Table 4. Number (%) of patients reporting adverse events leading to dose reduction, safety population

Parameter, *n* (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (*n* = 3)	1.8 mg/m^2 (*n* = 10)	Total (*n* = 13)
No. of patients with dose reductions			
No dose reductions	3 (100)	8 (80)	11 (85)
One or more dose reductions	0	2 (20)	2 (15)
No. of dose reductions per patient*			
One	0	2 (100)	2 (15)
Any adverse event leading to dose reduction†	0	2 (20)	2 (15)
Thrombocytopenia	0	1 (10)	1 (8)
Pleural effusion	0	1 (10)	1 (8)

*Percentages are based on number of patients with ≥1 dose reduction in each treatment group. †Totals at a higher level are not necessarily the sum of those at the lower levels since a patient was able to report two or more different adverse events within the higher level category.

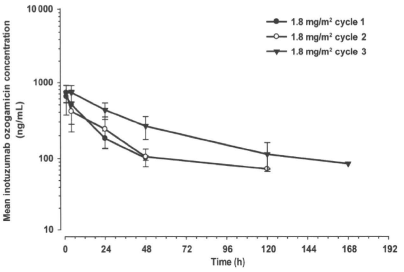

Fig. 1. Mean (SD) serum concentrations of inotuzumab ozogamicin after 1.8 mg/m^2 infusion of inotuzumab ozogamicin once every 4 weeks. Closed circle, cycle 1 (day 1, *n* = 10); open circle, cycle 2 (day 29, *n* = 8); closed triangle, cycle 3 (day 57, *n* = 5).

for patients who received 1.8 mg/m^2 are shown in Figures 1 and 2, respectively. The peak concentration of inotuzumab ozogamicin was generally observed at or shortly after the termination of infusion with moderate intersubject variability. The peak total calicheamicin concentrations were observed typically within 4 h after the start of inotuzumab ozogamicin infusion with small intersubject variability.

The mean pharmacokinetic parameters for inotuzumab ozogamicin and total calicheamicin are shown in Tables 5 and 6, respectively. The AUC of inotuzumab ozogamicin tended to increase with increased dose and period. The $t_{1/2}$ was prolonged with repeated treatment cycles. These were reflected by substantial decreases in clearances.

The mean total calicheamicin C_{max} appeared to increase with dose. The AUC of total calicheamicin increased with increased dose and period. No antibodies to inotuzumab ozogamicin were detectable in patients' serum during the course of the study. The pharmacokinetics data indicate that the disposition of inotuzumab ozogamicin and total calicheamicin following IV treatment was nonlinear with dose or number of doses.

Efficacy. The best tumor response is presented in Table 7. Antitumor activity was observed at both dose levels. In the 1.3 mg/m^2 cohort, two out of three patients had CR, and one patient had CRu for an ORR of 100% (95% CI, 29–100%). In the 1.8 mg/m^2 cohort, one out of 10 patients had CR, three patients had CRu, and four patients had PR for an ORR of 80% (95% CI, 44–98%).

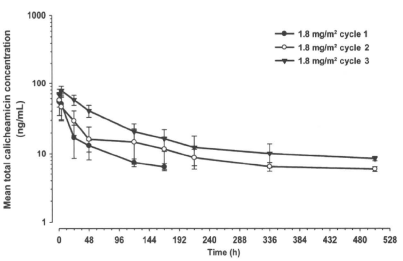

Fig. 2. Mean (SD) serum concentrations of total calicheamicin after 1.8 mg/m^2 infusion of inotuzumab ozogamicin once every 4 weeks. Closed circle, cycle 1 (day 1, *n* = 10); open circle, cycle 2 (day 29, *n* = 8); closed triangle, cycle 3 (day 57, *n* = 5).

Discussion

To improve the clinical outcome of patients with B-NHL who were pretreated with rituximab or rituximab-containing regimens, a number of new agents including antibodies, small mole-

Table 5. Serum pharmacokinetic parameters of inotuzumab ozogamicin

Dose (Once/4 weeks)	Treatment Day (*n*)	Number of cycles	C_{max} (ng/mL) (%)	$t_{1/2}$ (h) (%)	AUC (ng h/mL) (%)	CL (L/h) (%)	V_{ss} (L) (%)
1.3 mg/m^2	1 (3)	1	463 (8)	NC	NC	NC	NC
	29 (3)	2	610 (17)	29.7 (30)	24166 (29)	0.08 (32)	3.27 (11)
	57 (3)	3	524 (18)	43.6 (18)	31642 (21)	0.06 (22)	3.79 (12)
1.8 mg/m^2	1 (10)	1	657 (41)	13.0 (30)	14266 (32)	0.24 (40)	4.06 (21)
	29 (8)	2	727 (27)	35.8 (43)	34518 (46)	0.11 (54)	4.40 (20)
	57 (5)	3	763 (20)	44.0 (32)	39677 (41)	0.09 (56)	4.89 (19)

Data are expressed as mean, and percent coefficient of variance is expressed in parentheses. AUC, total area under the concentration-time curve; CL, clearance; C_{max}, peak concentration; NC, not calculated; $t_{1/2}$, terminal-phase elimination half-life (0.693/λz); V_{ss}, steady-state volume of distribution.

