Original Article

Bendamustine Is Effective Therapy in Patients With Rituximab-Refractory, Indolent B-cell Non-Hodgkin Lymphoma

Results From a Multicenter Study

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BACKGROUND: Bendamustine hydrochloride is a novel alkylating agent. In this multicenter study, the authors evaluated the efficacy and toxicity of single-agent bendamustine in patients with rituximab-refractory, indolent B-cell lymphoma. **METHODS:** Eligible patients (N = 100, ages 31-84 years) received bendamustine at a dose of 120 mg/m^2 by intravenous infusion on Days 1 and 2 every 21 days for 6 to 8 cycles. Histologies included follicular (62%), small lymphocytic (21%), and marginal zone (16%) lymphomas. Patients had received a median of 2 previous regimens (range, 0-6 previous regimens), and 36%were refractory to their most recent chemotherapy regimen. Primary endpoints included overall response rate (ORR) and duration of response (DOR). Secondary endpoints were safety and progression-free survival (PFS). **RESULTS:** An ORR of 75% (a 14% complete response rate, a 3% unconfirmed complete response rate, and a 58% partial response rate) was observed. The median DOR was 9.2 months, and median PFS was 9.3 months. Six deaths were considered to be possibly treatment related. Grade 3 or 4 (determined using National Cancer Institute Common Toxicity Criteria [version 3.0.19]. reversible hematologic toxicities included neutropenia (61%), thrombocytopenia (25%), and anemia (10%). The most frequent nonhematologic adverse events (any grade) included nausea (77%), infection (69%), fatigue (64%), diarrhea (42%), vomiting (40%), pyrexia (36%), constipation (31%), and anorexia (24%). **CONCLUSIONS:** Single-agent bendamustine produced a high rate of objective responses with acceptable toxicity in patients with recurrent, rituximab-refractory indolent B-cell lymphoma. ***Cancer*** **2010;116:106–14.** © *2010 American Cancer Society.*

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The anti-CD20 monoclonal antibody rituximab, either as a single agent or, particularly, in combination with chemotherapy, has changed the therapeutic landscape for patients with indolent B-cell lymphoma. In follicular lymphoma, which is the most common indolent non-Hodgkin lymphoma (NHL), rituximab combined with chemotherapy has led to notable improvements in response rates, progression-free survival (PFS), and overall survival (OS).[1-4] Treatment guidelines from the National Comprehensive Cancer Network now recommend a rituximab-based regimen as initial therapy for patients with B-cell lymphoma.[5] Unfortunately, patients tend to become refractory to rituximab over time. Although yttrium-90 ibritumomab tiuxetan and iodine-131 tositumomab have demonstrated activity in patients who are refractory

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to single-agent rituximab, their use has been limited by strict eligibility criteria and other factors.[6,7] Moreover, patients with indolent B-cell lymphoma currently are more likely to be treated with rituximab-chemotherapy combinations than with single-agent rituximab.[8] Consequently, rituximab resistance often develops within the context of generalized chemotherapy resistance, and innovative treatments are needed for this "rituximab-refractory" patient population.

Bendamustine (Treanda; Cephalon, Inc., Frazer, Pa) is a novel alkylator whose mechanisms of action involve induction of apoptosis through activation of DNA-damage stress responses, inhibition of mitotic checkpoints, and induction of mitotic catastrophe.[9] The compound also contains a benzimidazole ring, which may confer purine analogue-like properties in addition to the alkylating properties. In vitro studies indicate that the DNA repair mechanisms that operate after exposure to the drug are different from those evoked by other agents, potentially explaining observed antitumor effects in cell lines that are resistant to other alkylating agents.[10] Several German studies have evaluated its efficacy as a single agent or in combination with chemotherapy and/or rituximab in patients with recurrent, indolent B-cell lymphoma.[11-16] Bendamustine is indicated for the treatment of indolent lymphoma, multiple myeloma, and chronic lymphocytic leukemia (CLL) in Germany and was approved for the treatment of CLL in the United States in March 2008. A recent North American phase 2 multicenter study in patients with recurrent, rituximab-refractory, indolent B-cell lymphoma demonstrated that bendamustine produced durable objective responses with acceptable toxicity.[17] The purpose of the current phase 3 multicenter study was to further evaluate the effects of bendamustine in a larger group of patients with rituximab-refractory, indolent B-cell lymphoma and to provide the pivotal evaluation in this patient population.

MATERIALS AND METHODS

Study Design and Objectives

This multicenter, open-label, single-arm clinical trial was designed to investigate the efficacy and safety of bendamustine in patients with rituximab-refractory, indolent B-cell NHL. Primary endpoints included the overall response rate (ORR) and the duration of response (DOR). Secondary endpoints included progression-free survival (PFS) and the safety profile. The study was performed at 24 centers in the United States and at 4 centers in Canada. The protocol was approved by the institutional review board (IRB) at each site, and an IRB-approved consent form was signed by each patient before study enrollment.

