A phase I study of bendamustine hydrochloride administered once every 3 weeks in patients with solid tumors

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The present phase I trial was planned to assess the maximum tolerated dose, the dose-limiting toxicity and the pharmacokinetics of bendamustine hydrochloride in a once every 3 weeks schedule, and to recommend a safe dose for future phase II studies. Included were patients with refractory solid tumors. Bendamustine hydrochloride was administered as a short intravenous infusion over 30 min. The starting dose was defined at 160 mg/m^2 and dose escalation used increments of 20 mg/m^2. Plasma and urine samples were analyzed using validated high-pressure liquid chromatography/fluorescence assays. Twenty-six patients (14 men, 12 women) were enrolled for the study. At 280 mg/m^2, one out of four patients developed a thrombocytopenia grade 4, two experienced grade 3 fatigue and three experienced cardiac toxicity (grade 2). The latter toxicity was considered dose limiting also and further dose escalation was stopped. Plasma pharmacokinetics parameters of bendamustine hydrochloride and its metabolites were assessed in 15 patients. Mean pharmacokinetic parameters of bendamustine hydrochloride were a t_{max} of 32.3 min, a $t_{1/2}$ of 37.8 min, a volume of distribution of 14.2 l/m^2 and a clearance of 287.8 ml/min/m^2. No dose dependency of bendamustine hydrochloride was observed within the used dose range. The metabolites comprised only 23% of the overall area under the concentration–time curve. The maximum tolerated dose of bendamustine hydrochloride on day 1 q 3 weeks is 280 mg/m^2. Fatigue and cardiac toxicity were dose limiting. The plasma pharmacokinetics data of bendamustine and its metabolites were in accordance with previous reports. The recommended dose for future trials is 260 mg/m^2 every 3 weeks. *Anti-Cancer Drugs* 18:587–595 © 2007 Lippincott Williams & Wilkins.

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Introduction

Bendamustine (bendamustine hydrochloride; BM) has cytotoxic and immunosuppressing activities, and was synthesized in 1963 by Ozegowski and colleagues in Jena, Germany [1]. Chemically, BM (4-[5-[bis(2-chloro-ethyl)amino]-1-methyl-2-benzimidazolyl] butyric acid) is a cytotoxic agent that was developed to combine both alkylating, antimetabolite activity and solubility in water [2,3]. In-vitro data have suggested that it is only partially resistant with other alkylating agents and that the DNA double-strand breaks, induced by BM, are more long-lasting [4]. Its unique mechanism of action in comparison with other alkylators might be due to a specific gene signature regulated by BM [5] and the fact that DNA damage caused by BM is repaired by base excision repair rather than by the alkylguanine transferase mechanism. All of these characteristics could explain the lack of activity correlation between BM and other drugs present in the National Cancer Institute Developmental Therapeutics Program database [6]. Furthermore, other preclinical data suggest a concentration-dependent induction of apoptosis in lymphoma cells [7,8]. Evidence is available for synergism with fludarabine or cladarabine, which is thought to be associated with down-regulation of the inhibitors of apoptosis proteins, prostate-apoptosis-response-gene 4, death-associated protein and enforced caspase activity [9,10].

Following intravenous administration, a high percentage (> 95%) of the drug is bound to proteins, primarily albumin. Only free, unbound BM is active. The elimination of BM is biphasic, rapid and – for the unchanged agent and the hydrolysis products, hydroxybendamustine (OH-BM) and dihydroxybendamustine (DiOH-BM) – mostly renal [11–13]. Pharmacodynamic studies in rats have shown that the polar metabolites which are produced by hepatic metabolization have a biliary excretion pattern [14]. The main biotransformation products are a cytotoxic hydroxy-

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derivative (γ-hydroxybendamustine, γ-OH-BM) and *N*-demethylbendamustine (*N*-demethyl-BM) [2].

Since 1971, BM has been used in the treatment of various malignant diseases including chronic lymphocytic leukemia, Hodgkin's disease, non-Hodgkin's disease, multiple myeloma and breast cancer. All of these earlier studies have used BM, both in monotherapy and combination schedules, in arbitrary dosages.

Thus the clinical side effects of the drug, such as myelosuppression (leukopenia usually being more pronounced than thrombocytopenia), nausea, vomiting, loss of appetite, anticholinergic effects, allergic reactions and cardiotoxicity, have been described. Moreover, BM also demonstrated mutagenic, embryotoxic and teratogenic potential in animal studies [15,16].

Recently the compound has attracted renewed interest, and a whole range of preclinical and clinical trials were initiated. A multitude of phase II clinical studies evaluated BM's activity in hematologic malignancies [17–23]. Also in more recent studies, the activity of the compound was demonstrated in patients with solid tumors such as lung, breast and germ cell cancer [24–29].

As the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) had never been formally addressed in a 3-weekly dosing schedule, we studied this in a single-center phase I study. The present report summarizes our findings with respect to safety, tolerability, tumor response and clinical pharmacokinetics (PK) for BM given as a short intravenous infusion over 30 min at 3-week intervals.