Table 6. Serum pharmacokinetic parameters of total calicheamicin

Dose (Once/4 weeks)	Treatment Day (*n*)	Number of cycles	C_{max} (ng/mL) (%)	$t_{1/2}$ (h) (%)	AUC (ng h/mL) (%)	CL (L/h) (%)	V_{ss} (L) (%)
1.3 mg/m^2	1 (3)	1	44.6 (17)	17.0 (39)	987 (44)	2.35 (58)	49.44 (13)
	29 (3)	2	52.4 (22)	150.6 (45)	5754 (40)	0.38 (48)	62.86 (30)
	57 (3)	3	56.6 (26)	216.3 (55)	8060 (37)	0.27 (43)	60.17 (25)
1.8 mg/m^2	1 (10)	1	59.0 (31)	49.6 (77)	2329 (51)	1.61 (54)	72.3 (28)
	29 (8)	2	59.4 (15)	162.4 (34)	7100 (48)	0.54 (62)	89.18 (41)
	57 (5)	3	78.2 (15)	172.7 (48)	9225 (32)	0.37 (44)	68.37 (26)

Data are expressed as mean, and percent coefficient of variance is expressed in parentheses. AUC, total area under the concentration-time curve; CL, clearance; C_{max}, peak concentration; NC, not calculated; $t_{1/2}$, terminal-phase elimination half-life (0.693/λz); V_{ss}, steady-state volume of distribution.

Table 7. The best tumor response during treatment: number (%) of patients in efficacy population

Best tumor response	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (*n* = 3)	1.8 mg/m^2 (*n* = 10)	Total (*n* = 13)
CR, CRu	3 (100)	4 (40)	7 (54)
PR	0	4 (40)	4 (31)
OR	3 (100)	8 (80)	11 (85)
SD	0	2 (20)	2 (15)

CR, complete response; CRu, unconfirmed complete response; OR, overall response (CR + CRu + PR); PR, partial response; SD, stable disease.

cule, targeted agents, and chemotherapeutic drugs have been developed. However, new treatment modalities with improved toxicity profiles and better responses are needed. Inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapy agent, has demonstrated an acceptable toxicity profile and high activity against relapsed or refractory patients with FL who were pretreated with rituximab or rituximab-containing treatment.

In a recent phase I, multicenter, open-label, dose escalation study of inotuzumab ozogamicin administered IV as a single agent in the USA and the European Union, inotuzumab ozogamicin was found to be reasonably well-tolerated with the MTD of 1.8 mg/m^2 administered every 4 weeks and with the major toxicity of grade 3 or greater thrombocytopenia, which was manageable with careful monitoring and platelet transfusion. Response rates of 69% in patients with FL and 33% in patients with DLBCL in the expanded cohort of this trial were observed.[12]

In the present phase I dose escalation study in Japanese patients with relapsed or refractory FL, who were pretreated with rituximab, the MTD of inotuzumab ozogamicin was determined to be 1.8 mg/m^2 administered once every 28 days, a value that was the same as that observed for non-Japanese patients.

Most common inotuzumab ozogamicin related adverse events were thrombocytopenia, leukopenia, lymphopenia, neutropenia, elevated AST, anorexia, and nausea, a finding that was very similar to the non-Japanese study. Adverse events (AEs) leading to dose delays were neutropenia and thrombocytopenia.

The pharmacokinetic profiles of inotuzumab ozogamicin and total calicheamicin indicated that disposition was non-linear and was associated with increases in drug exposure with increasing dose or number of doses. The pharmacokinetic profiles of inotuzumab ozogamicin and total calicheamicin in Japanese patients were similar to the values for non-Japanese patients. The study population was very limited, thus no definite conclusion can be made for Japanese patients. However, nonlinearities in drug disposition are known for antibodies[16] and had been observed previously for gemtuzumab ozogamicin.[17] Saturable binding with target antigen is thought to influence antibody disposition, potentially leading to nonlinear distribution and elimination.

Potent antitumor activity for inotuzumab ozogamicin was observed at both the 1.3 and 1.8 mg/m^2 dose levels. In the 1.3 mg/m^2 cohort, all three patients had CR or CRu for an ORR of 100%. In the 1.8 mg/m^2 cohort, one out of 10 patients had CR, three patients had CRu, and four patients had PR for an ORR of 80%. Although the number of patients was limited, our preliminary ORR was greater in comparison to other reported antibody-based agents in the treatment of patients with FL and prior exposure to rituximab-containing regimens. For example, in a recent phase I/II study, veltuzumab, a humanized second-generation anti-CD20 monoclonal antibody, was reported to have an ORR of 44%.[18] In another phase I/II, single-agent, dose escalation study, galiximab, an anti-CD80 antibody, demonstrated an ORR of only 11%.[19] Fludarabine phosphate, one of the most effective drugs in the treatment of indolent B-NHL, had an ORR of 65%, when administered as a single agent.[20]

The FLIPI scores in this study were good predictors of favorable outcome. Of the five patients who had low scores (low risk) two demonstrated CR, two had CRu, and one had PR. Of the six patients who had intermediate scores, one had CR, two had CRu, one had PR, and two had SD. The two patients with high FLIPI scores demonstrated only PR.