Eligibility

Patients aged $\geq$18 years with a World Health Organization performance status $\leq$2 were eligible for study participation if they had documented rituximab-refractory, indolent B-cell lymphoma. Rituximab-refractory disease was defined as no objective response or documented progression within 6 months of 1) receiving the first dose of a full course of single-agent rituximab ($\geq$4 doses of 375 mg/m^2 weekly), 2) completion of rituximab maintenance therapy or progression before the next scheduled rituximab dose, or 3) completion of a full course of rituximab in combination with chemotherapy. Patients were required to have bidimensionally measurable disease with at least 1 lesion that measured $\geq$2.0 cm in a single dimension. Patients may have received from 1 to 3 previous chemotherapy regimens. Prior autologous stem cell transplantation was permitted. The baseline evaluation included a complete medical history, physical examination, radiographic imaging studies (computed tomography [CT] or magnetic resonance imaging [MRI] studies), bone marrow evaluation, electrocardiogram, and routine laboratory studies, including lactate dehydrogenase (LDH) levels. The following baseline laboratory parameters were required: absolute neutrophil count (ANC) $\geq$1000 cells/mm^3, platelet count $\geq$100,000 cells/mm^3 (or $\geq$75,000 cells/mm^3 in patients who had thrombocytopenia attributable to bone marrow involvement with NHL), creatinine clearance >30 mL per minute, and adequate hepatic function (<2.5 times the upper limit of normal [ULN] range for aspartate aminotransferase and alanine aminotransferase and <1.5 times the ULN for total bilirubin).

Patients were excluded from study participation for the following reasons: chemotherapy, immunotherapy, radioimmunotherapy, or investigational therapy within 28 days before the start of Cycle 1 or failure to recover from adverse events (AEs) associated with prior treatment; myeloid growth factor treatment within 14 days (chronic erythropoietic-stimulating agent was allowed); concurrent treatment with therapeutic doses

of systemic steroids within 14 days; transformed disease; history of prior high-dose chemotherapy with allogeneic stem cell support; concurrent, active malignancy (except nonmelanoma skin cancer, in situ cervical cancer, or localized prostate cancer treated with hormone therapy); central nervous system or leptomeningeal lymphoma; serious infection or another medical or psychiatric condition that might interfere with achieving the study objectives; pregnancy or lactation; or expected survival <3 months.

Treatment

Bendamustine at a dose of 120 mg/m^2 was infused intravenously over 60 to 120 minutes on Days 1 and 2 every 21 days. Treatment was planned for 6 to 8 cycles as long as a response or stable disease (SD) was observed. The development of grade 4 hematologic or grade 3/4 nonhematologic toxicities after any cycle led to a bendamustine dose reduction to 90 mg/m^2 for the subsequent cycle; if grade 4 hematologic or grade 3/4 nonhematologic toxicities were observed at the reduced dose level, then bendamustine was reduced further to a dose of 60 mg/m^2. All dose reductions were permanent. If further toxicity occurred, then study treatment was discontinued.

Subsequent cycles could be administered if nonhematologic toxicities resolved to grade $\leq$1 and if the ANC recovered to $\geq$1000 cells/mm^3 and the platelet count recovered to $\geq$75,000 cells/mm^3 by the time of the next scheduled dose. Dosing was delayed up to 4 weeks until these criteria were met. Patients who did not meet these criteria after a 4-week delay were removed from protocol therapy.

Primary prophylactic use of growth factors was not allowed during Cycle 1. Subsequent filgrastim or pegfilgrastim therapy was allowed for patients who had grade 4 neutropenia that lasted $\geq$1 week, failure of the white blood cell count to recover to grade $\leq$1 by the next scheduled dose, or febrile neutropenia in a previous treatment cycle. Low-dose corticosteroids ($\leq$10 mg daily of prednisone or equivalent) were allowed for non-neoplastic disorders; however, other on-study use of corticosteroids was not permitted (with the exception of $\leq$2 doses per cycle as an antiemetic). Any patient who demonstrated disease progression during therapy was removed from the study.

Criteria for Response and Toxicity

Response was evaluated by contrast-enhanced CT scans or MRI studies at Week 6, Week 12, and every 12 weeks thereafter until the end of treatment. An end-of-treatment scan was obtained within 28 days. Investigators used the International Working Group Response criteria for malignant lymphoma to determine response to treatment.[18] Patients underwent bone marrow aspiration and biopsy to confirm a complete response (CR) if the patient's bone marrow initially had been positive for lymphoma. LDH levels also were measured at each disease assessment. Tumor response was assessed by investigators and also by an independent review committee (IRC) (RadPharm, Princeton NJ). The ORR was defined as the proportion of patients who achieved as their best response a CR, an unconfirmed CR (CRu), and a partial response (PR). DOR was defined as the time from the first documentation of response until disease progression, death, or change of therapy. PFS was calculated as the time from the first dose of bendamustine administered until disease progression or death from any cause. Patients who remained progression free at the end of treatment were evaluated every 3 months until death, disease progression, or the start of a new anticancer therapy up to a maximum of 2 years after treatment. AEs were recorded and their severity was assessed according to the National Cancer Institute' Common Toxicity Criteria for Adverse Events (version 3.0).[19] Serious AEs (SAEs) were defined as those that were life-threatening, required hospitalization, or resulted in significant disability, congenital anomaly of offspring, or death.

