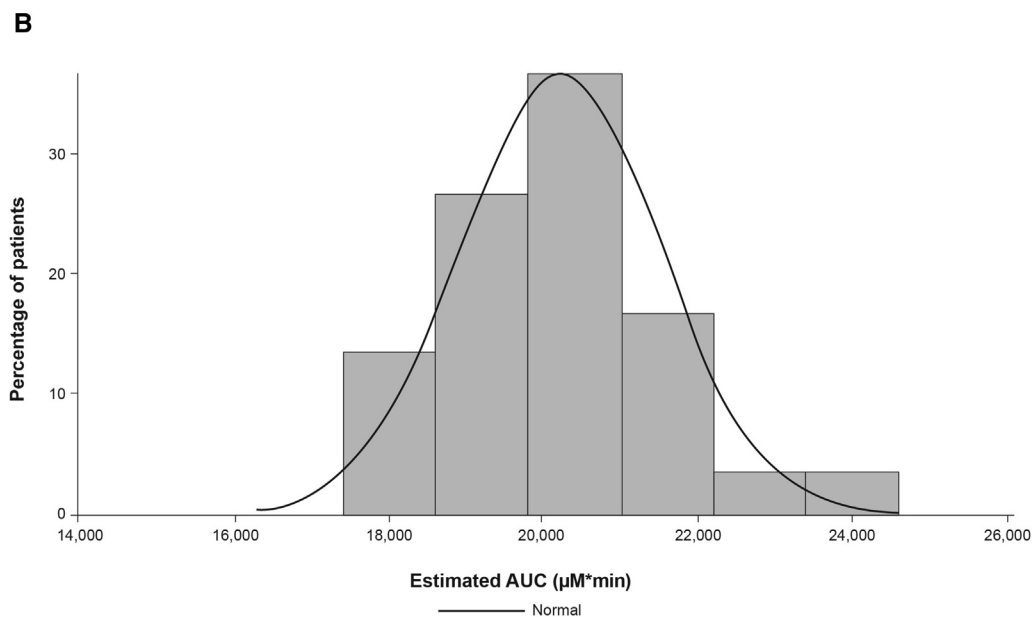
Bu clearance: median 2.81 ml/min/kg (range: 2.05–3.91)

Figure 3. (A) Preconditioning test pharmacokinetics (PK) results. After a test i.v. Bu dose of .8 mg/kg, 40% of patients had an area under the curve (AUC) outside of the expected range: n = 11 (<1000 µM × minute) or n = 1 (>1500 µM × minute). (B) Total estimated AUC from preconditioning test PK and 4-day conditioning. Histograms indicate total Bu AUC exposure from a test dose (.8 mg/kg) and from 4-day administration during the conditioning regimen using individualized doses of i.v. Bu. The total estimated AUC exposure over 5 days fell within the target range (AUC, 20,000 µM × minute ± 20%).

28 patients (93.3%) used the same Bu dose for 4 consecutive days. Two patients (6.7%) needed downward dose adjustment during the conditioning regimen because of decreased Bu clearance from test PK to confirmatory PK. These 2 patients would have had out-of-target AUC for the last 2 doses of Bu if confirmatory PK had not been carried out. Consequently, the total estimated AUC exposure over 5 days fell within the target range (AUC, 20,000 µM × minute ± 20%) in all patients (Figure 3B). The total mean dose of i.v. Bu (including the test dose and 4-day administration) was 14.2 mg/kg (standard deviation = 2.48; range, 8.7 to 19.2). This is approximately 11% more than 12.8 mg/kg, the dose recommended based on body weight.

Table 2
Incidence of Treatment-Related Adverse Events with Toxicity Grade ≥ Three Occurring in at least Two Subjects

Adverse Event	Grade Three n (%)	Grade Four n (%)	Total Events of All Grades n (%)
Febrile neutropenia	14 (46.7)	1 (3.3)	17 (56.7)
Stomatitis	12 (40.0)	1 (3.3)	28 (93.3)
Nausea	3 (10.0)	1 (3.3)	24 (80.0)
Fatigue	2 (6.7)	0 (0.0)	20 (66.7)
Hypokalemia	3 (10.0)	0 (0.0)	15 (50.0)
Hypotension	1 (3.3)	1 (3.3)	7 (23.3)
Hypophosphatemia	2 (6.7)	0 (0.0)	6 (20.0)
Pain in extremity	2 (6.7)	0 (0.0)	6 (20.0)
Hypoxia	1 (3.3)	1 (3.3)*	4 (13.3)
Hallucination	2 (6.7)	0 (0.0)	4 (13.3)
Renal failure acute	1 (3.3)	1 (3.3)	3 (10.0)
Sepsis	1 (3.3)	1 (3.3)	2 (6.7)

* Treatment-related death.