Patients and methods

Patient selection

Patients could be included in this phase I study if the following criteria were present: (1) histologically confirmed advanced cancer refractory to standard therapy or for which no standard therapy existed; (2) age older than 18 years; (3) World Health Organization performance status 0–2; (4) a life expectancy of ≥ 3 months; (5) a negative pregnancy test and use of effective means of contraception in fertile women; and (6) a hemoglobin (Hb) level of ≥ 8.0 g/dl, white blood cells $\geq 4.0 \times 10^9$/l and a platelet count $\geq 100 \times 10^9$/l. Exclusion criteria included: (1) liver enzymes more than two times the upper limit of normal [aspartate (AST) and alanine aminotransferases (ALT) and bilirubin]; (2) renal dysfunction with a serum creatinine more than two times the upper limit of normal or any other severe metabolic disorder; (3) chemotherapy or experimental medications within the last 4 weeks before the start of the study; (4) prior treatment with BM; (5) serious concomitant disease such as congestive heart failure, uncontrolled infection, epilepsy or peptic ulcer; (6) alcohol and/or drug dependency; and (7) suspected central nervous system involvement.

In accordance with the Declaration of Helsinki, International Conference on Harmonization Good Clinical Practice guidelines and applicable local laws, all patients provided signed informed consent. The Ethics Committee of the University Hospital of Antwerp approved the protocol.

Treatment schedule

Ribosepharm (Munich, Germany) supplied the study drug in sterile vials containing 100 mg BM and 120 mg of mannitol. The calculated dose had to be administered in a final volume of 500 ml normal saline over 30 min by a peripheral or central intravenous infusion. The treatment was given on an outpatient basis. BM was administered once every 3 weeks. The selected starting dose of BM was 160 mg/m^2. As higher doses of the drug had already been used in combination chemotherapy regimens, this starting dose appeared adequately safe and hence ethically acceptable. The dose increment per treatment group consisted of 20 mg/m^2, providing that no dose-limiting event occurred in the first cycle of the previous group. At least three patients were recruited at each given dose level.

Treatment continued until disease progression or unacceptable toxicity occurred, whatever came first. Supportive care was at the discretion of the treating physician. The concomitant use of other cytotoxic or experimental agents was not permitted. Also hematopoietic growth factors were not permitted.

Definition of dose-limiting toxicity, dose-escalation procedure and maximum tolerated dose

Initially, three patients were to be included at the first dose level. If no DLT occurred, dose escalation would continue. If one of three patients developed a DLT, another three would be enrolled at that same dose level. If then only one of the six patients had developed a DLT, dose escalation would proceed. If at least two of three patients or at least two of six patients had developed an identical DLT, that dose level was considered to be the MTD.

No intrapatient dose escalation was permitted. Once three patients completed the first cycle of treatment (defined as 21 days after the first BM administration) and had been observed for acute toxicity, patients were allowed to start treatment at the next dose level. DLT was defined as (1) any grade 3 or 4 nonhematologic toxicity, which was possibly, probably or definitely related to BM and occurring up to 3 weeks after BM administration; (2) any grade 4 anemia or thrombocytopenia or grade 4 leukopenia or neutropenia lasting for at least 5 days (lymphocytopenia was not considered dose limiting); and (3) febrile neutropenia. Nausea and vomiting were not considered as DLT, if antiemetics had not been used adequately or if it was considered disease related.

The protocol was amended, after the evaluation of three cardiac events (all grade 2) at the 280-mg/m^2 level. Although grade 2 nonhematologic toxicity was defined as significant toxicity, it was decided to consider ischemic cardiac changes grade 2 as dose limiting because of its clinical relevance.

Assessment of toxicity

Patients were monitored for safety and tolerability using the National Cancer Institute of Canada Common Toxicity Criteria (NCIC-CTC version December 1994). The evaluation of side effects was based on weekly outpatient visits with laboratory and clinical investigations, medical history, and a full physical examination. The following investigations were performed before each 3-week cycle: physical examination, toxicity assessment with pulse rate, blood pressure, body temperature and body weight; blood sedimentation rate, coagulation parameters, hematologic blood examination including: Hb, white blood cells, platelets and differential blood count; serum analysis including sodium, potassium, calcium, magnesium, phosphate, creatinine, blood urea nitrogen, uric acid, AST and ALT, alkaline phosphatase, γ-glutamyltranspeptidase, lactate dehydrogenase, bilirubin, creatine kinase (CK), CK-MB, protein, albumin, C-reactive protein, glucose, β-human chorionic gonadotropin (for women only), tumor markers and urine analysis with urinary sediment and creatinine clearance (calculated by the Cockroft–Gauld formula).

Physical examination, toxicity assessment, Hb, white blood cells, platelets, differential blood count, blood urea nitrogen, AST, ALT, CK, CK-MB, glucose, urine analysis and urinary sediment were evaluated weekly. For the assessment of cardiac toxicity, electrocardiograms were performed on days 1 and 8 of each cycle. The left ventricular ejection fraction was evaluated by technetium scan before the first administration of BM.