Statistical Methods

The primary efficacy and safety analyses were performed on all patients who received treatment with bendamustine (the primary analysis set). Patients were classified according to their best overall response at the completion of therapy. Response assessments were made by the investigator and an IRC, and the latter assessment informed the primary endpoint analysis. The number and percentage of patients in each response category (CR, CRu, PR, SD, or progressive disease [PD]) were summarized along with a 2-sided binomial exact 95% confidence interval (95% CI) for ORR.

The statistical criterion for success relative to the response outcome was evidence of a true response probability >40% with the trial powered for a response probability $\geq$60%. Therefore, the trial tested the null hypothesis that the true response probability was $\leq$40% with a planned trial size of 100 patients who had no major screening or eligibility violations.

Table 1. Patient Demographics and Disease Characteristics

Characteristic	No. of Patients (%)
No. of patients treated	100
No. of men/women	65/35
Median age [range], y	60 [31-84]
Disease stage	
I	8 (8)
II	16 (16)
III	33 (33)
IV	43 (43)
Histology	
Follicular	62 (62)
Grade 1	33 (33)
Grade 2	16 (16)
Grade 3	8 (8)
Unknown	5 (5)
Small lymphocytic lymphoma	21 (21)
Lymphoplasmacytic lymphoma	1 (1)
Marginal zone	16 (16)
Follicular Lymphoma Prognostic Index, n = 62	
Low risk: 0-1 risk factor	18 (29)
Intermediate risk: 2 risk factors	26 (42)
High risk: 3-5 risk factors	18 (29)

Table 2. Previous Therapies

Variable	No. of Patients (%)
No. of previous chemotherapy regimens	
0	1 (1)
1	41 (41)
2	36 (36)
3	14 (14)
>3	8 (8)
Median [range]	2 [0-6]
Type of previous therapy	
Single-agent rituximab	1 (1)
CHOP-like chemotherapy rituximab	37 (37)
CVP ± rituximab	38 (38)
Purine analogue-based combinations±rituximab	44 (44)
Radioimmunotherapy	24 (24)
External beam radiotherapy	20 (20)

CHOP indicates combined cyclophosphamide, doxorubicin, vincristine, and prednisone; ±, with or without; CVP, combined cyclophosphamide, vincristine, and prednisone.

The median DOR and PFS were assessed using the Kaplan-Meier method.[20] If the patient did not experience disease progression, death, or change of therapy at the time of the computation of the DOR or PFS, then the patient had a censored observation at the date of the most recent progression-free visit. The criterion for success with respect to the duration of response was demonstrating that the DOR was not significantly less than 6 months (defined as the lower end of the 95% CI for the median DOR of >4 months).

RESULTS

Patients

Between October 2005 and July 2007, 102 patients were enrolled at 28 institutions. Two patients did not receive treatment and were excluded from the study analysis. One hundred patients received at least 1 dose of bendamustine, and these patients comprise the current primary analysis set. Demographics and baseline characteristics of the 100 patients in the primary analysis set are summarized in Table 1. The median age was 60 years (range, 31-84 years), and 76% of patients had advanced-stage disease at enrollment. Histologies included follicular lymphoma (n = 63), small lymphocytic lymphoma (n = 21), lymphoplasmacytoid lymphoma (n = 1), and marginal zone lymphoma (n = 16). The patients who had follicular histologies were categorized according to the Follicular Lymphoma International Prognostic Index (FLIPI) as follows: low risk, 29%; intermediate risk, 42%; and high risk, 29%. Table 2 summarizes prior treatment history for all patients. The median number of prior chemotherapy regimens was 2 (range, 0-6 regimens). One patient had not received prior chemotherapy (having received only single-agent rituximab), and 8 patients had received >3 prior chemotherapy regimens. These 9 patients were in violation of the protocol, which mandated at least 1 but not more than 3 prior chemotherapy regimens. They were included in the primary analysis, consistent with prespecified analysis conditions. Prior treatments included single-agent rituximab, chemotherapy with or without rituximab, single-agent chemotherapy, radioimmunotherapy, and external beam radiation. Thirty-six patients (36%) had disease that was refractory to their most recent chemotherapy.

Tolerability and Safety

The median number of cycles completed was 6 (range, 1-8 cycles). Sixty patients (60%) received at least 6 cycles of bendamustine. Forty patients discontinued treatment early for the following reasons: AEs (n = 27), disease progression (n = 10), patient decision (n = 1), bone marrow transplantation referral (n = 1), and an excessive treatment delay (n = 1) (Table 3). Twenty-four patients (24%) had dose reductions because of AEs: Twenty

Table 3. Patient Disposition and Study Drug Tolerability

Variable	No. of Patients (%)
No. of patients enrolled	102
No. of patients treated	100
No. of patients completing ≥6 cycles	60 (60)
Median no. of cycles completed [range]	6 [1-8]
Reasons for early termination*	
Total no. of AEs	27
Thrombocytopenia	9
Fatigue	6
Neutropenia	4
Infusion-related reaction	1
Nausea	1
Renal failure	1
Cough	1
Pulmonary alveolar hemorrhage	1
Decline in performance status	1
Increase in platelet count	1
Leukopenia	1
Disease progression	10
Patient preference for reason other than AE	1
Other†	2

AE indicates adverse event.
*Forty patients completed <6 treatment cycles.
†Other reasons for early termination included referral for bone marrow transplantation in 1 patient and a treatment delay >4 weeks in 1 patient.

