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ORIGINAL ARTICLE

Bendamustine pharmacokinetic profile and exposure–response relationships in patients with indolent non-Hodgkin's lymphoma

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Abstract
Purpose The pharmacokinetic profiles of bendamustine and active metabolites were defined in patients with rituximab-refractory, relapsed indolent B-cell non-Hodgkin's lymphoma, and supported understanding of exposure–response relationships for efficacy and safety.
Methods Bendamustine was administered as a 60-min 120 mg/m^2 intravenous infusion on days 1 and 2 of six 21-day cycles. Pharmacokinetic models were developed, with covariate assessment. Correlations between bendamustine exposure and responder status or occurrence of neutropenia, thrombocytopenia, fatigue, nausea, and vomiting were examined.
Results Following a single dose of bendamustine HCl, concentrations declined in a triphasic manner, with rapid distribution, intermediate, and slow terminal phases. The intermediate $t_{1/2}$ (40 min) was considered the pharmacologically relevant (beta elimination) $t_{1/2}$ since the initial phases accounted for 99% of the AUC. Age, sex, mild/moderate renal, or mild liver impairment did not alter pharmacokinetics. Metabolite concentrations were low relative to parent. No correlation was observed between exposure and safety or efficacy measures because of the limited range of exposures after 120 mg/m^2 administration, except bendamustine C_{max} was a significant (P value = 0.013) predictor of the probability of nausea in patients, most of whom were pretreated with antiemetics.
Conclusions The BSA-based dosing regimen for bendamustine achieved the targeted exposure and was associated with a high incidence of therapeutic response. Given the short $t_{1/2}$ and low concentrations of bendamustine observed by 12 h after dosing, the single-dose profile for bendamustine described by these analyses is expected to be representative of the multiple-dose profile. The occurrence of nausea was significantly related to bendamustine exposure, with the probability of nausea increasing as bendamustine C_{max} increases.

Keywords Bendamustine · Pharmacokinetics · Exposure–response · Nausea · Metabolite

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Introduction

Bendamustine (Treanda®, Cephalon, Inc., Frazer, PA, USA) is a novel chemotherapeutic agent comprised of a bifunctional mechlorethamine alkylating group, a purine-like benzimidazole ring, and a butyric acid side chain. The drug has been shown to be a potent cytotoxic agent, with in vitro studies demonstrating extensive and durable DNA damage [1, 2]. In addition to direct DNA damage and apoptosis, other mechanisms for bendamustine action include inhibition of mitotic checkpoints and induction of mitotic

catastrophe [1]. These mechanistic characteristics may explain bendamustine's activity in drug-resistant cancer cells and refractory lymphoma patients [2, 3]. The contribution of the benzimidazole group to the overall anti-tumor activity of bendamustine is not currently known.

This agent has shown clinical activity against various hematologic malignancies and has been commercially available in Germany for many years [3–7]. Bendamustine was recently approved for marketing by the US Food and Drug Administration for chronic lymphocytic leukemia (CLL) and indolent non-Hodgkin's lymphoma (NHL) that has progressed during or within 6 months of treatment with rituximab or a rituximab-containing regimen. In a Phase 2 trial of bendamustine monotherapy for rituximab-refractory indolent (80%) and transformed (20%) NHL, the most frequent non-hematologic adverse effects included nausea, vomiting, fatigue, and constipation [3]. Grade 3 or 4 reversible hematologic toxicities included neutropenia (54%), thrombocytopenia (24%), and anemia (12%).

The primary route of bendamustine metabolism in humans is hydrolysis to the inactive metabolites monohydroxy (HP1) and dihydroxy (HP2) bendamustine. Two metabolites of bendamustine (gamma-OH-bendamustine (M3) and *N*-desmethyl-bendamustine (M4)) have cytotoxic activity and are formed via the minor CYP1A2 oxidative pathway [8]. Relative potency was considered 1/5 for M4 and 1 for M3, compared to bendamustine [8]. Bendamustine is >95% protein bound, but this binding is not affected by age (>70 years), low serum albumin levels (<31 g/L), or the presence of advanced tumors (data on file, Cephalon, Inc).

Although bendamustine has been in clinical use for many years, little information has been published regarding the pharmacokinetics (PK) of bendamustine. The goals of these analyses were to define the PK profile for bendamustine and its two active metabolites based on data obtained from patients with indolent NHL refractory to rituximab. Individual patient estimates of bendamustine exposure [area under the plasma concentration versus time curve (AUC) and maximum plasma concentration (C_{max})] were generated using the PK model to perform exposure–response analyses characterizing the efficacy (duration of response, progression-free survival) and safety (occurrence of neutropenia, fatigue, nausea, and vomiting) of bendamustine in these patients. Factors influencing either the PK of bendamustine or response to this therapy were also assessed.

Materials and methods

Study design, blood sampling, and bioanalysis

Data were obtained from a Phase 3, multicenter, open-label, 6-treatment cycle, single-agent study designed to investigate the safety, efficacy, and PK profile of bendamustine in patients with indolent NHL refractory to rituximab treatment [9]. Patients were men and women at least 18 years of age who had a World Health Organization (WHO) performance score of 0 through 2 [10]. Patients had adequate liver function ($\leq$2.5 × upper limit laboratory normal for AST (SGOT), ALT (SCPT), and alkaline phosphatase, $\leq$1.5 × upper limit laboratory normal for total bilirubin). Estimated creatinine clearance was greater than 30 mL/min, [11] and an absolute neutrophil count of greater than 1,000 cell/mm^3.

Patients included in the analyses had NHL in the WHO classifications of small lymphocytic lymphoma, lymphoplasmacytic lymphoma, splenic marginal zone B-cell lymphoma (with or without villous lymphocytes), extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type, nodal marginal zone lymphoma (with or without monocytoid B cells), and follicular lymphoma.