Every two cycles, patients were evaluated for tumor response by imaging. In this phase I study, the World Health Organization criteria were used to evaluate tumor response: complete response was defined as complete disappearance of the tumor for at least 4 weeks; partial response as a tumor reduction of more than 50% (calculated as the product of largest diameter and its perpendicular measurement); minimal response as a reduction of 25–50%, stable disease was defined as a reduction of less than 25% or a tumor progression of less than 25% and progressive disease was a tumor expansion of more than 25%.

Statistical analyses

For the interpretation of the clinical data, the SPSS 11.5 statistical software (SPSS, Chicago, Illinois, USA) was used.

Results are described in absolute numbers or as group medians with the range or mean values and standard deviation (SD) or standard error as indicated.

Pharmacodynamics and pharmacokinetic measurements

Venous blood samples were taken from individual patients and collected into heparinized tubes at the following time points: 0 (predose baseline), 10, 20, 30 (end of infusion), 35, 40, 50, 60, 75, 90, 105, 120, 180, 280, 360 and 480 min. Immediately upon collection, the samples were transferred in ice water and centrifuged (4–6°C) at 2000*g* for 4 min. The plasma was then divided into three aliquots and immediately deep-frozen at −70°C. Before the first treatment, a 2-ml predose urine sample was collected from each patient. The urine produced after drug infusion (first micturition) was collected throughout cycles 1 and 2, therefore sampling periods differ from each other. Two 1-ml aliquots of each collection were stored at −70°C immediately after micturition.

Plasma and urine samples were analyzed using validated high-performance liquid chromatography and fluorescence assays. The lowest limit of detection in plasma samples for BM was 2 ng/ml. Those for the metabolites varied considerably (100, 500, 2 and 2 ng/ml for OH-BM, DiOH-BM, γ-OH-BM and *N*-demethyl-BM, respectively). The lowest levels of quantification in urine samples for BM, OH-BM, DiOH-BM, γ-OH-BM and *N*-demethyl-BM were 7, 68, 160, 7 and 7 ng/ml, respectively. The concentrations of OH-BM and DiOH-BM were not quantifiable in some urine samples because of interfering matrix peaks.

PK calculations were performed by the PK software package WinNonlin Pro 4.0 (Pharsight Corporation, Mountain View, California, USA; 2002). Parameters were determined by noncompartmental analysis. The noncompartmental analysis was based on a model requiring a constant infusion of the drug (duration of infusion: 30 min). The area under the plasma concentration–time curve (AUC) was calculated using a log-linear trapezoidal method from the first to the last measurable concentration (AUC_{all}) and extrapolated to infinity (AUC_{inf}) using the ratio of the last measured concentration to the terminal slope. The peak plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were read directly from the concentration–time data. The terminal elimination half-life ($t_{1/2\beta}$) was calculated using a log-linear regression of the concentration data. The volume of distribution (V_d) and the clearance (Cl) were calculated by standard methods and normalized to body surface area. Mean values were compared with the *t*-test for unpaired small samples.

Results

Between August 2000 and November 2002, 26 patients were enrolled in this phase I study. They all gave written informed consent.

Patient characteristics

Patient characteristics are given in Table 1. All patients were Caucasian.

Three patients were chemonaive whereas the remaining 23 patients had been treated earlier with a median of two different chemotherapy regimens (range 0–7). Only five patients had been treated with nitrosoureas. More often prior treatment consisted of platinum salts, antimetabolites and taxanes. Few patients had been treated with anthracyclines, vinca alkaloids or topoisomerase inhibitors before study entry. At the 220-mg/m^2 dose level a fourth patient was enrolled, although no DLTs were evaluated in the previous three patients, this was carried out for PK evaluation at that dose level.

Bendamustine treatment

The number of patients treated at each dose level studied is given in Table 2. Sixty cycles were given and the cumulative dose of BM ranged from 296 to 3088 mg. Three patients declined further study treatment after one cycle, 20 patients stopped because of progressive disease and three patients died while on treatment. These deaths were not considered related to BM treatment.

Table 1 Patient characteristics ($n=26$)

Age (years)	
Median	60
Range	35–75
Sex (*n*)	
Male	14
Female	12
Performance status (WHO)	
0	7
1	13
2	6
Primary tumor (*n*)	
Primary unknown	4
Colorectal cancer	4
Melanoma	3
Urinary tract cancer	3
Pancreatic cancer	3
Breast cancer	2
Soft tissue sarcoma	2
Renal cell cancer	2
Thyroid cancer	1
Gastric cancer	1
Head and neck cancer	1
Number of previous chemotherapeutic regimens per patient (*n*)	
0	3
1	6
2	7
3	5
>3	5
Previous radiotherapy	
Yes	11
No	15

WHO, World Health Organization.

Table 2 Bendamustine administration (number of cycles=60)

Treatment	Number of patients
Dose level (mg/m^2)	
160	3
180	3
200	3
220	4
240	3
260	6
280	4
Cycles per patient	
1	5
2	17
≥ 3	4
Reason for treatment discontinuation	
Progressive disease (radiological)	18
Progressive disease (clinical)	2[a]
Refusal	3[b]
Death	3[c]
Adherence to protocol	
Delay due to hematologic toxicity	0
Delay due to nonhematologic toxicity	3[d]
Dose reduction	0

[a]Two patients with jaundice and worsened general condition.
[b]Two disease-related, one unknown.
[c]Considered not to be therapy-related.
[d]Three patients with flu-like symptoms.