Table 4. Incidence of Adverse Events by Severity (N = 100)

	No. of Patients (%)		
AE	All Grades	Grade 3	Grade 4
Hematologic AEs*			
Anemia	94 (94)	7 (7)	3 (3)
Thrombocytopenia	88 (88)	19 (19)	6 (6)
Neutropenia	83 (83)	38 (38)	23 (23)
Febrile neutropenia	6 (6)	5 (5)	1 (1)
Nonhematologic AEs†			
Nausea	77 (77)	4 (4)	0
Infection	69 (69)	15 (15)	6 (6)
Fatigue	64 (64)	12 (12)	2 (2)
Diarrhea	42 (42)	5 (5)	0
Vomiting	40 (40)	2 (2)	0
Fever	36 (36)	1 (1)	0
Constipation	31 (31)	0	0
Anorexia	24 (24)	3 (3)	0
Headache	21 (21)	0	0
Stomatitis	21 (21)	0	0
Infusion reaction	14 (14)	1 (1)	1 (1)

AE indicates adverse event.
*Severity was determined using National Cancer Institute Common Toxicity Criteria (version 3.0.19).
†Listed are common nonhematologic AEs that occurred in >20% of patients and all grade 3/4 nonhematologic AEs that occurred in >1 patient.

percent involved reductions from 120 mg/m^2 to 90 mg/m^2, and 4% were reduced further to 60 mg/m^2. Overall, 68 patients either had dose reductions or dose delays or did not receive both doses in any given cycle. Neutropenia and thrombocytopenia were the most common reasons for dose reductions or delays. The mean relative dose intensity was 88%.

Toxicities are summarized in Table 4. Grade 3/4 neutropenia was noted in 61% of patients over the course of the study and led to filgrastim or pegfilgrastim administration in 38% of patients. Grade 3/4 thrombocytopenia was noted in 25% of patients and was the second most common reason for dose delays or reductions. Failure to recover platelet counts to a threshold of 75,000/mm^3 was the most common reason for premature treatment discontinuation (9%).

Infections (any grade) were reported in 69 patients. The most frequently reported infections included urinary tract infections (n = 11), upper respiratory tract infections (n = 9), pneumonia (n = 9), and sinusitis (n = 8). Twenty-two grade 3 infections were documented in 15 patients. Eight grade 4 infections were reported in 6 patients and included pneumonia (n = 2), sepsis (n = 1), *Clostridium difficile* infection (n = 1), septic shock (n = 1), mycobacterial infection (n = 1), tuberculosis (n = 1), and noncharacterized infection (n = 1). Five episodes of cytomegalovirus (CMV) infection were reported. Nonhematologic AEs predominantly involved the gastrointestinal tract, and most were grade 1 or 2 in severity.

Secondary malignancies were reported in 2 patients. The first was a man aged 63 years who developed myelodysplastic syndrome (MDS) on Day 470 of the study. Prior therapies for this patient included combined rituximab, fludarabine, mitoxantrone, and dexamethasone (R-FND) and I-131 tositumomab. Cytogenetic testing was not performed at baseline. MDS was considered by the investigator to be possibly related to bendamustine treatment. The second patient was a man aged 70 years who underwent excision of a squamous cell carcinoma on Day 185 that was considered unrelated to bendamustine.

There were 2 episodes of tumor lysis syndrome (1 grade 3 and 1 grade 4), which resolved with appropriate supportive care, and both patients were able to continue therapy. Infusion-related or hypersensitivity reactions were relatively infrequent. Twelve patients experienced grade 1 or 2 events within 24 hours of bendamustine infusion, including chills, fever, rash, back or shoulder pain, pruritus, hypotension, and swelling. One grade 3 and 1 grade 4 hypersensitivity reaction occurred after Day 1 of Cycle 3 and after Day 1 of Cycle 2, respectively, and resolved with discontinuation of bendamustine.

Table 5. Response Rates (in Percentages) According to Non-Hodgkin Lymphoma Histology

Histology	No. of Patients	ORR	CR	CRu	PR	SD	PD	Unknown
Follicular	62	74	15	5	55	15	10	2
Small lymphocytic	21	71	5	0	67	19	5	5
Lymphoplasmacytic	1	100	0	0	100	0	0	0
Lymph node marginal zone	9	78	11	0	67	22	0	0
Extralymph node marginal zone	7	86	43	0	43	14	0	0
Total	100	75*	14	3	58	16	7	2

ORR indicates overall response rate (complete responses plus unconfirmed complete responses plus partial responses); CR, complete response; CRu, unconfirmed complete response; PR, partial response; SD, stable disease; PD, progressive disease.
*The 95% confidence interval was from 65% to 83%.