In conclusion, the results from this phase I study suggest that inotuzumab ozogamicin is safe, well tolerated, and shows promising efficacy in Japanese patients with relapsed or refractory FL pretreated with rituximab-containing therapy. In addition, pharmacokinetics and efficacy in this study are comparable with those in preceding studies in non-Japanese patients. These results therefore warrant further investigation of inotuzumab ozogamicin in relapsed or refractory B-NHL.

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Disclosure Statement

This study was funded by Wyeth which was acquired by Pfizer, Inc., in October 2009. Dr. Junko Ohata was an employee of Wyeth K.K. at the time of the study. Dr. Chiho Ono is an employee of Wyeth K.K. No other potential conflict of interest relevant to the article is reported.

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ORIGINAL PAPER

Management of venous thromboembolism in colorectal cancer patients treated with bevacizumab

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Abstract Venous thromboembolism associated with use of a central venous access system is an urgent problem in patients treated with bevacizumab (bev). We investigated the effectiveness of Doppler ultrasound imaging (DUS) in the early detection of catheter-related thrombosis for avoidance of severe venous thromboembolism. Patients with metastatic colorectal cancer received either FOLFOX-4 + bev or FOLFIRI + bev. DUS was performed on the deep venous system for detection of thrombus formation during the initial cycle of treatment, followed by re-evaluation after the third cycle in patients with asymptomatic thrombus formation. All patients were followed up until treatment was interrupted. Median duration of follow-up was 484 days (range 72–574). Among 41 enrolled patients, curable symptomatic thrombosis occurred in one, and asymptomatic thrombosis in 21 (51.2%). Of 21 patients undergoing re-evaluation, thrombi remained without progression in 17 patients, and enlargement in 4 patients. In two of the patients in whom there was progression, pulmonary embolism occurred after the sixth cycle. In the asymptomatic group, no thrombi developed as far as the superior vena cava in any patient. In the cases of progression, thrombotic enlargement was observed in all the 4 patients, with decreased vascular flow in 2. Using DUS, we were able to detect asymptomatic thrombosis in the early cycles of treatment, indicating its potential in the monitoring of venous thrombi. In the event of an enlarging asymptomatic thrombosis developing into the superior vena cava along with decreased vascular flow, careful follow-up and appropriate anticoagulant therapy may be recommended without increased risk of bleeding.

Keywords Venous thromboembolism · Bevacizumab · Colorectal cancer

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Introduction

Bevacizumab (bev) is a recombinant, humanized monoclonal antibody against vascular endothelial growth factor (VEGF). The combination of bev and chemotherapy for first- and second-line treatment of metastatic colorectal cancer has been shown to improve survival [1–4]. Furthermore, a large observational study indicated that use of bev beyond first progression correlated with improved survival [5]. However, use of bev in conjunction with chemotherapy is associated with an increased risk of arterial thromboembolism, and there is also some controversy as to whether bev contributes to the development of venous thromboembolism (VTE) [6]. Pulmonary embolism (PE) occurs in 2–5% of cases where bev and chemotherapy are

used together [1, 3]. A recent meta-analysis of 15 randomized controlled trials [7] found that bev significantly increased risk of VTE in cancer patients and anticoagulant therapy is indicated in the event of VTE.

An implantable central venous access system (CVAS) is a risk factor for VTE [8]. Many VTEs, although asymptomatic, can be as serious as PEs in terms of morbidity [9, 10]. Based on the results of studies on the prevention of catheter-related thrombosis, anticoagulant prophylaxis is not generally recommended with a CVAS [11–13].

In our hospital, symptomatic venous thrombosis associated with a CVAS occurred in patients treated with bev plus chemotherapy during the initial cycle. Appropriate screening and management of patients after detection of either symptomatic or asymptomatic VTE remain to be clarified.

We evaluated the effectiveness of Doppler ultrasound imaging (DUS) in the early detection of CVAS-associated venous thrombosis to determine its potential in the prevention of further development into severe VTE.

Patients and methods

Study design

This was a prospective cohort study conducted at a single institute. Patients were enrolled from July 2007 onward after approval of bev in June 2007 in Japan. The study protocol, including the use of DUS, was approved by the institutional review board of our institute. All the patients provided written informed consent before treatment.

The study design is shown in Fig. 1. DUS was performed on the deep venous system in the upper extremities where catheterization had been carried out to detect thrombus formation during the early cycles of chemotherapy. The first DUS was performed after the initial cycle of bev. Only patients with asymptomatic thrombus formation underwent follow-up evaluation by DUS after the third cycle of bev. During DUS, location and dimension of thrombus, vascular flow, and collateral vascular flow were evaluated and diagnosed by a radiologist at our institute. In addition, dimension of thrombus, location, whether it extended as far as the junction of the external jugular vein (EJV), suprascapular vein (SSV) or subclavian vein (SCV), collateral vascular flow, and retention of peripheral vascular flow were evaluated as important factors directly affecting vascular flow.