Toxicity

Hematologic toxicity

Hematologic toxicity per patient and cycle is shown in Table 3. Preexisting cancer-related anemia was present in five patients; two patients developed anemia grade 3 during treatment (dose level 160 and 240 mg/m^2). One patient experienced thrombocytopenia grade 4, 22 days after the first administration of 280 mg/m^2 of BM, for which no platelet transfusion was required. Leukopenia and neutropenia were rarely severe. Leukopenia and neutropenia grade 3 were seen in only five and two patients, respectively.

A severe decrease in the absolute number of lymphocytes (grade 4 lymphocytopenia) was present at every dose level. In total, 25 patients and 78% of all cycles (47 of 60 cycles) demonstrated severe lymphocytopenia. Often this lymphocytopenia was already present after the first cycle of BM. Of notice, 13 of 26 patients presented with some depletion of lymphocytes before the start of treatment (seven patients grade 3 and six patients grade 4). None of the patients suffered from neutropenic fever or opportunistic infections.

Nonhematologic toxicity

Nonhematologic toxicities were observed at all dose levels (Table 4). Among the most frequent were nausea and vomiting, dryness of mouth and diarrhea. Although no episodes of febrile neutropenia or bacteremia occurred, some patients – at every dose level – developed mild fever and flu-like symptoms. No neurologic toxicity was observed.

Some gastrointestinal complaints (vomiting, nausea, anorexia and diarrhea) were described as severe and

Table 3 Hematologic toxicity (NCIC-CTC version December 1994) per cycle/patient

Toxicity (NCIC–CTC)	Dose level (mg/m^2)						
	160 (cycles = 11/pts = three)	180 (cycles = five/pts = three)	200 (cycles = 13/pts = three)	220 (cycles = eight/pts = four)	240 (cycles = five/pts = three)	260 (cycles = 11/pts = six)	280 (cycles = seven7/pts = four)
Hemoglobin							
3	1/1	–/–	–/–	–/–	1/1	–/–	–/–
4	–/–	–/–	–/–	–/–	–/–	–/–	–/–
1–4	2/1	2/2	7/2	7/4	3/2	6/4	6/4
Thrombocytopenia							
3	–/–	–/–	–/–	–/–	–/–	–/–	–/–
4	–/–	–/–	–/–	–/–	–/–	–/–	1/1
1–4	–/–	1/1	8/3	3/3	–/–	2/2	4/3
Leukopenia							
3	1/1	–/–	1/1	1/1	–/–	–/–	2/2
4	–/–	–/–	–/–	–/–	–/–	–/–	–/–
1–4	6/2	1/1	11/2	6/4	–/–	6/4	4/2
Neutropenia							
3	3/1	–/–	–/–	1/1	–/–	–/–	1/1
4	–/–	–/–	–/–	–/–	–/–	–/–	–/–
1–4	4/2	1/1	3/2	3/2	–/–	2/2	2/2
Lymphopenia							
3	4/2	1/1	1/1	2/2	–/–	–/–	–/–
4	6/3	4/3	11/3	6/3	4/3	10/6	6/4
1–4	10/3	5/3	12/3	8/3	5/3	10/6	6/4

NCIC-CTC, National Cancer Institute of Canada Common Toxicity Criteria; pts, patients.

scored as grade 3 toxicities at lower dose levels (at 200 and 220 mg/m^2, respectively). These events were not considered dose limiting as they were of short duration and did not seem related to BM administration. Indeed, at higher dose levels these events were not severe or did not occur.

No DLT was observed until the dose level of 280 mg/m^2. At this dose level, two patients experienced grade 3 fatigue and three patients had grade 2 cardiologic events (Table 5). Three out of four patients had electrocardiogram (ECG) changes compatible with ischemic cardiac lesions (ST-segment and T-wave changes).

One 60-year-old woman using antihypertensive medication entered the trial with a normal ECG, but showed anterolateral ischemia on ECG before the start of the second cycle. No clinical symptoms of angor were noted nor was there any biochemical change to suspect cardiac infarction. She received the second treatment cycle, but refused further follow-up investigations by day 15. Her general condition deteriorated fast and she died on day 30 of the second cycle. The ischemic ECG changes were noted until the last investigations before her death.

The second patient was a 47-year-old man with bladder cancer, who in addition had arterial hypertension and diabetes. Previously, he had been treated with anthracyclines. His ECG demonstrated a T-wave inversion after the first administration of BM (on day 8), at which time he experienced grade 2 anemia. These nonsymptomatic ECG changes recuperated by day 15. The third patient with cardiac toxicity was a 67-year-old man with disseminated rectal cancer, who developed asymptomatic ischemic ST deviations during the first cycle, which remained similar throughout the treatment period (two cycles). In this case, no relationship with anemia was suspected as this only developed by day 15 of the second cycle. Before the therapy the left ventricular ejection fraction had been normal in all these patients.

Although the patients did not experience clinical symptoms of cardiac ischemia, these ECG changes were felt to be important enough to consider them as dose limiting.