All studies were conducted in accordance with the principles of the Declaration of Helsinki and were approved by the Human Investigational Review Board of each study center. Informed consent was obtained from each subject after explanation of the potential risks and benefits, as well as the investigational nature of the study.

Bendamustine was administered as a 60-min intravenous (IV) infusion at a dose of 120 mg/m^2 on day 1 and day 2 of six consecutive 21-day treatment cycles. A dose reduction was required for study drug-related grade 4 hematologic toxicity or grade 3 or 4 non-hematologic toxicity [12]. The 120 mg/m^2 dose was selected based on previous ex vivo studies indicating that the median lethal dose for bendamustine in CLL cells produces concentrations ranging from 4.3 to 7.4 μg/mL [13].

Sparse samples (up to 4 per patient) for PK analysis were collected from eighty-eight patients within the following time windows on day 1 or day 2 of cycle 1 or cycle 2: before infusion and at 0.25 to 0.5, 0.5 to <1, 1 to <3, 3 to <7, and 7 to <10 h after infusion. In addition, full PK profiles were obtained in a subset (n = 12) of the patient population at the following time points in cycle 1: immediately before start of the IV infusion, at 30 min after start of infusion, at end of infusion (1 h), and post infusion at 15, 30, and 45 min and 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 h. Sparse sampling was also performed in this subset (n = 12) of the patient population within the following time windows in cycle 2: immediately before start of the IV infusion and more than 0.25 to <0.5 h and more than 1 to <3 h after start of infusion.

Whole blood (5 mL) was drawn into evacuated tubes containing EDTA and immediately placed on ice. Within 30 min, samples were centrifuged at 2,000 rpm for 10 min at 4°C, and plasma withdrawn. Within 30 min of separation, plasma samples were transferred to a freezer at −20°C until shipment.

Plasma concentrations of bendamustine, M3, and M4 were determined by validated high-performance liquid chromatography tandem mass spectrometry (HPLC MS/MS) methodology. The lower limit of quantification (LLOQ) was 0.10 ng/mL for bendamustine and M4, and 0.11 ng/mL for M3. The inter-day coefficients of variation for the assay of bendamustine, M3, and M4 metabolite concentrations were ≤10.9, ≤12.3, and ≤9.2%, respectively.

Efficacy response measures

Assessment of NHL was performed within 28 days before cycle 1, day 1 (baseline), and at week 6, week 12, and every 12 weeks thereafter until the patient completed or discontinued treatment. The end-of-treatment scan was performed within 28 days after the last dose of study drug unless the patient had experienced a delay due to toxicity, in which case the evaluation was performed within 2 weeks of the decision to withdraw the patient from treatment.

The International Workshop Response Criteria (IWRC) for NHL was used to classify each patient's response to bendamustine treatment [14]. Minor modifications were made to these criteria in order to clarify certain parameters that were not fully defined in the published criteria. Assessments were based on computed tomography scans of neck, chest, abdomen, and pelvis, plus palpation of lymph nodes and organs, and assessments of lactate dehydrogenase and bone marrow involvement. Response at each assessment was classified as: complete response (CR), complete response/unconfirmed (CRu), partial response (PR), stable disease, relapsed disease (RD), or progressive disease. Best response was defined as the best tumor outcome assessment observed for a patient (CR, CRu, or PR). Responder patients were defined as those patients who achieved a best response during the study according to the IWRC for NHL based on the response assessments provided by an independent review committee.

Duration of response (DR) was defined as the interval from the date of first observation of a response (CR, CRu, or PR) until the date of progression, change of therapy, or death. Progression-free survival (PFS) was defined as the time interval from the date of first study drug dose to the first documentation of disease progression, change of therapy, or death regardless of cause.

Safety response measures

Safety endpoints of interest included neutropenia, thrombocytopenia, nausea, vomiting, and fatigue. The occurrence of any of these adverse events included any instance reported during treatment (observed events from the start of the first dose until 28 days after the last dose) and classified as definitely, possibly, or probably related to study medication. Neutropenia and thrombocytopenia were determined based on weekly laboratory hematology measurements. For each patient cycle, the lowest laboratory absolute neutrophil counts (ANC) measurement was graded based on Common Terminology Criteria for Adverse Events, version 3.0 [12]. Only patients with a grade of 3 or 4 were classified as having neutropenia or thrombocytopenia in this analysis. Cytokine use was discouraged for the first cycle to stimulate WBC production, but was allowed in any patient who demonstrated a need for their use.

Statistical methods and population pharmacokinetic model development

Population PK and pharmacokinetic/pharmacodynamic (PK/PD) analyses were performed using NONMEM software, version 6, level 1.0 [15]. First-order conditional estimation (FOCE) was used for PK models and Laplacian estimation with the LIKELIHOOD option was used for PK/PD models.

Separate population PK models were developed for bendamustine, M3, and M4. The population PK models were developed using an index dataset, evaluated using a smaller test dataset, and then the model parameters were re-estimated using a total dataset. For brevity, since the test performance was adequate, this manuscript will detail only the final models.

The PK profile for bendamustine plasma concentrations declined from peak in a triphasic manner. A three-compartment model was parameterized using central clearance (CL), central (V_c) and peripheral compartment volumes of distribution (V_{p1}, V_{p2}), and intercompartmental clearances (Q_1, Q_2). For M3 and M4, one- and two-compartment models were evaluated with zero-order input following a lag time. Interindividual variability (IIV) and residual variability (RV) were described using an exponential and a proportional error model, respectively. Point estimates of half-life were obtained algebraically using the final, typical parameters of the model.

Covariate analysis was performed for bendamustine only, using a forward selection ($\alpha = 0.05$) followed by backward elimination ($\alpha = 0.001$) process. Patient covariates explored included sex, age, race, weight, body surface area (BSA), creatinine clearance (CrCL) (modified by the use of ideal body weight (IBW), as proposed by Peck), and concentrations of alanine aminotransferase, aspartate aminotransferase, total bilirubin, and serum albumin [11, 16, 17].