One or more SAEs were reported in 39 patients. In addition, 7 patients experienced SAEs that resulted in death: CMV pneumonia (considered to be related to bendamustine); pneumonia, diffuse intra-alveolar hemorrhage, and thrombocytopenia (related to bendamustine); pneumonia and respiratory failure (most likely related to bendamustine); pneumonia and sepsis (most likely related to bendamustine); respiratory failure (possibly related to bendamustine); worsened chronic obstructive pulmonary disease with neutropenia (possibly related to bendamustine); and cardiopulmonary arrest (considered unrelated to bendamustine). Four additional deaths were attributed to disease progression.

Efficacy

Responses to therapy are summarized in Table 5. In the 100 patients who received at least 1 dose of bendamustine, an ORR of 75% (95% CI, 65-83%), as assessed by the IRC, was achieved. In patients with follicular histologies (n = 62), the ORR was 72%, 77%, and 72% for patients who had FLIPI low-risk, intermediate-risk, and high-risk disease, respectively. Response rates did not vary appreciably by histology.

The ORR in patients who were sensitive to their last chemotherapy regimen (ie, patients who had at least a PR; n = 51) was 88%, whereas patients who were refractory to their last chemotherapy regimen (ie, patients who had no response; n = 36) demonstrated an ORR of 64%. Among alkylator-sensitive patients (n = 51), the ORR was 86% and, among alkylator-refractory patients (n = 30), the ORR was 60%. The responses rates among patients who had bulky disease ($\geq$10 cm) and nonbulky disease ($<$10 cm) were 50% and 80%, respectively.

The median DOR in patients who achieved an objective response (n = 75) was 9.2 months (95% CI, 7.1-10.8 months) (Table 6). The DOR was 10 months in chemosensitive patients compared with 6.3 months in chemorefractory patients. On the basis of a median follow-up of 11.8 months, the median PFS for the overall study population was 9.3 months (95% CI, 8.1-11.9 months) (Fig. 1 Top). The median PFS for patients who were sensitive (n = 51) and refractory (n = 36) to their last chemotherapy regimen was 11.8 months (95% CI, 9-13 months) and 7.5 months (95% CI, 4.4-12 months), respectively (Fig. 1 Bottom). Among patients who were sensitive (n = 51) and refractory (n = 30) to previous alkylator therapy, the median PFS was 11.8 months (95% CI, 8.4-13 months) and 7.5 months (95% CI, 4.4-12 months), respectively.

Table 6. Median Response Duration in Responders and Subgroups*

Patient Group	No. of Responders	Duration of Response (95% CI), mo
Overall	75	9.2 (7.1-10.8)
Chemosensitive	45	10.0 (8.4-11.7)
Chemorefractory	23	6.3 (4.9-NA)
Alkylator sensitive	44	9.7 (8.3-11.7)
Alkylator refractory	18	7.7 (4.9 to NA)

95% CI indicates 95% confidence interval; NA, not available.
*Subgroups do not total 75 patients, because some patients could not be characterized as sensitive versus refractory.

DISCUSSION

Bendamustine is a unique cytotoxic compound. Its chemical structure contains a 2-chloroethyl (nitrogen mustard) group that confers alkylating (DNA-damaging) properties and a benzimidazole ring. The precise contribution of the benzimidazole ring to the activity of the drug has not been defined, but it may affect the types of cross-links formed and the susceptibility to DNA repair.[9,10]

Two previous German studies have demonstrated substantial single-agent activity in recurrent, indolent lymphoma. Heider and Niederle conducted a study of