Blood Progenitor Cell Infusion and Engraftment

Twenty-nine patients received blood progenitor cells and 1 patient received bone marrow. Twenty-eight patients underwent transplantation with progenitor cells that were harvested before the first transplantation, 1 subject used progenitor cells harvested before the second transplantation, and 1 subject used both. The median number of infused $CD34^+$ cells was 3.8×10^6/kg (range, 2.1 to 13.8). Post-transplantation granulocyte-colony stimulating factor was used in 27 patients (90.0%). Median times to neutrophil count >500/μL and platelet count >20,000/μL were 11 days. Median time to platelet count >50,000/μL was 14 days.

Toxicity

Incidence of all observed AEs with extramedullary toxicity grade ≥ 3 occurring in at least 2 cases are listed in Table 2. One treatment-related death occurred on day 5 after transplantation in a 54-year-old male patient with multiple comorbidities, who died of pneumonitis. Normal Bu exposure was observed in this case as the total estimated AUC was 17,798 μM × minute from the total administered Bu dose of 14.6 mg/kg (.8 mg/kg for test PK plus 3.4 mg/kg/day × 4 days).

The most frequently observed grade 3 or 4 AEs were febrile neutropenia in 15 patients (50.0%), stomatitis in 13 patients (43.3%), and nausea in 4 patients (13.3%). There was no clear correlation between Bu exposure and the incidence and severity of stomatitis (data not shown). No cases of SOS were diagnosed as defined by the Baltimore criteria.

No new cases of sensory neuropathy were observed. Of 20 patients who had sensory neuropathy at baseline, only 1 patient experienced worsening of the neuropathy from grade 1 to 2. The neuropathy improved in 8 patients and no change in neuropathy was reported in 10 patients. One case was not evaluable because of early death. No instances of seizure were reported, as all patients took lorazepam and/or levetiracetam as seizure prophylaxis from the night before starting Bu until 1 day after the last dose of Bu.

Three consecutive ECGs on day −1 showed no significant QTc prolongation compared with the ECG at study entry.

Disease Response

One patient died of transplantation-related complications and another patient withdrew consent less than 3 months

Table 3
Disease Response in Individual Subjects after Second Autologous Transplantation

Response at Study Entry	Response at Three Months	Response at Six Months	Response at Early Termination	Best Response after Second ASCT	Months from First to Second ASCT
VGPR	SD	Allo after 3 months	SD	SD	20
VGPR	VGPR*	sCR*		NE	21
VGPR	SD	SD*		SD	25
VGPR	Transplantation-related death on day 5				26
VGPR	CR	CR*		CR	39
VGPR	VGPR	VGPR		VGPR	49
VGPR	SD	PD		SD	59
PR	PD		PD	PD	18
PR	PR	SD		PR	19
PR	SD	SD		SD	21
PR	VGPR	VGPR		VGPR	21
PR	PR	PD*		PR	22
PR	PD		PD	PD	24
PR	VGPR	VGPR		VGPR	27
PR	PD		PD	PD	41
PR	SD*	LFU		−	48
PR	PR	PD	PD	PR	49
PR	Lost follow-up before 3 months				70
PR	SD	PD		SD	73
SD	PD		PD	PD	32
SD	PD		PD	PD	38
PD	PD		PD	PD	12
PD	PD		PD	PD	15
PD	PD		PD	PD	18
PD	PD			PD	21
PD	VGPR	PD	PD	VGPR	38
PD	SD	SD		SD	29
PD	CR	VGPR*		CR	45
PD	SD	SD		SD	48
PD	PR	SD		PR	119

ASCT indicates autologous stem cell transplantation; Allo, allogeneic transplantation; NE, not evaluable; sCR, stringent complete response; LFU, lost to follow-up.
* Response assessment after administering maintenance therapy.