Model qualification through assessment of predictive performance was conducted. The fit of the model to the data was evaluated graphically and through measurements of prediction error. The differences between the measured and predicted bendamustine concentrations were evaluated for

bias (percent individual prediction error (%IPE)) and precision (absolute percent individual prediction error (|%IPE|)) according to the methods of Sheiner and Beal [18].

In addition, the final model for bendamustine was evaluated using a simulation-based predictive check method. A visual predictive check (VPC) was performed generating 100 simulations from the final model. An overlay of the original data on a prediction interval based on the simulated replicate datasets was prepared.

Determination of exposure measures

Bayesian estimates of PK model parameters for each patient were generated and used to compute measures of exposure. These exposure measures included bendamustine, M3, and M4 cycle 1 AUC and cycle 1 C_{max}, as well as composite AUC and C_{max}. Composite measures were weighted sums of the exposures based on the potency of the moiety.

Summary statistics of exposure measures (i.e., AUC and C_{max}) were evaluated by categories of sex, age, race, BSA, hepatic function, and renal function. Age was classified into three categories: 16–64 years, 65–74 years, and greater than or equal to 75 years. Body surface area was classified into quartiles. Classifications for hepatic function were defined as: normal [total bilirubin and aspartate aminotransferase (AST) ≤upper limit of normal (ULN)], mild (total bilirubin >ULN to 1.5 × ULN or AST > ULN), moderate (total bilirubin >1.5 to 3 × ULN, any AST), and severe (total bilirubin >3 × ULN, any AST) [19]. Classifications for renal function were defined as: normal (CrCL > 80 mL/min), mild renal impairment (50 mL/min < CrCL ≤ 80 mL/min), moderate renal impairment (30 mL/min < CrCL ≤ 50 mL/min), and severe renal impairment (CrCL ≤ 30 mL/min) [20].

Exposure–response model development

Logistic regression analysis was used to statistically assess the relationship between various exposure measures and covariates and responder status or the occurrence of adverse events (fatigue, nausea, and/or vomiting). Residual variability was considered using an additive error structure. Although the protocol allowed for bendamustine dose reduction due to adverse events, statistical comparison of bendamustine, M3, M4, or composite exposures in patients requiring dose reduction relative to those without dose reduction was not performed as no relationship was observed during exploratory graphical assessment.

The influence of various covariates was only examined for the exposure measure with the most significant relationship with responder status or the occurrence of fatigue, nausea, or vomiting. The following covariates were evaluated in the exposure–response analyses: sex, age, race, BSA, number of prior treatment courses (non-bendamustine; at baseline), indolent NHL subtype, and WHO performance grade. Prophylactic antiemetic use was also allowed in this clinical trial and was considered for the covariate assessment.

Diagnostic plots were evaluated to determine whether the functional form of the relationship between the estimated logit parameter and the covariate was linear. Non-linear functional forms of continuous covariates were transformed or grouped to meet the linear logit assumption.

A univariate analysis of each patient covariate was performed. Each patient covariate was added to the new base logistic regression model, one at a time, and was tested for significance using a likelihood ratio test ($\alpha = 0.05$).

Graphical analysis of duration of response and progression-free survival

The relationships between measures of exposure and efficacy response were explored graphically. Displays of DR and PFS versus exposure stratified by the covariates of interest including sex, age, race, number of prior treatment courses, and indolent NHL subtype were constructed. Kaplan–Meier plots of DR (responders only) and PFS were constructed by categorizing each exposure into two groups using the median exposure measure.

Results

Pharmacokinetic data

The total dataset consisted of 347 bendamustine plasma concentrations from 78 subjects, 302 M3 plasma concentrations from 77 subjects, and 254 M4 plasma concentrations from 74 subjects. The data from 22 subjects were removed as their PK samples were assayed outside the validated stability period. Patient characteristics for the total dataset stratified by analyte are presented in Table 1.

Bendamustine population pharmacokinetic model

A three-compartment, open model with zero-order input, first-order elimination, and IIV terms on CL, *V*c, *V*p1, and *V*p2 reasonably described the plasma concentration data for bendamustine. Residual variability was expressed with a proportional error model. No covariate factors were found to significantly influence the observed variability in the PK of bendamustine. A schematic diagram of the final bendamustine model is shown in Fig. 1.

The population PK parameters are shown in Table 2. The population estimate for clearance of bendamustine

Table 1 Summary statistics of patient characteristics, stratified by analyte: total

Patient characteristic		Bendamustine ($N = 78$)	M3 ($N = 77$)	M4 ($N = 74$)
Baseline age (year)	Median	58.5	58.0	57.5
	Min, max	31, 84	31, 84	31, 84
Baseline alanine aminotransferase (U/L)	Median	24	240	23
	Min, max	9, 115	9, 115	9, 115
Baseline aspartate aminotransferase (U/L)	Median	22	22	22
	Min, max	13, 135	13, 135	13.00, 135
Baseline creatinine clearance (mL/min)	Median	93.3	93.3	92.5
	Min, max	40.7, 150	40.7, 150	40.7, 150
Baseline serum albumin (g/L)	Median	42	42	42
	Min, max	24, 49	20, 48	20, 48
Baseline total bilirubin (μmol/L)	Median	8.55	8.55	8.78
	Min, max	1.71, 29.1	1.71, 29.1	1.71, 29.1
Baseline weight (kg)	Median	85.0	86.6	86.8
	Min, max	44, 151	44, 151	44, 151
Body surface area (m^2)	Median	2.0	2.0	2.0
	Min, max	1.33, 2.72	1.33, 2.72	1.33, 2.72
Race, *n* (%)	Black	5 (6.4)	5 (6.5)	5 (6.8)
	Caucasian	69 (88.5)	68 (88.3)	66 (89.2)
	Hispanic	1 (1.3)	1 (1.3)	0 (0.0)
	Other	2 (2.6)	2 (2.6)	2 (2.7)
Sex, *n* (%)	Male	50 (64.1)	50 (64.9)	47 (63.5)
	Female	28 (35.9)	27 (35.1)	27 (36.5)