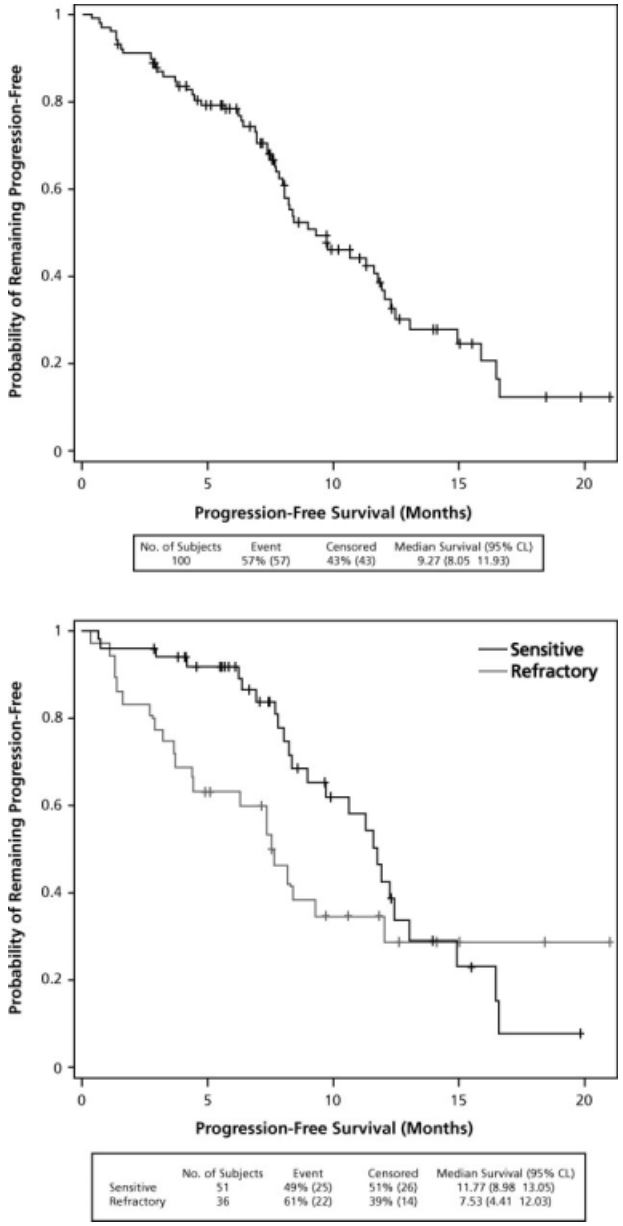

Figure 1. Kaplan-Meier curves for progression-free survival in patients who received bendamustine are shown both (*Top*) overall (N = 100) and (*Bottom*) for patients who were sensitive versus those who were refractory to prior chemotherapy. 95% CL indicates 95% confidence limits.

single-agent bendamustine at a dose of 120 mg/m^2 on Days 1 and 2 repeated every 21 days.[11] Despite a high frequency of alkylator-resistant disease, the ORR in that study was 73%, and the median DOR was 16 months. Bremer evaluated single-agent bendamustine in a similar patient population but at a dose and schedule of 60 mg/m^2 daily for 5 days every 4 to 6 weeks and observed an ORR of 82%.[12] A recently published, multicenter North American study confirmed the efficacy of single-agent bendamustine in recurrent NHL.[17] Seventy-six patients with rituximab-refractory indolent and transformed B-cell lymphoma were treated using a dose and schedule of 120 mg/m^2 on Days 1 and 2 every 21 days. An ORR of 77% was observed, and the median DOR was 6.7 months. The median DOR was only 2.3 months in the transformed population, but limiting the analysis to the patients who had indolent histology resulted in a median DOR of 9 months. Toxicities were mainly hematologic and reversible.

The current trial confirms and expands upon the results observed in the previous North American study.[17] Our trial used the same dose and schedule of bendamustine in a very similar patient population and produced an ORR of 75% and a median DOR of 9.2 months. When evaluating these results, it is important to consider the patient population under study. This trial included 100 patients who had received a median of 2 prior chemotherapy regimens (range, 0-6 regimens), and almost all of them (97 of 100 patients) were refractory to rituximab. Most patients (91 of 100) had received prior alkylating-agent therapy, 44 patients had received prior purine analogue therapy, and 24 patients had received prior radioimmunotherapy. Thirty-six patients were deemed chemotherapy-refractory (30 patients were alkylator-refractory) based on the lack of an objective response to their most recent chemotherapy treatment. Despite the heavily pretreated nature of this population, single-agent bendamustine demonstrated encouraging efficacy, including an ORR of 64% and a PFS of 7.5 months in the chemotherapy-refractory population. These clinical data support the in vitro findings of bendamustine's activity in cell lines that are resistant to other alkylating agents.[9,10] The efficacy appears to be comparable in the different indolent histologic subtypes. For example, the ORR was 74% among the 62 patients who had follicular lymphoma and 71% among the 21 patients who had small lymphocytic lymphoma.

All of the patients in this study were refractory to rituximab, and most developed rituximab resistance after they received rituximab-chemotherapy combinations rather than single-agent rituximab. Preclinical data suggest that the biologic basis of rituximab resistance may vary as a function of the prior therapies received.[21,22] Thus, it is important to establish benchmarks of activity in this unique and growing patient population for which there are no published trials evaluating other agents or regimens. The closest comparison that can be made is to radioimmunotherapy studies in a rituximab-refractory

population, which was defined as no response or a time to progression of <6 months after single-agent rituximab. Witzig et al[6] reported an ORR of 74% and a median DOR of 6.8 months using yttrium-90 ibritumomab tiuxetan, and Horning et al[7] reported an ORR of 65% and a median DOR of 10.4 months using iodine-131 tositumomab in such patients.[6,7]