Table 4
Retrospective Studies for Second ASCT to Salvage Relapsed Myeloma

Study Group	Princess Margaret Hospital	University of Pennsylvania	University of Texas, San Antonio	San Bortolo Hospital, Italy	BSBMT Registry	CIBMTR Registry
No. subjects	81	41	25	26	148	187
Age, median (range), yr	55 (30-67)	54 (28-73)	58 (39-73)	NA	53 (26-75)	59 (28-74)
Interval between first and second ASCT, median (range), mo	39 (median time to relapse after the first transplantation)	37 (3-91)	39 (4-74)	20.4 (3-91)	NA	32 (6-122)
Regimen for second HSCT	MEL (n = 78) MEL/TBI/etoposide (n = 1) Others (n = 2)	MEL (n = 23) MEL/TBI (n = 14) BU/CY(n = 3) CY/TBI (n = 1)	MEL (n = 25)	Oral Bu 12 mg/kg + MEL 120 mg/m^2	Multiple TBI = 11 No TBI = 133	MEL (n = 158) Others (n = 29)
Response at Second ASCT	CR 0% VGPR 12.5% PR 73.8% Less than PR 13.8%	NA (37% had responsive disease at second ASCT)	CR 0% PR 24% MR/NR 28% PD 48%	NA	NA	CR/PR 40% MR/NR/SD 46% Relapse/PD 14%
Response after second ASCT	CR 7.7% VGPR 39.7% PR 50% SD 1.3% PD 1.3%	CR 5% VGPR 10% PR 37% SD 27% PD 15%	CR 20% PR 44% MR/NR 12% PD 8%	CR 3.8% VGPR 11.5% PR 53.8% MR 19.2% SD/PD 11.5%	CR 26% PR 37%	CR 25% PR 43% MR 6% SD 16% PD 10%
PFS, median, mo	16.4	8.5	12	14.8	32% at 4 yrs	11.2
OS, median, mo	53	20.7	19	38.1	NA	30
TRM	2.6% (all death)	7% (at day 100)	8%	0%	8% (at day 100)	2% (at Year 1)
Reference	[2]	[3]	[1]	[4]	[5]	[6]

BSBMT indicates British Society for Blood and Marrow Transplantation; CIBMTR, Center for International Blood and Marrow Transplant Research; NA, not available; HSCT, hematopoietic stem cell transplantation; MEL, melphalan; TBI, total body irradiation; Cy, cyclophosphamide; MR, minimal response; NR, no response.

after transplantation and was not evaluable for response (Table 3). Assessment of response excluded response after initiating maintenance therapy because it did not necessarily reflect the efficacy of the conditioning regimen. At 3 months after transplantation, 10 of 26 patients attained a PR or better response, including 2 patients who attained a complete response (CR). Of 9 patients who had PD at second transplantation, 3 experienced at least a PR at 3 months after transplantation, including 1 CR and 1 very good PR. At 3 months after transplantation, 9 patients had experienced PD, 2 of whom died.

Seventeen patients remained on the study 6 months after transplantation, 5 of whom received maintenance chemotherapy. Of the remaining 12 patients, 5 patients maintained their response and 7 patients experienced PD.

Maintenance therapy was initiated in 2 patients within 3 months after transplantation: 1 patient attained stringent CR at month 6 after transplantation and the other 1 was lost to follow-up.

Four patients started maintenance therapy between 3 and 6 months after transplantation. Two patients did not experience any change in the status of their disease and 2 patients experienced PD.

PFS and OS

Median PFS was 191 days, whereas median OS was not reached during the study period. The interval duration between transplantations did not correlate with PFS. Specifically, the median PFS was 183 days for patients with an interval $\geq$ 24 months (n = 18) compared with 191 days for patients with an interval of < 24 months (n = 11). Those with $\geq$ 10% of plasma cell percentage in bone marrow at second transplantation (n = 10) had a median PFS of 92 days, whereas the median PFS was not reached in those with a plasma cell percentage in bone marrow <10% (n = 17). Because of the small number of the sample, statistical analysis is not reported.