At our institute, time to treatment from implantation of a CVAS is usually just 2 days. This made it difficult to perform DUS prior to initiation of treatment and we could not delay treatment for that purpose in the patients enrolled in this study. Therefore, as a subordinate examination, we performed additional pre-treatment DUS between implantation of the CVAS and induction of bev in those patients who consented to the procedure.

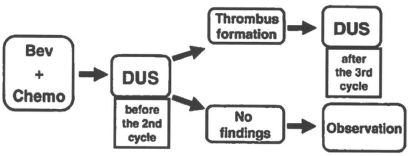

Fig. 1 Timing of DUS: study schema

Patients

All the patients conformed to the following criteria: histologically confirmed colorectal cancer; advanced metastatic colorectal cancer; age $\leq$ 70 years; Eastern Cooperative Oncology Group performance status of 0, 1, or 2; no history of thromboembolic events; no prior use of bev; no increased risk factors for bleeding; hypertension, if present, controlled with a single agent.

All the patients received the initial cycle of treatment when they were in the hospital, and additional treatment cycles at an ambulatory center. Complete blood count, international normalized ratio (INR), and D-dimer were measured biweekly or before treatment in all the patients. Deficiencies of protein C, protein S, and antithrombin III as congenital risk factors for thrombosis were examined, as well as the presence of acquired risk factors before initial bev administration.

Chemotherapy treatment

Treatment regimens were as follows: FOLFOX-4 + bev (biweekly cycles of 85-mg/m^2 intravenous oxaliplatin for 2 h on day 1 plus 100-mg/m^2 L-leucovorin (L-LV) for 2 h and 400-mg/m^2 bolus 5-FU, followed by a 22-h infusion of 600 mg/m^2 5-FU on days 1 and 2, plus 5–10-mg/kg bev on day 1 every 2 weeks); or 5-mg/kg FOLFIRI + bev (biweekly cycles of 150-mg/m^2 intravenous irinotecan for 1.5 h on day 1 plus 200-mg/m^2 L-LV for 2 h and 400-mg/m^2 bolus 5-FU, followed by a 46-h infusion of 1200 mg/m^2 5-FU on days 1 and 2 plus 5-mg/kg bev on day 1 every 2 weeks). Treatment continued until progression, unmanageable toxic effects, or patient refusal. Antiemetic agents were provided at the discretion of the treating physician. No prophylactic use of colony-stimulating factor was permitted. No anticoagulant therapy before initial evaluation by DUS was permitted. If an asymptomatic thrombus that could potentially cause a PE was identified on DUS, anticoagulant therapy was permitted. The anticoagulant treatment regimen was at the discretion of the physician.

Evaluation of toxicity and efficacy

Patient data were recorded and reviewed on electronic clinical records. Adverse effects were graded in all the patients biweekly or before treatment using the National Cancer Institute Common Toxicity Criteria, version 3.0. Cancer response was assessed every 12 weeks using computed tomography according to the response evaluation criteria for solid tumors. Data on toxicity and tumor evaluation were analyzed using electronic medical records and by examination of films of each patient. A radiologist examined the films and made an assessment of status, and the evaluations were recorded in electronic medical records.

Differences in baseline characteristics and clinical features between patients with and without catheter-related thrombosis were analyzed. We used the Chi-square test (without the Yates correction) and Fisher's exact probability test for categorical comparisons of data. Differences in the means of continuous measurements were tested by the Student's t test and checked by Mann–Whitney U test. Quantitative variables such as time between two points were summarized using medians. A two-way repeated-measures analysis of variance was used to evaluate differences between sequential continuous variables. A P value of <0.05 was considered significant.

Results

Characteristics of patients

Forty-one patients were enrolled in the study. The baseline characteristics of the patients are shown in Table 1. Median follow-up time from the date of initial bev administration was 484 days (range 72–574 days). Eight patients (19.5%) received an antihypertensive agent at baseline. In addition, no congenital factors for thrombosis were seen, but anticardiolipin antibody IgG and lupus anticoagulant were observed in 5 (12.2%) and 1 (2.4%) patients, respectively.

Effectiveness of DUS

The results of DUS are shown in Table 2. Catheter-related thrombosis was observed on initial DUS in 22 patients (53.7%), including both asymptomatic ($n = 21$ [51.2%]) and symptomatic ($n = 1$ [2.4%]) thrombi. No thrombus formation was detected in 19 patients (46.3%). Twenty-two patients received a follow-up DUS, one of whom received anticoagulant therapy after initial DUS. Thrombi disappeared completely in 3 (13.6%) of these 22 patients without anticoagulant therapy.