The major toxicities associated with bendamustine were reversible myelosuppression, gastrointestinal toxicity, and infection. Some of the infections observed suggested a degree of immunosuppression beyond what would be anticipated from transient neutropenia, including 12 episodes of herpes zoster and 5 episodes of CMV infection. The CMV reactivation was unexpected, because this infection has not been reported in other bendamustine trials. No clear risk profile for CMV reactivation emerged from the data (of the 5 patients with CMV, 2 had received prior purine nucleoside analogues, 2 had CLL/small lymphocytic lymphoma, and 3 had follicular lymphoma). Given the absence of CMV in other bendamustine trials, we do not advocate monitoring CMV viral load in asymptomatic individuals who are receiving bendamustine, but we do recommend a low threshold for testing in individuals who develop signs or symptoms compatible with CMV reactivation. It is important for clinicians to be aware of the potential risk for CMV infection, because complications of CMV infection largely can be prevented with early recognition and appropriate therapy.[23] The 6 deaths that occurred during the current study were considered at least possibly related to treatment. Although this rate is higher than expected, a previous study of single-agent bendamustine that used the same dose and schedule in a virtually identical patient population reported no treatment-related deaths among 76 patients.[17] It is unclear why this difference was observed between the 2 trials; however, when estimating the risk of serious complications from single-agent bendamustine, it is worthwhile to consider the entire body of literature.

A legitimate concern associated with DNA-damaging agents is the risk of secondary myelodysplasia (MDS)/acute myelogenous leukemia (AML). It is estimated that the cumulative risk in this patient population approaches 5% after chemotherapy or radioimmunotherapy.[24-27] Only 1 episode of MDS was noted during the reporting period for the current study; however, given the natural history of treatment-related MDS/AML, longer follow-up of this patient cohort will be needed before the risk can be fully assessed.

Some of the myelosuppression and associated infectious complications noted in the current trial may have been a function of the dose and schedule selected for study. There are now 3 reports of bendamustine treatment in indolent lymphoma and mantle cell lymphoma using a dose of 90 mg/m^2 on Days 1 and 2 every 28 days in combination with rituximab. A multicenter trial that was conducted in Germany noted that 16% of patients had grade 3/4 neutropenia, 3% had grade 3/4 thrombocytopenia, and there were no treatment-related deaths.[13] A recently completed North American trial that involved 66 patients reported that 36% had grade 3/4 neutropenia, 9% had grade 3/4 thrombocytopenia, and there was 1 death because of toxic epidermal necrolysis (which was considered possibly related to therapy).[28] A second interim analysis of an ongoing randomized clinical trial comparing bendamustine-rituximab (BR) with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) plus rituximab (CHOP-R) as initial therapy for indolent lymphoma and mantle cell lymphoma recently was reported.[29] Response rates and PFS were comparable in the 2 arms. Toxicity appeared to be less with BR compared with CHOP-R for alopecia (0% vs 89%), grade 3/4 leukocytopenia (19% vs 36%), and infectious complications (25% vs 37%). These encouraging results support further study to determine the optimal bendamustine dose and schedule.

The current results confirmed the promising clinical activity of bendamustine with acceptable toxicity in patients with rituximab-refractory, indolent B-cell lymphoma and provided the basis for approval by the United States Food and Drug Administration in October 2008. These data, combined with the emerging worldwide experience for bendamustine, strongly suggest that bendamustine is a valuable addition to the treatment armamentarium for this patient population, which has limited treatment options.

CONFLICT OF INTEREST DISCLOSURES

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REFERENCES

1. Marcus R, Imrie K, Belch A, et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. *Blood*. 2005;105:1417-1423.
2. Hiddemann W, Kneba M, Dreyling M, et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced-stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective