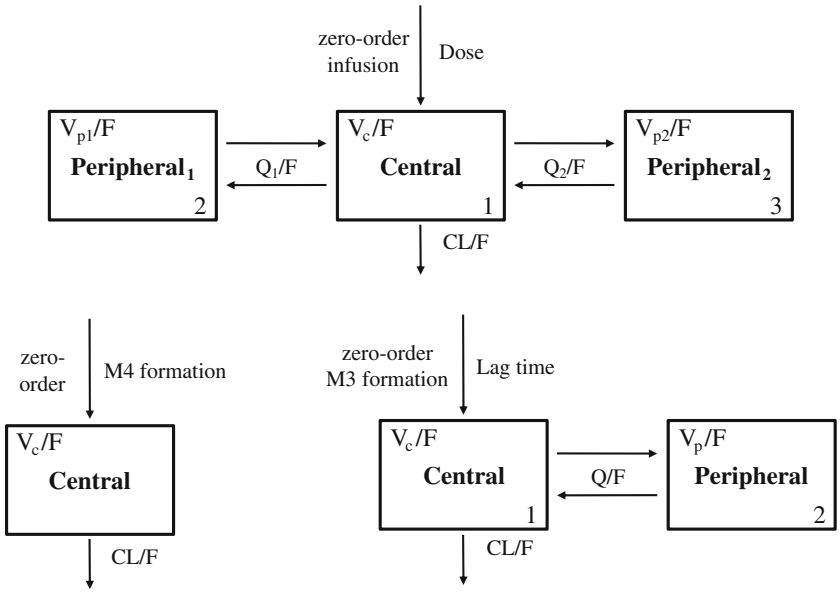

Fig. 1 Schematics of the bendamustine, M4, and M3 final pharmacokinetic models

(31.7 L/h) was similar to that reported previously in patients with chronic lymphocytic leukemia and solid tumors (approximately 27 L/h/1.73 m^2) based on non-compartmental PK analysis [21, 22]. The overall volume of distribution reported here (approximately 40 L) is larger than reported previously (approximately 20 L); however, the duration of full-profile post-dose PK sampling after bendamustine administration was limited in previous investigations.

Estimates of $t_{1/2}$ α, $t_{1/2}$ β, and $t_{1/2}$ γ for bendamustine were 0.29, 0.7, and 110 h, respectively, consistent with a rapid distribution phase, intermediate phase, and a slow terminal decline of plasma concentrations after a single intravenous dose. The AUC for the terminal phase accounted

Table 2 Population mean (%SEM) estimates of the pharmacokinetic parameters for Bendamustine, M3, and M4

	Bendamustine	M3[b]	M4[b]
CL (L/h)	31.7 (6.6)	347 (6.5)	3,890 (8.5)
V_1 (L)	14.1 (5.7)	209 (8.4)	3,490 (12.8)
Q_1 (L/h)	0.989 (9.2)	6.60 (12.1)	NA
V_2 (L)	0.92 (8.0)	26.1 (15.9)	NA
Q_2 (L/h)	0.159 (24.5)	NA	NA
V_3 (L)	25.2 (33.6)	NA	NA
Duration of metabolite formation (h)	NA	1.07 (3.3)	1.27 (0.3)
Formation lag time (h)	NA	0.198 (3.4)	NA
IIV on CL (%CV)	33.32 (21.9)	19.21 (26.9)	62.85 (26.3)
IIV on V_1 (%CV)	15.56 (69.4)	19.60 (43.2)	88.49 (33.6)
IIV on V_2 (%CV)	22.29 (40.8)	38.73 (29.4)	NA
IIV on V_3 (%CV)	66.78 (51.6)	NA	NA
Covariance between IIV on CL/*F* and IIV on *V*/*F*[a]	NA	NA	0.527 (31.1)
RV (%CV)	35.64 (18.2)	36.74 (17.9)	34.79 (12.1)

All parameters for M3 and M4 are apparent values based on f, the fraction metabolized to each moiety

CL clearance, *CV* coefficient of variation, *IIV* interindividual variability, *NA* not applicable, Q_1 intercompartmental clearance 1, Q_2 intercompartmental clearance 2, *RV* residual variability, V_1 central distribution volume for bendamustine and M3, V_2 peripheral distribution volume for bendamustine (compartment 2) and M3, V_3 peripheral distribution volume for bendamustine (compartment 3)

[a] $r^2 = 0.898$

[b] Clearance and volume terms for M3 and M4 are relative to the fraction converted to each metabolite (for example, CL/F_m)

for less than 1% of the total AUC. Therefore, the $t_{1/2}$ of the β phase is considered to be reflective of bendamustine elimination half-life. Further, the predicted concentration at 12 h (C_{12}) after the first dose was 0.27 ng/mL. The ratio of C_{12} to C_{max} had a mean value of 1:25,000. Thus, accumulation is not expected and the single-dose PK profile is considered representative of the multiple-dose profile. The median AUC and C_{max} for bendamustine were 13,635 ng × h/mL and 5,839 ng/mL, respectively.

The mean and median values of %IPE and |%IPE| (6.5 and 18.2%, respectively) measures demonstrated that the model was essentially unbiased and had a reasonable predictive performance. Observed bendamustine concentrations overlaid with median typical value population-predicted concentrations (Fig. 2) indicate that the model adequately described the central tendencies in the bendamustine concentration–time data.