No further dose escalation was applied beyond the 280 mg/m^2 dose level and this dose level was considered the MTD with fatigue and cardiac toxicity as DLTs.

In total, six patients were treated with BM at the 260-mg/m^2 dose level and no DLTs occurred. Therefore, this level was considered to be the recommended dose for future phase II trials.

Tumor response

Tumor response was evaluable in 18 patients; all received at least two cycles. One patient with a metastatic melanoma showed a complete response for eight treatment cycles (four evaluations). Stable disease was observed in three other patients (with unknown primary, renal cell cancer and thyroid cancer, respectively) for the duration of two, two and four treatment cycles, respectively.

Table 4 Nonhematologic toxicity (NCIC-CTC, version December 1994) per evaluable patient

	160 mg/m^2 (n=3 pts)			180 mg/m^2 (n=3 pts)			200 mg/m^2 (n=3 pts)			220 mg/m^2 (n=4 pts)			240 mg/m^2 (n=3 pts)			260 mg/m^2 (n=6 pts)			280 mg/m^2 (n=4 pts)		
Toxicity grade	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Alopecia	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fatigue	–	1	–	2	1	–	1	2	–	–	2	–	1	1	–	3	2	–	–	1	2[b]
Anorexia	2	–	–	2	1	–	1	–	1	2	1	–	3	–	–	4	2	–	1	2	–
Nausea	–	2	–	–	1	–	2	–	–	2	–	1	–	1	–	3	2	–	2	–	–
Vomiting	–	3	–	1	–	–	2	–	–	2	–	1	–	1	–	5	–	–	1	1	–
Constipation	1	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	–
Diarrhea	–	–	–	–	–	–	–	–	–	–	–	2	3	–	–	1	–	–	–	–	–
Dry mouth	1	–	–	2	–	–	–	–	–	1	–	–	1	–	–	1	–	–	–	–	–
Tachycardia	1	–	–	1	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	–
Arrhythmia	–	1	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Peripheral neuropathy	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	1	1	–	–	1	–
Myocardial ischemia	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	3[b]	–
Fever	1	–	–	–	1	–	1	–	–	1	–	–	3	–	–	1	–	–	2	–	–
Infection	–	1	–	–	–	–	–	–	1[a]	–	1	–	1	–	–	–	–	1	1	1	–

NCIC-CTC, National Cancer Institute of Canada Common Toxicity Criteria; pts, patients.
[a]Bronchopneumonia, leading to death (not therapy-related).
[b]Dose-limiting toxicity.

Table 5 Cardiotoxicity per patient in three patients receiving a dose of 280 mg/m^2

Patient number	Cycle number	Cardiac event	ECG changes	NCIC-CTC
20	2	Antero-lateral ischemia	ST and T-wave deviations	2
21	1	Sinustachycardia, inferior and anterior ischemia	Nonspecific T-wave inversion	2[a]
23	2	Sinus bradycardia, mild ischemia	Infero-lateral ischemic ST-changes	2

NCIC-CTC, National Cancer Institute of Canada Common Toxicity Criteria.
[a]Patient was pretreated with doxorubicin.

Pharmacokinetics

The plasma PK of BM and its metabolites OH-BM, DiOH-BM, γ-OH-BM and *N*-demethyl-BM were investigated after the first infusion of the study drug in 15 patients. Noncompartmental PK parameters AUC_{all}, C_{max}, V_d and CL normalized for body surface area are shown in Table 6.

BM is rapidly eliminated from the plasma with a mean $t_{1/2\beta}$ of 37.8 min (SD: 2,6). The mean V_d was 14.2 l/m^2 with a SD of 6.6 l/m^2 and the mean Cl of BM was 287.8 ml/min/m^2 with a SD of 166.4. As expected, maximum plasma concentrations of BM were found at the end of the 30 min lasting infusion period.

In this study, all mean values of t_{max} and $t_{1/2\beta}$ estimated for BM and its metabolites OH-BM, DiOH-BM, γ-OH-BM and *N*-demethyl-BM were in range from 31 to 49 min. No valid evaluation could be performed for DiOH-BM beause of few data. The mean AUC_{all} of BM was 77 and 66% for the patients receiving 160 and 260 mg/m^2, respectively. These percentages are expressed as percentages of the sum of the AUCs of all compounds determined in the study and indicated lack of dose dependency. A similar conclusion could be made when BM Cl was correlated with the given dose. Furthermore, no significant correlation between dose and $t_{1/2\beta}$ of BM and its metabolites could be found. In this study, the AUC_{all} was calculated to be 76.6, 3.1, 12.2, 7.2 and 1.0% for BM, OH-BM, DiOH-BM, γ-OH-BM and *N*-demethyl-BM, respectively, expressed as mean percentage of the sum of the parent compound and all detected metabolites. The mean total amount of BM and its metabolites recovered in the first micturition was 9.8%, expressed as percentage of the administered dose. The amounts of BM and its metabolites excreted via the urine in the first micturition were found in the order: BM (41.5%) > OH-BM (30.3%) > DiOH-BM (18.5%) > γ-OH-BM (8%) > *N*-demethyl-BM (1.9%) expressed as mean percentage of the sum of the parent compound and identified metabolites.