Comparisons of initial and follow-up DUS findings in asymptomatic thrombi are shown in Table 3. In 21 patients with asymptomatic thrombi, none of the thrombi extended to the superior vena cava, and complete disappearance was seen in 3. Thrombus size was <20 mm in 16 patients (76.2%) on initial DUS, compared to in 16 patients (76.2%) on follow-up DUS. Decreased vascular flow was observed

Table 1 Baseline characteristics of patients ($N = 41$)

Characteristics	*N* (%)
Sex: male/female	17/24
Mean age (range), years	58.4 (16–69)
Chemotherapy regimen	
FOLFOX4 + bev 5 mg/kg	28 (68.3)
FOLFOX4 + bev 10 mg/kg	1 (2.4)
FOLFIRI + bev 5 mg/kg	12 (29.3)
ECOG performance status	
0	39 (95.1)
1	2 (4.9)
Treatment set as systemic chemotherapy for metastatic disease	
First-line	28 (68.3)
Second-line	13 (31.7)
Prior adjuvant fluorouracil	5 (12.2)
No. of involved organs	
1	16 (39)
2	19 (46.3)
3	5 (12.2)
4	1 (2.4)
Sites of metastases	
Liver	19 (46.3)
Lung	20 (48.8)
Peritoneum	8 (19.5)
Nodes	17 (41.5)
Local recurrence	7 (17.1)
Bone	1 (2.4)
Previous history/complication	
Thromboembolic events	0
Hypertension	8 (19.5)
Diabetes	2 (4.9)
Hyperlipidemia	1 (2.4)
Hyperuricemia	1 (2.4)
Liver function failure	1 (2.4)
Congenital risk factors	
Protein C deficiency	0
Protein S deficiency	0
Antithrombin III deficiency	0
Acquired risk factors	
Anticardiolipin antibody IgG	5 (12.2)
Anticardiolipin antibody β2-glycoprotein 1	0
Lupus anticoagulants	1 (2.4)

Bev bevacizumab, *ECOG* Eastern Cooperative Oncology Group, *INR* international normalized ratio, *CRP* C-reactive protein, *IgG* immunoglobulin G

Table 2 Results of DUS ($N = 41$)

Median length, days (range)	
IP-CVAS—induction of bev	7 (2–695)
IP-CVAS—initial DUS	18 (7–700)
Induction of bev—initial DUS	7 (4–14)
Induction of bev—follow-up DUS	35 (14–49)
Results of initial DUS, *n* (%)	
Thrombus formation	22 (53.7)
Symptomatic thrombosis	1 (2.4)
Asymptomatic thrombosis	21 (51.2)
No thrombus formation	19 (46.3)
Results of follow-up DUS ($n = 22$), *n* (%)	
Thrombus formation	19 (86.4)
No thrombus formation (disappeared)	3 (13.6)

IP-CVAS implantation of central venous access system, *bev* bevacizumab

in 3 patients (14.3%) on initial DUS that showed no progression on follow-up DUS; however, another 2 patients in whom adequate vascular flow was detected on initial DUS showed decreased vascular flow on follow-up DUS. We defined overall improvement as at least one improved finding without progression in location, maximum size, or (collateral) vascular flow and progression as at least one progressed finding; those fitting neither category were defined as the remainder. Overall, thrombi improved or remained stable in 17 patients (81%), and progressed without symptoms in 4 patients (19%). Correlations between vascular flow and other findings are shown in Table 4. Incidence of thrombi extending into the junction of the SCV, ECV, and SSV was significantly higher in patients with inadequate vascular flow than in patients with adequate vascular flow on initial DUS (66.7 vs. 5.6%, respectively; $P = 0.0414$) and on follow-up DUS (80 vs. 0%, respectively; $P = 0.0016$).

Symptomatic thrombosis was revealed on DUS in 1 patient (a DUS image with a diagram is shown in Fig. 2). This thrombus extended into the superior vena cava, was >40 mm in diameter, and resulted in decreased vascular flow. This patient received anticoagulant therapy after initial DUS and re-started bev after a follow-up DUS revealed that the thrombus had not progressed.

Characteristics of patients with and without thrombi are shown in Table 5. Median follow-up time from date of initial bev administration was 518 days (range 232–574 days) and 487 days (72–559), respectively, in these patients. No significant difference was observed in performance status or age. Presence of acquired risk factors showed no effect on thrombus formation or outcome in patients with asymptomatic thrombus. Incidence of thrombus formation was significantly higher in the FOLFOX + bev treatment group than in the FOLFIRI + bev group ($P = 0.0047$). Median length of time between implantation of CVAS and induction of bev was significantly shorter in patients with thrombus formation than in patients without thrombus formation (5 vs. 107.5 days, respectively; $P = 0.0048$). Similarly, median length of time between implantation of CVAS and initial DUS was significantly shorter in patients with thrombus formation than in patients without thrombus formation (13.5 vs. 116 days, respectively; $P = 0.0059$). In further follow-up after completion of the protocol, 1 patient in whom no thrombus was detected in the planned DUS experienced asymptomatic thrombosis of the inferior vena cava during the 12th cycle. However, we were able to continue FOLFOX in this patient without bev using warfarin.

A comparison of patients with improved thrombus findings on follow-up DUS ($n = 5$) with those showing thrombus progression ($n = 4$) revealed that median follow-up time from date of initial bev administration was 505 days (range 446–563 days) and 395.5 days (range 256–484 days), respectively. No significant differences were observed in findings on initial DUS, median time to induction of bev from implantation of CVAS (5 vs. 6 days; $P = 0.9004$), or laboratory data between the two groups. The results of a two-way repeated-measures analysis of variance to evaluate differences between sequential continuous variables such as platelet count, INR, and D-dimer showed no significant differences. Changes in thrombus size, as well as decreased vascular flow, were mainly related to outcomes of thrombus on initial DUS. However, two patients showing thrombus progression developed pulmonary embolism requiring urgent treatment with a thrombolytic agent followed by warfarin, after which, they were able to continue with FOLFOX without bev until progression of disease. None of the patients experienced sequelae, including post-thrombotic syndrome, and there were no deaths related to thromboembolic events or anticoagulant therapy.