DISCUSSION

The purpose of this study was to determine the safety and efficacy of a novel combination of i.v. Bu and bortezomib for patients undergoing a second autologous transplantation for MM. As the i.v. Bu exposure was untested in patients receiving this combination, Bu was initially targeted to 20,000 μM $\times$ minute AUC, an exposure used frequently in hematologic malignancies. We demonstrated that this Bu exposure was safe after no CLTs were observed during the initial part of this study.

The toxicity of this regimen was acceptable. Only 1 patient died of a treatment-related pulmonary complication. The most common severe toxicities were febrile neutropenia and stomatitis, which occurred in 50% and 40% of patients, respectively. These toxicities are frequently experienced after high-dose chemotherapy but the frequency of stomatitis was higher in our study compared with that reported after high-dose melphalan [1]. In our study, 93% of patients developed mucositis, 40% of them grade 3. Of 20 patients who had sensory neuropathy at study entry, only 1 patient experienced worsening of the neuropathy from grade 1 to 2. Neuropathy improved in 8 patients despite the use of bortezomib as part of the conditioning regimen. Of importance is the fact that no patient developed SOS, a toxicity that has limited the use of oral Bu in conditioning regimens for hematologic malignancies.

This study demonstrated that the combination of i.v. Bu and bortezomib is active in myeloma, even in relapse after a preceding transplantation. Despite the fact that most patients had received bortezomib and immunomodulatory drugs and one third of patients had PD at the time of the transplantation, more than one third of evaluable patients had at least a PR 3 months after the second transplantation. The true response rate to this regimen is probably understated, as in this study any disease response after initiating maintenance therapy was excluded from the response analysis. At 6 months after transplantation, 5 of 12 evaluable

patients had maintained or improved their response. Median PFS in this study was 191 days, whereas median OS was not reached during the study period.

The role of maintenance therapy after a salvage auto-transplantation is unknown. For this reason, it is hard to ascertain the influence of maintenance therapy in the outcome of patients in this study. Of 2 patients who started maintenance therapy before the 3-month evaluation, 1 attained stringent CR at 6 months after transplantation and the other was lost to follow-up. Of 4 patients who started maintenance therapy between 3 and 6 months after transplantation, 2 remained with stable disease and the other 2 experienced PD.

In this study, PK analysis after a test dose of i.v. Bu allowed for optimization of the i.v. Bu dose utilized in the conditioning regimen. After the test dose of i.v. Bu, 40% of patients had an AUC outside of the expected range. The total AUC from these patients during the conditioning regimen would have fallen outside the target total AUC ($<$16,000 or $>$24,000 μM $\times$ minute) if a fixed dose of i.v. Bu based on the patient's weight had been used. Confirmatory Bu PK analysis performed during the first day of the conditioning regimen demonstrated that only 2 of 30 patients needed dose adjustment of i.v. Bu during the last 2 days of the conditioning regimen to attain the target AUC. Despite the large numbers of samples required for PK analysis and the fact that a central reference laboratory was used, our multicenter study demonstrates that this approach is feasible and could be implemented widely.

When compared with other reports of salvage auto-transplantation for patients with MM, our patients had a much shorter time interval between transplantations (28 months) compared with most studies, in which the time intervals were 32 to 39 months, suggesting that our patients had more aggressive disease (Table 4) [1,3,6]. Only 1 study from San Bartolo Hospital had a shorter interval between transplantations than our study [4]. Another indication that our study population had very aggressive disease was that, at the time of enrollment, there were no patients in complete remission and more than one third of the patients had PD. On the other hand, we excluded patients with adverse cytogenetic features whereas other studies did not. Despite these adverse clinical factors, 38% of evaluable patients attained a partial remission or better response after the second transplantation, including 2 patients who attained CR.

In summary, this study demonstrated that a Bu AUC of 20,000 μM $\times$ minute is safe in patients with MM undergoing autotransplantation with Bu and bortezomib. This novel combination induced a PR or better in one third of heavily pretreated patients who failed a previous auto-transplantation with acceptable toxicity. Further studies are warranted to evaluate the combination of PK dose-targeted i.v. Bu with other active agents in patients with MM. These studies should include patients with more favorable characteristics and explore using higher doses of bortezomib or other chemotherapeutic agents. The use of a uniform post-transplantation therapy should facilitate the assessment of novel regimens.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.08.007.

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