Table 6 Individual pharmacokinetic parameters of bendamustine in plasma

Number	Subject number	Dose (mg/m^2)	$t_{1/2\beta}$(min)	t_{max}(min)	C_{max}(ng/ml)	AUC_{all} (min/ng/ml)	V_d(l/m^2)	Cl (ml/min/m^2)
1	1	160	58.4	35	12567.5	626860.5	21.5075	255.1
2	2	160	25.1	30	10476.0	419019.5	13.8143	381.7
3	3	160	42.1	35	9156.9	446151.3	21.7878	358.5
4	4	180	24.7	30	20778.8	1149347.8	5.5917	156.6
5	7	200	42.3	35	18990.4	1175780.0	10.373.6	170.1
6	9	200	28.9	35	12638.5	594530.0	14.0140	336.4
7	10	220	32.6	30	9081.3	374857.5	27.6118	586.7
8	15	220	47.4	30	12634.2	640694.3	23.4599	343.3
9	13	240	35.5	35	18091.4	1075470.3	11.4156	233.1
10	16	240	44.0	30	23939.7	1748762.8	8.7080	137.1
11	17	260	54.0	30	42981.4	3309375.3	6.1116	78.4
12	18	260	27.9	30	18765.0	904573.1	11.5868	287.4
13	24	260	40.9	30	20825.4	1170014.3	13.1175	222.2
14	25	260	47.3	35	46337.9	2246363.3	7.897.7	115.7
15	22	280	16.0	35	12544.8	417270.8	15.366.1	665.2
Mean			37.8	32.3			14.1576	287.8
SD			11.9	2.6			6.6436	166.4

AUC_{all}, first to the last measurable concentration; Cl, clearance; $t_{1/2\beta}$, terminal elimination half-life; V_d, volume of distribution.

Discussion

The present phase I study with BM used a 3-weekly schedule and a short (30-min) intravenous infusion. It showed that thrombocytopenia, fatigue and cardiac events were dose-limiting at 280 mg/m^2 and indicated that a dose of 260 mg/m^2 might be a safe dose for further phase II studies.

The clinical development of BM has not followed the present standards of good clinical practice and evidence-based medicine. Almost all previously published clinical trials used arbitrary dosages and schedules. Despite this, BM's value in the treatment of hematologic malignancies such as multiple myeloma, chronic lymphocytic leukemia and non-Hodgkin's lymphoma, and more recently metastatic breast cancer, has been established, underlining the importance of the compound.

This study is one of a series of more recently performed phase I trials with this agent. Schöffski *et al.* [30,31] studied BM in a weekly schedule and in a day 1+8 every 4 weeks schedule. The DLTs observed in both schedules were dryness of the mouth and fatigue, which occurred at 80 mg/m^2 in the first and at 160 mg/m^2 in the second schedule.

As mentioned, next to thrombocytopenia and fatigue, cardiac events were recorded in our study. This is not unique; disturbances in cardiac rhythm had been observed in the phase I studies reported by Schöffski *et al.* also [30,31]. In those studies these cardiac events, however, were not considered as dose limiting. They described a transient tachyarhythmia (atrial flutter) in a patient receiving the weekly schedule (at 60 mg/m^2/week) [30] and a reversible total atrio-ventricular block in a patient with the day 1+8 schedule, already after the first dose of 160 mg/m^2 [31].

Furthermore, in several other studies cardiac events in patients treated with BM have been reported [24,26,32]. The highest frequency of cardiac events has been reported in a single-center phase II study in which advanced lung cancer patients were treated with BM in a 70 mg/m^2/day × 4 every 4 weeks schedule. Seven of the 43 included patients (16.3%) showed intermittent cardiac arrhythmias [24]. In our study, we observed cardiac ischemia in addition to disturbances in the rhythm. These cardiac events were serious enough to be considered as DLT.

The potential clinical relevance of BM-induced cardiologic toxicity clearly deserves further investigation.

Mean values of the PK parameters of BM as found in this study are quite comparable with previously reported PK data [11,32]. It should be noted, however, that variable hydrolysis of BM might alter concentrations for DiOH-BM as well as for BM itself resulting in altered PK parameters. Therefore, the calculated PK parameters should be interpreted with caution. The results, however, provide strong evidence that a considerable part of the BM moiety underwent chemical hydrolysis in any period of drug or sample management.

BM is rapidly eliminated from the plasma ($t_{1/2\beta}$ 37.8 min) and the V_d of 14.2 l/m^2 is closely related to blood volume indicating no strong binding of the native substance to peripheral tissues. No dose dependency was observed in this study in the dose range of 160–280 mg/m^2, when the dose was correlated with either $t_{1/2\beta}$, AUC or Cl of BM. The mean percentual AUC of BM and its hydrolysis products OH-BM and DiOH-BM was 77, 3 and 12%,

respectively, expressed as percentage of the sum of the AUCs of all compounds. With a mean percentage of 7 and 1%, respectively, γ-OH-BM and *N*-demethyl-BM represented only a minor part of the overall AUC. Similar proportions were found in the urine. The mean percentage of the administered dose as BM and its metabolites recovered in the first micturition was 9.8%, which is comparable to the results obtained by Teichert *et al.* [33] These investigators recovered 8.5 ± 5.2% of the administered BM dose in urine during a 0–24 h interval. Other PK analysis by the same investigators, however, demonstrated higher levels of recovery of BM and its metabolites in urine (20 and 14%, respectively) [34,35]. The different sampling periods ('first micturition') and the varying chemical hydrolysis – resulting in large interpatient variability – are likely to influence the quantification of BM and its hydrolysis products in urine.