Discussion

In this study, we assessed the effectiveness of DUS in the early identification of catheter-related thrombosis and variations in asymptomatic venous thrombosis under use of bev.

According to the American Society of Clinical Oncology [14], the presence of a central venous catheter is a risk factor for VTE in cancer patients. Active chemotherapy [15, 16] and antiangiogenic therapy [2, 3] also carry risk of VTE. With the newer antiangiogenic agents, the use of a prophylaxis for VTE is controversial [2, 3, 17, 18].

Although a CVAS makes it easier to administer chemotherapy in ambulatory patients, its use is associated with

Table 3 Findings associated with asymptomatic thrombosis ($n = 21$)

Findings	Initial DUS	Follow-up DUS
Location, *n* (%)		
Distal (not extended to SVC)	21 (100)	18 (85.7)
Central (extended to SVC)	0	0
Comparison	Improved (disappeared) in 3 patients (14.3)	
Maximum size, *n* (%)		
0–<10 mm	14 (66.7)	12 (57.1)
10–<20 mm	2 (9.5)	4 (19)
20–<30 mm	3 (14.3)	3 (14.3)
>30 mm	2 (9.5)	2 (9.5)
Comparison	Improved in 5 patients (23.8) (disappeared in 3 and reduced in 2)	
	Progressed in 4 patients (19)	
Vascular flow, *n* (%)		
Adequate	18 (85.7)	13 (61.9)
Inadequate	3 (14.3)	5 (23.8)
Comparison	Improved in 3 patients (14.3)	
	Progressed in 2 patients (9.5)	
Collateral vascular flow, n (%)		
Adequately increased	2 (9.5)	2 (9.5)
Inadequately increased	1 (4.8)	3 (14.3)
Comparison	Progressed in 2 patients (9.5)	
Overall evaluation[a]	Improved in 5 patients (23.8)	
	Stable in 12 patients (57.1)	
	Progressed in 4 patients (19)	

[a] Overall improvement was defined as at least one improved finding without progression in location, maximum size, or (collateral) vascular flow and progression as at least one progressed finding; those fitting neither category were defined as the remainder. One patient receiving anticoagulant therapy after initial DUS showed a thrombus 45 mm in diameter that developed into the brachiocephalic vein; no progression was noted. One patient with thrombus progression experienced a symptomatic pulmonary embolism after the sixth cycle, and one progressed patient experienced an asymptomatic pulmonary embolism after the sixth cycle

SVC superior vena cava

Table 4 Correlation between vascular flow and other findings of asymptomatic thrombosis ($n = 21$)

Findings on DUS	Initial DUS ($n = 21$)		Follow-up DUS ($n = 18$)	
	Adequate ($n = 18$)	Inadequate ($n = 3$)	Adequate ($n = 13$)	Inadequate ($n = 5$)
Location, *n* (%)				
SCV–ECV–SSV junction[a]	1 (5.6)	2 (66.7)	0	4 (80)
P value	0.0414		0.0016	
Maximum size, *n* (%)				
<30 mm	18(100)	1 (33.3)	13 (100)	3 (60)
≥30 mm	0	2 (66.7)	0	2 (40)
P value	0.0143		0.0654	

[a] Thrombi extended into junction of SCV, ECV, and SSV in two inadequate patients at initial DUS; both thrombi sizes were ≥30 mm

DUS Doppler ultrasound imaging, *SCV* subclavian vein, *ECV* external jugular vein, *SSV* suprascapular vein, *N.A* not applicable

an increased risk for VTE and PE [8–10]. According to a review by Vescia et al. [19], the incidence of catheter-related thrombosis varied from 12 to 64% in four retrospective studies [20–24]. In a recent prospective trial using phlebography in patients with a CVAS, Verso et al. [25] found that the incidence of thrombosis in two groups receiving low molecular weight heparin (LMWH) or placebo for 6 weeks was 14.1 and 18%, respectively (95% CI 0.47–1.31; $P = 0.35$), with symptomatic upper limb thrombosis seen only in 1.0% of the LMWH group and 3.1% of the placebo group (hazard ratio 0.32; 95% CI 0.07–1.66). In another trial by Couban et al. [12], the rate of symptomatic thrombosis in a group received 1-mg warfarin for 9 weeks was 4.6% when compared with 4.0% in the placebo group (hazard ratio 1.20; 95% CI 0.37–3.94).

We summarized thromboembolic events reported in nine pivotal studies of bev plus chemotherapy [1–4, 17, 18, 26–28]. According to the results, the incidence of thromboembolism ranged from 3 to 26% in these studies, and PE was reported in 1–4% of cases. Prophylactic anticoagulant treatment was not permitted in any study, except for maintenance of CVAS in four studies [1, 2, 4, 18].