In addition, a VPC was performed using the final pharmacokinetic model, simulating 100 replications of the analysis dataset. Figure 3 illustrates the 90% prediction interval, derived from the 100 simulated datasets, overlaid on the observed bendamustine concentration versus time relative to dosing data. The majority of the observed sparse data falls within the prediction interval. Overall, the VPC indicates no apparent biases in the overall model fit by comparing the simulated data (based on the model) to the observed data.

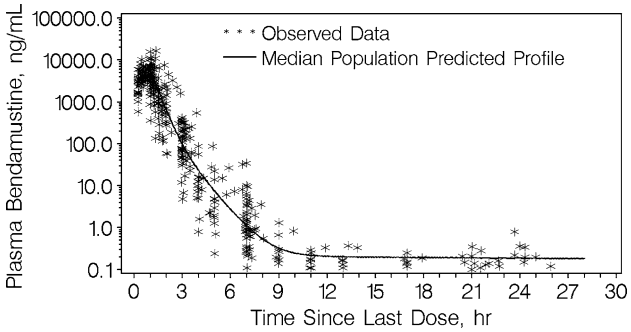

Fig. 2 Measured plasma bendamustine concentration versus time since last dose with the bendamustine model typical value population-predicted profile overlaid

Metabolite population pharmacokinetic models

The population PK models for M3 and M4 were a two- and one-compartment model, respectively (Table 2). Schematic diagrams of the final models for M3 and M4 are shown in Fig. 1. The estimated values of M3 $t_{1/2}$ α and $t_{1/2}$ β were 0.41 and 2.80 h, respectively, the estimated $t_{1/2}$ of M4 was 0.62 h, which was similar to that of the β phase of bendamustine. The median M3 and M4 AUC values were 1,252 and 115 ng h/mL, or approximately one-tenth and one-hundredth that of bendamustine, respectively.

Observed M3 concentrations overlaid with median typical value population-predicted concentrations (Fig. 4, upper

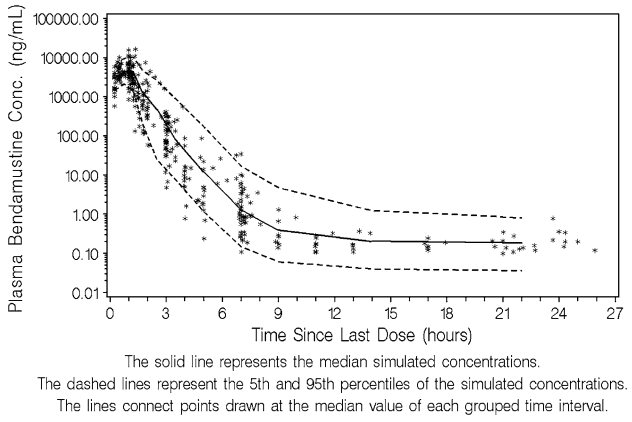

Fig. 3 Percentiles of simulated data from the visual predictive check of the final bendamustine pharmacokinetic model overlaid on the observed concentration data

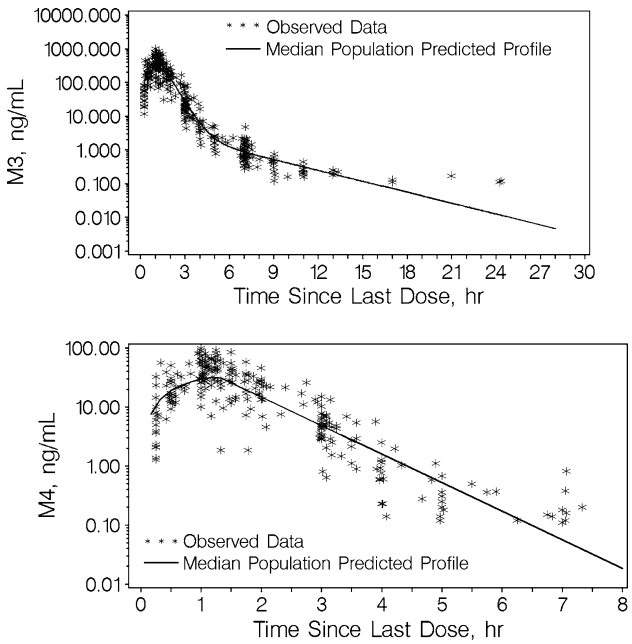

Fig. 4 Measured plasma M3 (*upper panel*) and M4 (*lower panel*) concentration versus time since last dose with the model typical value population-predicted profiles overlaid

panel) indicate that the model adequately described the central tendencies in the M3 concentration–time data. A similar pattern is seen for observed M4 concentrations as shown in Fig. 4, lower panel.

Determination of exposure

Individual patient exposures (AUC and C_{max}) for bendamustine, M3, and M4 were estimated using the population PK models. Though none of the covariate factors evaluated on bendamustine PK model parameters reached statistical significance, exposure estimates were stratified by covariate categories in order to assess the presence of trends for exposure differences between groups. No notable differences between groups were present for sex, age, or race.

A visual trend for increasing exposure with increasing BSA was observed, with maximum differences in median values across quartiles of BSA being approximately 30% for both AUC and C_{max}.

No patients with PK data were classified as having moderate or severe hepatic dysfunction or severe renal dysfunction. Median bendamustine AUC and C_{max} showed little difference between the normal ($N = 52$) and mild ($N = 26$) hepatic dysfunction groups or between the normal ($N = 47$), mild ($N = 23$), and moderate ($N = 8$) renal dysfunction groups. This assessment supports the lack of significance of mild hepatic dysfunction and mild or moderate renal dysfunction as a predictor of PK variability.