In summary, this study indicates that a dose of 260 mg/m^2 of BM given by short infusion every 3 weeks is the recommended dose for further use as a single agent in phase II settings. A cardiologic evaluation as part of the pretreatment workup seems advisable.

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References

1 Ozegowski W, Krebs D. w-[bid-(β-chlorethyl)-amino-benzimazolyl-(2)]-propionic or butyric acids as potential cytotoxic agents. *J Prakt Chem* 1963; **20**:178–186.
2 Gandhi V. Metabolism and mechanisms of action of Bendamustine: rationale for combination therapies. *Semin Oncol* 2002; **29 (Suppl 13)**:4–11.
3 Barmam Balfour JA, Goa KL. Bendamustine. *Drugs* 2001; **61**:631–640.
4 Strumberg D, Harstrick A, Doll K, Hoffman B, Seeber S. Bendamustine hydrochloride activity against doxorubicin-resistant human breast carcinoma cell lines. *Anti-cancer Drugs* 1996; **7**:415–421.
5 Leoni LM, Bailey B, Reiffert J, Niemeyer C, Bendall H, Dauffenbach L, Kerfoot C. *In vitro* and *ex vivo* activity of SDX-105 (bendamustine) in drug-resistant lymphoma cells. *Proc Am Assoc Cancer Res* 2004; **45**:[Abstract 1215].
6 Niemeyer C, Bailey B, Reiffert FJ, Bendall H, Corbeil J, Leoni LM. SDX-105 (Bendamustine) is a clinically active chemotherapeutic agent with a distinct mechanism of action. *Proc Am Assoc Cancer Res* 2004; **45**: [Abstract 1129].
7 Konstantinov SM, Kostovski A, Topashka-Ancheva M, Genova M, Berger MR. Cytotoxic efficacy of bendamustine in human leukaemia and breast cancer cell lines. *J Cancer Res Clin Oncol* 2002; **128**:271–278.
8 Schwänen C, Hecker T, Hübinger G, Wölfe M, Rittgen W, Bergmann L, Karakas T. *In vitro* evaluation of bendamustine induced apoptosis in B-chronic lymphocytic leukemia. *Leukemia* 2002; **16**:2096–2105.
9 Chow KU, Boehrer S, Geduldig K, Krapohl A, Hoezer D, Mitrou PS, Weidmann E. *In vitro* induction of apoptosis of neoplastic cells in low-grade non-Hodgkin's lymphomas using combinations of established cytotoxic drugs with bendamustine. *Haematologica* 2001; **86**:485–493.
10 Chow KU, Nowak D, Boeher S, Ruthardt M, Knau A, Hoelzer D, *et al.* Synergistic effects of chemotherapeutic drugs in lymphoma cells are associated with down-regulation of inhibitor of apoptosis proteins (IAPs), prostate-apoptosis-response-gene 4 (Par-4), death-associated protein (Daxx) and with enforced caspase activation. *Biochem Pharmacol* 2003; **66**:711–724.
11 Preiss R, Sohr R, Matthias M, Brockmann B, Hüller H. Untersuchungen zur Pharmakokinetik von Bendamustine (Cytostasan) am Menschen. *Pharmazie* 1985; **40**:782–784.
12 Bräunlich H, Stolze G, Stolze R. Renale wirkungen von Bendamustin (Cytosan®) bei Ratten. *Pharmazie* 1998; **43**:265–268.
13 Weber H, Amlacher R, Preiss R, Hoffmann H. Pharmakokinetik von Bendamustin (Cytostasan®) in B6D2F$_1$-Mäusen. *Pharmazie* 1991; **46**:589–591.
14 Bezek S, Scasnár V, Trnovec T, Grupe R. Hepatobiliary elimination of bendamustin (Cytosan®) in rats. *Pharmazie* 1991; **46**:810–811.
15 Güttner J, Burns G, Juingstand W. Onkogene Wirkung von χ-[1-Methyl-5-bis-(β-chloräthyl)-aminobenzimidazolyl-(2)]-buttersäuerhydrochlorid (Cytostasan®) bei der Maus. *Arch Geschulstforsch* 1974; **43**:16–21.
16 Heinecke H, Klaus S. Embryotoxic and teratogenic action of the nitrogen mustard derivatives IMET 3393 and IMET 3106 in micer. *Zentralbl Pharm Pharmacother Laboratoriumdiagn* 1971; **110**:1067–1076.
17 Rummel MJ, Mitrou PS, Hoelzer D. Bendamustine in the treatment of non-Hodgkin's lymphoma: results and future perspectives. *Semin Oncol* 2002; **29**:27–32.
18 Cohen P, Cheson B, Friedberg J, Robinson KS, Foran J, Fayad L, *et al.* The novel alkylator, Trenada™ (Bendamustine HCl), is active in both rituximab-refractory and rituximab-sensitive relapsed indolent NHL with acceptable toxicity. *Proc Am Soc Clin Oncol* 2005; **23 (Suppl 16)**:576s [Abstract 6564].
19 Borchmann P, Schnell R, Diehl V, Engert A. New drugs in the treatment of Hodgkin's disease. *Ann Oncol* 1998; **9 (Suppl 5)**:S103–S108.