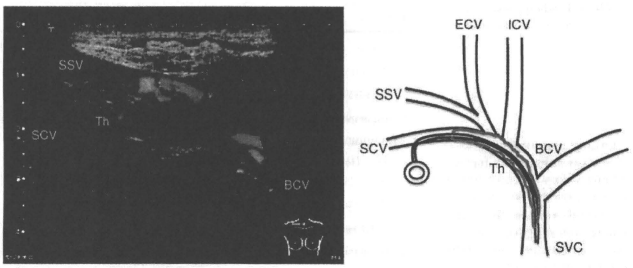

Fig. 2 Findings of DUS image and illustration in symptomatic case. This thrombus (Th) extended into superior vena cava (SVC) through brachiocephalic vein (BCV), was >40 mm in diameter, and resulted in clearly decreased vascular flow

Table 5 Comparison between patients with and without thrombus formation

	With thrombus (n = 22)	Without thrombus (n = 19)	P value
Sex: male/female	9/13	8/11	>0.9999
Mean age (range), years	62 (16–69)	60.1 (47–69)	0.9896
ECOG performance status, n (%)			
0	22 (100)	17 (89.5)	0.2308
1	0	2 (10.5)	
Chemotherapy regimen, n (%)			
FOLFOX4 + bev	20 (90.9)	9 (47.4)	0.0047
FOLFIRI + bev	2 (9.1)	10 (52.6)	
Prior treatment, n (%)			
FOLFOX	2 (9.1)	10 (52.6)	0.0047
Hepatic arterial infusion	3 (13.6)	4 (21.1)	0.6847
Radiation	3 (13.6)	0	0.2354
No. of involved organs, n (%)			
1/2/3/4	10/10/2/0	6/9/3/1	0.3899
≥3	2 (9.1)	4 (21.1)	
Baseline laboratory data, mean ± SD			
Platelets ($\times 10^4$ μl)	22.6 ± 5.68	18.89 ± 6.84	0.0652
INR	1.05 ± 0.49	1.05 ± 0.12	0.9811
D-dimer	1.15 ± 1.52	1.21 ± 0.83	–
Acquired risk factors, n (%)			
Anticardiolipin antibody IgG	3 (13.6)	2 (10.5)	>0.9999
Lupus anticoagulants	1 (4.5)	0	>0.9999
Median length (range), n (%)			
IP-CVAS—induction of bev	5 days (2–252)	107.5 days (2–695)	0.0048
IP-CVAS—initial DUS	13.5 days (7–259)	116 days (7–700)	0.0059

ECOG Eastern Cooperative Oncology Group, *bev* bevacizumab, *INR* international normalized ratio, *IP-CVAS* implantation of central venous access system, *DUS* Doppler ultrasound imaging, *SD* standard deviation

According to these studies, the incidence of thromboembolic events was not high and routine prophylactic anticoagulant treatment for thromboembolism did not appear necessary.

Patient characteristics in our study were similar to those in previous reports, and no specific characteristics related to thrombus formation were seen. However, we observed a higher rate of thrombi than expected using DUS and almost all of them were asymptomatic. Indeed, this study was designed to detect diagnostic findings, not clinical findings.

This study had a number of limitations. First, there was no control population (with no administration of bev). In other words, thromboembolic events may have been due to prior chemotherapy rather than bev, as only small doses of bev had been given at first screening. Second, the study protocol did not provide true baseline DUS at pre-treatment, as the time to treatment from implantation of the CVAS was usually just 2 days or more. Therefore, it was difficult to establish whether there was a correlation between the treatment drugs and catheter-related thrombosis, or when

thrombus formation occurred, as a CVAS itself is a risk factor for VTE.

However, we did perform DUS at pre-treatment between implantation of the CVAS and induction of bev in a limited number (17) of the patients. The characteristics of these 17 patients showed no differences to those of the other enrolled patients. Of these 17 patients, asymptomatic thrombosis was detected in 5 (29.4%). Of the other 12 patients, 5 showed asymptomatic thrombosis on initial DUS. Treatment with bev was probably associated with thrombus formation in these 5 patients, with incidence lower than that in the total study population (41.7 vs. 53.7%). The characteristics of these 5 patients were also similar to those of the general study population, and their outcomes consisted of a stable thrombus in 3 and asymptomatic progression in 2. The results indicate that a CVAS-associated thrombus prior to induction of bev was not necessarily a significant risk factor for severe thromboembolism.