Exposure–response data

The efficacy and safety analysis datasets consisted of data from a total of 80 patients (78 patients with bendamustine concentrations and 2 patients with metabolite concentrations only). A total of 78 patients had bendamustine exposure estimates, 77 patients had M3 exposure estimates, and 74 patients had M4 exposure estimates. A total of 71 patients had each of the analytes, and thus a composite AUC and C_{max}. Patient characteristics for the efficacy/safety dataset are presented in Table 3.

Efficacy results

Of the 80 patients in the exposure–response analysis of efficacy, 68 patients (85%) were responders after treatment with bendamustine. No exposure measure was a significant predictor of responder status.

No relationship between DR and exposure was identified by exploratory graphical or Kaplan–Meier analysis. Exploratory graphical analyses suggested an initial trend for a relationship between PFS and bendamustine C_{max} up to 60 weeks, and between bendamustine AUC, composite AUC, and composite C_{max} up to 30 weeks, followed by no difference. However, Kaplan–Meier analysis resulted in no statistically significant relationship between PFS and any exposure measure. The Kaplan–Meier plot for progression-free survival based on cycle 1 bendamustine AUC separated by AUC values above and below the median value is shown in Fig. 5 (P value = 0.3025).

Safety results

Across all cycles of the study, there were 237 patient cycles with the occurrence of grade 3 or 4 neutropenia and 179 patient cycles without grade 3 or 4 neutropenia. The mean (SD) AUCs in patients with and without neutropenia were 13,547 (5,261) ng × h/mL and 12,459 (3,654) ng × h/mL.

Table 3 Summary statistics of patient characteristics for patients included in the efficacy and safety analyses

Patient characteristic		*N* = 80
Sex, *n* (%)	Male	50 (62.5)
	Female	30 (37.5)
Baseline age (year)	Median	57.50
	Min, max	31.0, 84.0
Race, *n* (%)	Caucasian	71 (88.8)
	Black	5 (6.3)
	Asian	1 (1.3)
	Hispanic	1 (1.3)
	Other	2 (2.5)
Body surface area (m^2)	Median	2.00
	Min, max	1.3, 2.7
Number of prior treatment courses	Median	3.00
	Min, max	1.0, 10.0
Indolent NHL subtype, *n* (%)	B-cell chronic lymphocytic leukemia/ small lymphocytic lymphoma	12 (15)
	Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type	8 (10)
	Follicular lymphoma, follicular	53 (66)
	Lymphoplasmacytic lymphoma	1 (1)
	Nodal marginal zone lymphoma (±Monocytoid B cells)	6 (8)
Baseline WHO performance grade, *n* (%)	0	39 (48.8)
	1	37 (46.3)
	2	3 (3.8)
	Unknown	1 (1.3)
Prophylactic antiemetic use, *n* (%)	No	16 (20)
	Yes	64 (80)
Lowest BSA-based dose, mg/m^2, *n* (%)	60	3 (3.8)
	90	17 (21.3)
	120	60 (75.0)
Patient dose reduced, *n* (%)	No	60 (75.0)
	Yes	20 (25.0)

BSA body surface area, *NHL* non-Hodgkin's lymphoma, *SD* standard deviation, *WHO* World Health Organization

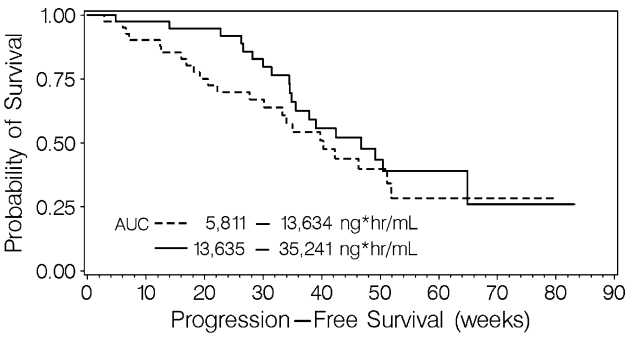

Fig. 5 Kaplan–Meier plot of progression-free survival, stratified by median cycle 1 bendamustine AUC

Thus, though neutropenia occurred frequently even with some use of cytokines, there did not appear to be a strong relationship with the exposure level to bendamustine.

A total of 45 patients (56%) had at least one occurrence of fatigue during the treatment period; however, no exposure measures or patient factors were statistically significant predictors of the probability of fatigue.

A total of 59 patients (74%) had at least one occurrence of nausea during the treatment period. The majority of the patients (64 of 80 patients) received antiemetic therapy (primarily 5-HT3 receptor antagonists) during the trial. This high percentage of prophylactic antiemetic use precluded statistical assessment of the influence of this factor on the relationship between bendamustine exposure and nausea in this analysis. Bendamustine C_{max} was a statistically significant (P value = 0.013) predictor of the probability of nausea. The relationship between the predicted probability of nausea and bendamustine C_{max} is shown in Fig. 6, upper panel.