randomized study of the German Low-Grade Lymphoma Study Group. *Blood*. 2005;106:3725-3732.
3. Herold M, Haas A, Srock S, et al. Rituximab added to first-line mitoxantrone, chlorambucil, and prednisolone chemotherapy followed by interferon maintenance prolongs survival in patients with advanced follicular lymphoma: an East German Study Group Hematology and Oncology Study. *J Clin Oncol*. 2007;25:1986-1992.
4. Foussard C, Mounier N, Van Hoof A, et al. Update of the FL2000 randomized trial combining rituximab to CHVP-interferon in follicular lymphoma patients [abstract]. *J Clin Oncol*. 2006;18S(June 20 suppl):424. Abstract 7508.
5. National Comprehensive Cancer Network (NCCN). NCCN Clinical Practice Guidelines in Oncology. Non-Hodgkin's Lymphomas. Version 3.2008. 04/10/08. Available at: http://www.nccn.org/professionals/physician_gls/PDF/nhl.pdf Accessed January 13, 2009.
6. Witzig TE, Flinn IW, Gordon LI, et al. Treatment with ibritumomab tiuxetan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's lymphoma. *J Clin Oncol*. 2002;20:3262-3269.
7. Horning SJ, Younes A, Jain V, et al. Efficacy and safety of tositumomab and iodine-131 tositumomab (Bexxar) in B-cell lymphoma, progressive after rituximab. *J Clin Oncol*. 2005;23:712-719.
8. Friedberg JW, Huang J, Dillon H, et al. National LymphoCare Study Investigators. Initial therapeutic strategy in follicular lymphoma (FL): an analysis from the National Lymphocare Study (NLCS) [abstract]. *J Clin Oncol*. 2006;18S(June 20 suppl):428. Abstract 7527.
9. Leoni LM, Bailey B, Reifert J, et al. Bendamustine (Treanda) displays a distinct pattern of cytotoxicity and unique mechanistic features compared with other alkylating agents. *Clin Cancer Res*. 2008;14:309-317.
10. Strumberg D, Harstrick A, Doll K, Hoffmann B, Seeber S. Bendamustine hydrochloride activity against doxorubicin-resistant human breast carcinoma cell lines. *Anticancer Drugs*. 1996;7:415-421.
11. Heider A, Niederle N. Efficacy and toxicity of bendamustine in patients with relapsed low-grade non-Hodgkin's lymphomas. *Anticancer Drugs*. 2001;12:725-729.
12. Bremer K. High rates of long-lasting remissions after 5-day bendamustine chemotherapy cycles in pre-treated low-grade non-Hodgkin's-lymphomas. *J Cancer Res Clin Oncol*. 2002; 128:603-609.
13. Rummel MJ, Al-Batran SE, Kim SZ, et al. Bendamustine plus rituximab is effective and has a favorable toxicity profile in the treatment of mantle cell and low-grade non-Hodgkin's lymphoma. *J Clin Oncol*. 2005;23:3383-3389.
14. Weide R, Hess G, Koppler H, et al. High anti-lymphoma activity of bendamustine/mitoxantrone/rituximab in rituximab pretreated relapsed or refractory indolent lymphomas and mantle cell lymphomas. A multicenter phase II study of the German Low Grade Lymphoma Study Group (GLSG). *Leuk Lymphoma*. 2007;48:1299-1306.
15. Herold M, Schulze A, Niederwieser D, et al. Bendamustine, vincristine and prednisone (BOP) versus cyclophosphamide, vincristine and prednisone (COP) in advanced indolent non-Hodgkin's lymphoma and mantle cell lymphoma: results of a randomised phase III trial (OSHO £19). *J Cancer Res Clin Oncol*. 2006;132:105-112.
16. Koenigsmann M, Knauf W, Herold M, et al. Fludarabine and bendamustine in refractory and relapsed indolent lymphoma—a multicenter phase I/II trial of the East German Society of Hematology and Oncology (OSHO). *Leuk Lymphoma*. 2004;45:1821-1827.
17. Friedberg JW, Cohen P, Chen L, et al. 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*. 2008;26:204-210.
18. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. *J Clin Oncol*. 1999;17:1244-1244.
19. National Cancer Institute. Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE). Published August 9, 2006. Available at: http://ctep.cancer.gov/proto colDevelopment/electronic_applications/docs/ctcaev3.pdf Accessed January 13, 2009.
20. Kaplan EL, Meier P. Nonparametric estimation from incomplete observation. *J Am Stat Assoc*. 1958;53:457-481.
21. Czuczman MS, Olejniczak S, Gowda A, et al. Acquirement of rituximab resistance in lymphoma cell lines is associated with both global CD20 gene and protein down-regulation regulated at the pretranscriptional and posttranscriptional levels. *Clin Cancer Res*. 2008;14:1561-1570.
22. Olejniczak SH, Hernandez-Ilizaliturri FJ, Clements JL, Czuczman MS. Acquired resistance to rituximab is associated with chemotherapy resistance resulting from decreased Bax and Bak expression. *Clin Cancer Res*. 2008;14:1550-1560.
23. Wade JC. Viral infections in patients with hematological malignancies. *Hematology Am Soc Hematol Educ Program*. 2006:368-374.
24. Bennett JM, Kaminski MS, Leonard JP, et al. Assessment of treatment-related myelodysplastic syndromes and acute myeloid leukemia in patients with non-Hodgkin lymphoma treated with tositumomab and iodine I131 tositumomab. *Blood*. 2005;105:4576-4582.
25. Armitage JO, Carbone PP, Connors JM, Levine A, Bennett JM, Kroll S. Treatment-related myelodysplasia and acute leukemia in non-Hodgkin's lymphoma patients. *J Clin Oncol*. 2003;21:897-906.
26. McLaughlin P, Estey E, Glassman A, et al. Myelodysplasia and acute myeloid leukemia following therapy for indolent lymphoma with fludarabine, mitoxantrone, and dexamethasone (FND) plus rituximab and interferon alpha. *Blood*. 2005;105:4573-4575.
27. Czuczman MS, Emmanouilides C, Darif M, et al. Treatment-related myelodysplastic syndrome and acute myelogenous leukemia in patients treated with ibritumomab tiuxetan radioimmunotherapy. *J Clin Oncol*. 2007;25:4285-4292.
28. Robinson KS, Williams ME, van der Jagt RH, et al. Phase II multicenter study of bendamustine plus rituximab in patients with relapsed indolent B-cell and mantle cell non-Hodgkin's lymphoma. *J Clin Oncol*. 2008;26:4473-4479.
29. Rummel MJ, von Gruenhagen U, Niederle N, et al. Bendamustine plus rituximab versus CHOP plus rituximab in the first-line treatment of patients with follicular, indolent and mantle cell lymphomas. Results of a randomized phase III study of the Study Group Indolent Lymphomas (StiL) [abstract]. *Blood (ASH Annual Meeting Abstracts)*. 2008;112: 900. Abstract 2596.