20 Avaido M, Schulte K, Henze LM, Burger J, Finke J, Haas R Bendamustine in the treatment of chronic lymphocytic leukemia: results and future perspectives. *Semin Oncol* 2002; **29 (Suppl 13)**:S19–S22.
21 Knauf WU, Lissitchkov T, Herbrecht R, Loscertales J, Aldaoud A, Merkle K. Bendamustine versus chlorambucil in treatment – naïve B-CLL patients. *Blood* 2004; **104**:287b [Abstract 4817].
22 Pönisch W, Niederwieser D. Bendamustine in the treatment of multiple myeloma: results and future perspectives. *Semin Oncol* 2002; **29 (Suppl 13)**:S23–S26.
23 Ponisch W, Mitrou PS, Merkle K, Hrold M, Assmann M, Wilhelm G, *et al.* Treatment of Bendamustine and prednisone in patients with newly diagnosed multiple myeloma results in superior complete response rate prolonged time to treatment failure and improved quality of life compared to treatment with melphalan and prednisone a randomized phase III study of the East German Study Group of Hematology and Oncology (OSHO). *J Cancer Res Clin Oncol* 2006; **132**:204–212.
24 Reck M, Haering B, Koschel G, Kaukel E, Van Pauwel J, Gatzemeier U. Chemotherapie des fortgeschrittenen nicht-kleinzelligen und kleinzelligen Bronchialkarzinoms mit Bendamustin- Eine Phase II Studie. *Pneumologie* 1998; **52**:571–574.
25 Koester W, Stamatis G, Niederle N, Heider A, Avramidis K, Wilke H, *et al.* Phase II study with bendamustine/carboplatin in untreated patients with extensive disease small cell lung Cancer. *J Clin Oncol* 2004; **22**:7265.
26 Höffken K, Merkle KH, Schönfelder M, Anger G, Brandtner M, Ridwelski K, Seeber S. Bendamustine as salvage treatment in patients with advanced progressive breast cancer: a phase II study. *J Cancer Res Clin Oncol* 1998; **124**:627–632.
27 Zulkowski K, Kath R, Semrou R, Merkle K, Höffken K. Regression of brain metastases from breast carcinoma after chemotherapy with bendamustine. *J Cancer Res Clin Oncol* 2002; **128**:111–113.
28 von Mickwitz G, Chermozemsky I, Sirakova L, Chilingrirov P, Souchon R, Marschner N, *et al.* Bendamustine prolongs progression-free survival in metastatic breast cancer (MBC): a phase III prospective, randomized, multicenter trial of bendamustine hydrochloride, methotrexate and 5-fluorouracil (BMF) versus cyclophosphamide, methotrexate and 5-fluorouracil (CMF) as first-line treatment of MBC. *Anticancer Drugs* 2005; **16**:871–877.
29 Kollmannsberger C, Gerl A, Scheuler N, Beyer J, Kuczyk M, Rick O, *et al.* Phase II study of bendamustine in patients with relapsed or cisplatin-refractory germ cell cancer. *Anticancer Drugs* 2000; **11**:535–539.
30 Schöffski P, Seeland G, Engel H, Grünwald V, Paul H, Merkle K, *et al.* Weekly administration of bendamustine: a phase I study in patients with advanced progressive solid tumors. *Ann Oncol* 2000; **11**:729–734.
31 Schöffski P, Hagedorn T, Grünwald V, Paul H, Merkle K, Kowalski R, *et al.* Repeated administration of short infusions of bendamustine: a phase I study in patients with advanced progressive solid tumors. *J Cancer Res Clin Oncol* 2000; **126**:41–47.
32 Matthias M, Preiss R, Sohr R, Possinger K. Pharmacokinetics of bendamustine in patients with malignant tumors. *Proc Am Soc Clin Oncol* 1995; **14**:485 [Abstract 1476].
33 Teichert J, Baumann F, Chao Q, Franklin C, Bailey B, hennig L, *et al.* Characterization of two phase I metabolites of bendamustine in human liver

microsomes and in cancer patients treated with bendamustine hydrochloride. *Cancer Chemother Pharmacol* 2006; [Epub ahead of print].
34 Teichert J, Mockel J, Pönisch W, Seidel A, Lotfi M, Matthias M, *et al.* Influence of hepatic and renal function on the pharmacokinetics of bendamustine. *Int J Clin Pharmacol Ther* 2003; **41**:564.
35 Teichert J, Sohr R, Baumann F, Henning L, Merkle K, Caca K, *et al.* Synthesis and characterisation of some new phase II metabolites of the alkylator bendamustine and their identification in human bile, urine and plasma from patients with cholangiocarcinoma. *Drug Metab Dispos* 2005; **33**: 984–992.