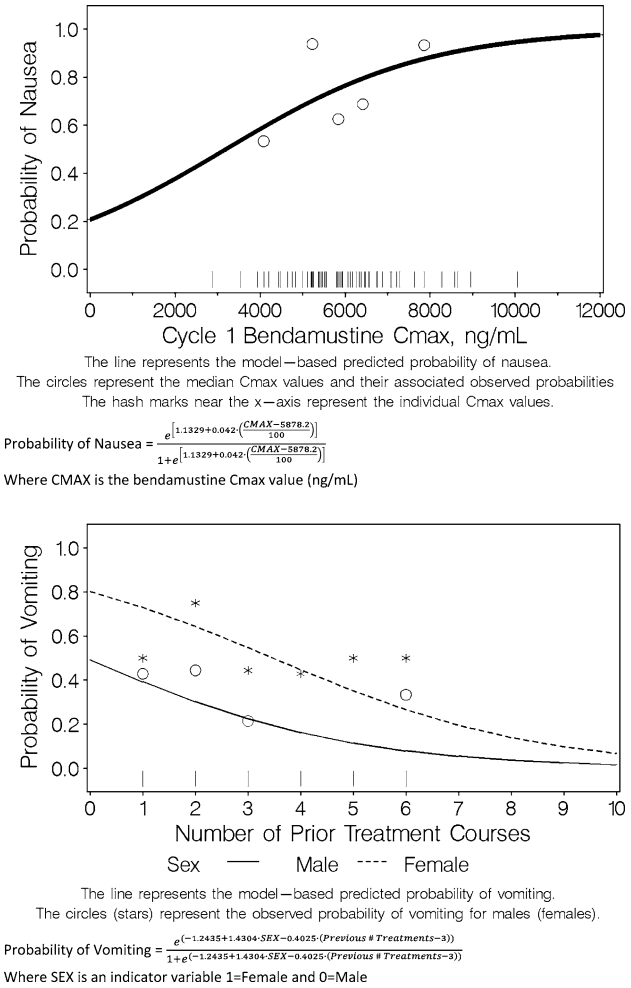

Fig. 6 Observed and model-predicted probabilities of adverse events: occurrence of nausea versus bendamustine C_{max} (*upper panel*) and occurrence of vomiting versus the number of prior treatment courses (*lower panel*)

As bendamustine C_{max} increases, the predicted probability of nausea increases. The odds ratio of 1.00043 (95% CI = 1.00000, 1.00088) indicates that for a 100-unit (ng/mL) increase in bendamustine C_{max}, the probability of a nausea adverse event is 1.043 times more likely. For a patient with a C_{max} value of 5,001.20 ng/mL (25th percentile), the probability of nausea is 0.68, whereas, a patient with a C_{max} value of 6,567.20 ng/mL (75th percentile) would have a probability of nausea of 0.81. The Hosmer-Lemeshow goodness-of-fit statistic was 13.01 with 8 degrees of freedom (P value = 0.1115). The area under the receiver-operator characteristic (ROC) curve was 0.63, indicating an adequate fitting and marginally predictive model.

A total of 25 patients (31%) had at least one occurrence of vomiting during the treatment period. Sex and the number of prior treatment courses (1–10) were statistically significant predictors of the probability of vomiting. No exposure measures were significant predictors of vomiting. Due to the high percentage of patients that received prophylactic antiemetics, the influence of this factor could not be statistically assessed. The predicted probability of vomiting versus the number of prior treatment courses with observed probabilities from each number of prior treatment courses is shown in Fig. 6, lower panel. As the number of prior treatment courses increases, the probability of vomiting decreases. The observed probability of vomiting for each number of prior treatment courses generally corresponds with the model-based predicted probabilities. Females were also shown to have a higher probability of vomiting when compared to males.

When only grade 3 or 4 level thrombocytopenia, fatigue, or nausea were considered, there was no significant differences in bendamustine exposure (AUC) between patients experiencing these toxicities at grade levels 3 or 4, and those patients without occurrence these events. There were no patients with grade 3 or 4 vomiting that had available PK information.

Discussion

Based on the PK profile of bendamustine, a dose of 120 mg/m^2 produced C_{max} concentrations of approximately 6 μg/mL in these patients with NHL. Ex vivo studies have previously demonstrated that the median lethal dose for bendamustine in CLL cells ranges from 4.3 to 7.4 μg/mL [13]. These ex vivo studies also demonstrated that high concentrations of bendamustine are more efficient than prolonged exposure and that a single exposure to bendamustine was sufficient to initiate apoptosis in cancer cells, with the proportion of dead cells increasing over 72 h.

Individual patient exposures were estimated for bendamustine and its metabolites using the final population PK models. In order to address clinical pharmacology labeling issues, the exposures were summarized across categories of patient factors, laboratory measures, and special populations to evaluate potential differences in estimated exposures between groups. Mild or moderate renal impairment, mild hepatic impairment, or differences in age or sex did not affect systemic exposure to bendamustine. These results are limited, however, and therefore bendamustine should be used with caution in patients with mild or moderate renal impairment, or mild liver impairment [23]. Conclusions regarding the effect of race/ethnicity on exposure to bendamustine in this analysis cannot be drawn due to the limited available data.

Across the full range of data, a trend for increasing exposure with increasing BSA was observed suggesting that BSA-based dosing did not normalize exposures. However, the relationship between AUC and BSA was flat over 80%

of BSA values suggesting that BSA-based dosing is appropriate to achieving a consistent exposure level. Given the narrow range of BSA-based doses in this trial, a definitive conclusion cannot be drawn regarding the effect of BSA-based dosing on bendamustine exposure based on the present data.

The lack of a statistically significant relationship between PFS and exposure may not be surprising since there was a high degree of response across a narrow range of exposures. The BSA-based dosing utilized in this study achieved the intent of targeting an exposure associated with a high incidence of therapeutic response across patients. Perhaps an exposure–response relationship could not be characterized because the exposures were clustered within the effective range. Nausea was the only safety or efficacy endpoint that was significantly related to bendamustine exposure, with the probability of nausea increasing as bendamustine C_{max} increased. This conclusion assumes the presence of concurrent prophylactic antiemetic use as most patients received this therapy during the trial. Perhaps it is not surprising that an exposure–response relationship for fatigue was not observed, since fatigue occurs commonly in patients with cancer, and its complex etiology makes it difficult to determine consistent correlates of fatigue in this patient population [24]. The ability of these analyses to detect potential exposure–response relationships for safety endpoints was limited, however, by the narrow range of exposures resulting from administration of BSA-based dosing of bendamustine at a single dose level.

Female patients were more likely to develop vomiting than male patients. This finding is consistent with previous reports indicating that female gender is a risk factor for chemotherapy-induced nausea and vomiting [25, 26]. As the number of prior treatment courses increased, the predicted probability of vomiting decreased. The reason for this finding is unclear, but is potentially related to more optimal antiemetic use based on prior experience, or to chemotherapy dose adjustment.

In summary, despite many years of clinical experience with bendamustine, only limited PK information has been published. This paper describes the population PK profile of bendamustine, and its active metabolites in patients with NHL. No patient factors (including BSA) or laboratory measures were found to significantly influence the PK of bendamustine. In addition, estimation of individual patient exposures allowed for the evaluation of the exposure–response relationships for safety and efficacy of bendamustine. The occurrence of nausea was related to bendamustine C_{max}, such that an increasing C_{max} results in an increased predicted probability of nausea, even in the presence of antiemetic therapy. No exposure–response was evident for measures of efficacy (responder status and progression-free survival), and though they occurred frequently, no exposure–response relationships were found for the safety measures neutropenia, fatigue, or vomiting. Sex and prior treatment courses were statistically significant predictors of the occurrence of vomiting. Overall, the results of these analyses facilitate understanding of the PK and exposure–response relationships for bendamustine demonstrated during clinical development for NHL.

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References

1. Leoni LM, Bailey B, Reifert J et al (2008) Bendamustine (Treanda) displays a distinct pattern of cytotoxicity and unique mechanistic features compared with other Alkylating agents. Clin Cancer Res 14:309–317
2. Strumberg D, Harstrick A, Doll K et al (1996) Bendamustine hydrochloride activity against doxorubicin-resistent human breast carcinoma cell lines. Anticancer Drugs 7:415–421
3. Friedberg JW, Cohen P, Chen L, Robinson KS, Forero-Torres A, La Casce AS et al (2008) Bendamustine in patients with rituximab-refractory indolent and transformed non-Hodgkin's lymphoma: results from a phase II multicenter, single-agent study. J Clin Oncol 26:204–210
4. Bergmann MA (2006) Efficacy of bendamustine in patients with relapsed or refractory chronic lymphocytic leukemia: results of a phase I/II study of the German CL Study Group. Haematologica 90:1357–1364
5. Ponisch W, Mitrou PS, Merkle K, Herold M, Assmann M, Wilhelm G et al (2006) 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 132:205–212
6. von Minckwitz G, Chernozemsky I, Sirakova L, Chilingirov P, Souchon R, Marschner N et al (2005) 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 16:871–877
7. Schmittel A, Knodler M, Hortig P, Schulze K, Thiel E, Keilholz U (2007) Phase II trial of second-line bendamustine chemotherapy in relapsed small cell lung cancer patients. Lung Cancer 55:109–113
8. Teichert J, Baumann F, Chao Q, Franklin C, Bailey B, Hennig L et al (2007) Characterization of two phase I metabolites of bendamustine in human liver microsomes and in cancer patients treated with bendamustine hydrochloride. Cancer Chemother Pharmacol 59:759–770
9. Kahl BS, Bartlett NL, Leonard JP, Chen L, Ganjoo K, Williams ME, et al (2009) Bendamustine is effective therapy in patients with rituximab-refractory indolent B-cell non-Hodgkin's lymphoma: results from a multicenter study. Cancer Nov 4 (Epub ahead of print)
10. World Health Organization (1998) Executive summary from the world symposium on primary pulmonary hypertension. World Health Organization, Evian, France
11. Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16:31–41

12. National Cancer Institute. Cancer Therapy Evaluation Program (2006) Common terminology criteria for adverse events v3.0 (CTCAE). http://www.ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf. Accessed 01 Aug 2007
13. Schwänen C, Hecker T, Hubinger G, Wolfle M, Rittgen W, Bergmann L et al (2002) In vitro evaluation of bendamustine induced apoptosis in B-chronic lymphocytic leukemia. Leukemia 16:2096–2105
14. Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM et al (1999) Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol 17:1244
15. Beal SL, Sheiner LB, Boeckmann AJ (1989–2006) NONMEM users guides. Ellicott City, Maryland
16. Mosteller RD (1987) Simplified calculation of body-surface area. N Engl J Med 317:1098
17. Peck CC, Conner DP, Murphy MG (1989) Bedside clinical pharmacokinetics: simple techniques for individualizing drug therapy. Vancouver, Washington
18. Sheiner LB, Beal SL (1981) Some suggestions for measuring predictive performance. J Pharmacokinet Biopharm 9:503–512
19. Patel H, Egorin MJ, Remick SC, Mulkerin D, Takimoto CHM, Doroshow JH et al (2004) Comparison of Child-Pugh (CP) criteria and NCI organ dysfunction working group (NCI-ODWG) criteria for hepatic dysfunction (HD): Implications for chemotherapy dosing. J Clin Oncol 22(14S suppl):6051
20. Food and Drug Administration (1998) Guidance for industry pharmacokinetics in patients with impaired renal function—study design, data analysis, and impact on dosing and labeling. Docket #97D-0214
21. Rasschaert M, Schrijvers D, Van den BJ, Dyck J, Bosmans J, Merkle K et al (2007) A phase I study of bendamustine hydrochloride administered day 1 + 2 every 3 weeks in patients with solid tumours. Br J Cancer 96:1692–1698
22. Rasschaert M, Schrijvers D, Dyck J, Bosmans J, Merkle K et al (2007) A phase I study of bendamustine hydrochloride administered once every 3 weeks in patients with solid tumors. Anticancer Drugs 18:587–595
23. Cephalon Inc (2009) Treanda (bendamustine) for injection. Cephalon Inc, Fraser PA (product information)
24. National Cancer Institute (2007) Fatigue (PDQ) health professional version. National Cancer Institute, Bethesda
25. Hesketh PJ (1999) Defining the emetogenicity of cancer chemotherapy regimens: relevance to clinical practice. Oncologist 4:191–196
26. National Cancer Institute (2007) Nausea and vomiting (PDQ) health profession version. National Cancer Institute, Bethesda