When comparing the thrombus group with the non-thrombus group, the shorter the time between implantation of CVAS and induction of bev, the greater the risk of thrombus formation, regardless of whether it was symptomatic or asymptomatic. Moreover, a statistically significant difference in thrombus formation was observed between FOLFOX and FOLFIRI (90.9 vs. 9.1%; $P = 0.0047$). However, we do not believe that variation of drugs in FOLFOX versus FOLFIRI was associated with incidence of catheter-related thrombosis, as FOLFOX was used as first-line therapy with implantation of the CVAS, and FOLFIRI as second-line therapy in patients who already had a CVAS. No significant difference in laboratory data was observed between patients receiving FOLFOX and those receiving FOLFIRI; moreover, a shorter time between implantation of the CVAS and induction of bev showed no correlation with poor prognosis of thromboembolism. This point is of particular importance for the physician in treating patients with a bev-based regimen. Therefore, we hypothesized as follows: inhibition of either VEGF or cyclooxygenase (COX)-2-dependent prostacyclin (PGI2) biosynthesis associated with bev may have abolished a tonic protective pathway, thereby increasing the risk of thrombosis. VEGF binds to its major endothelial receptor, kinase insert domain-containing receptor (KDR) or VEGF receptor-2, triggering activation of endothelial nitric oxide synthase (eNOS) and COX-2, enzymes that mediate production of nitric oxide (NO) and PGI2. Bev would interrupt the pathway by which NO and PG12 inhibit platelet aggregation and proliferation of vascular smooth muscle cells, thus increasing risk of thrombosis and arterial wall thickening [29, 30]. Fibroblast growth factor (FGF-2) is quickly released during the wound-healing process, providing an early stimulus for endothelial cell proliferation in the acute phase immediately after injury. FGF-2 appears to be able to up-regulate VEGF production and acts synergistically in stimulating angiogenesis. Platelet-derived growth factor, transforming growth factor-3 and local hypoxia may also regulate VEGF production. Consequently, VEGF increases gradually from the third day after injury onward, providing a sustained stimulus for endothelial cell migration and differentiation into new capillary tubes [31]. Based on these previous reports, we believe that induction of bev in the early phase after implantation of a CVAS may be associated with high risk of thrombus formation due to a low level of VEGF production.

The strength of this study is its prospective assessment of catheter-related thrombus formation using DUS, a highly sensitive and non-invasive strategy. Routine prophylactic anticoagulant treatment at baseline, or if asymptomatic thrombosis was detected, was not permitted; this provided us with the opportunity to evaluate asymptomatic thrombus formation without the influence of prophylactic drugs. The results showed that outcomes in patients with asymptomatic thrombosis mainly depended on changes in thrombus size, as well as decreased vascular flow. In addition, vascular flow appeared to deteriorate with increase in thrombus size.

Our findings indicate that an enlarging thrombus, or large thrombus (>40 mm in diameter), along with decreased venous flow, is a risk factor for symptomatic thromboembolism or PE. Accordingly, we have started to administer prophylactic anticoagulant treatment in such patients at this facility. Further examination of venous flow revealed that thrombi extending into the junction of the SCV, ECV, or SSV strongly affected vascular flow. This finding may furnish an indirect marker of decreased vascular flow.

The American Society of Clinical Oncology provides guidelines on the prevention of recurrent VTE in oncology patients [14]. LMWH is the preferred initial approach for established VTE, and is also preferred in long-term prevention (>6 months). Vitamin K antagonists are an option when LMWH is not available. In Japan, LMWH has not been approved, and unfractionated heparin is used as initial therapy, followed by long-term warfarin therapy with a targeted INR of 2–3.

In conclusion, we propose that routine prophylactic anticoagulant treatment should not be used in patients treated with bev, as bev can increase the risk of bleeding. Therefore, it is important to assess eligibility for bev before treatment and during routine follow-up using available strategies to prevent severe thromboembolism. The results of this study indicate that a period of 1 week or more should be left between introduction of an IP-CVAS to administration of bev to reduce thrombus formation. DUS may offer the optimum strategy for detection of asymptomatic thrombosis in the early cycles of treatment. Moreover,

detection of an enlarging asymptomatic thrombosis developing into the superior vena cava along with decreased vascular flow or extending into the junction of the SCV, ECV, or SSV by DUS may be predictive of subsequent severe symptomatic thromboembolism. Large randomized controlled trials are needed to investigate the mechanism of VTE associated with bev and optimal management of this problem.

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CORRESPONDENCE

Is Statin Use Really Associated With Efficacy of Rituximab?

TO THE EDITOR: We would like to raise several issues regarding the recent article in *Journal of Clinical Oncology* by Nowakowski et al,[1] "Statin Use and Prognosis in Patients With Diffuse Large B-Cell Lymphoma and Follicular Lymphoma in the Rituximab Era."

This report focused on the clinical impact of statin use on outcomes of patients with diffuse large B-cell lymphoma (DLBCL) or follicular lymphoma (FL) receiving rituximab treatment based on the experimental results that statins impair the efficacy of rituximab. However, we do not think it can be concluded with certainty that statin use was associated with the efficacy of rituximab.

First, the authors have mentioned the limitation of medication compliance, the timing of the opening for statin use, and the type of statin, which was collected retrospectively using medical records; however, they do not provide any information about the serum cholesterol level. In a laboratory study, statins were found to significantly decrease rituximab-mediated complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity against B-cell lymphoma cells.[2] This study suggested that statins, through the depletion of serum cholesterol, induce conformational changes in CD20 molecules that result in impaired binding of rituximab.[2] Thus, we think the difference in serum cholesterol levels in the statin group versus no statin group should be compared. If the statin use group had an advantage for higher total cholesterol level despite statin use, it would be difficult to know whether statin use with lowered cholesterol levels may have a different impact. Our recent analysis showed that statin use was not correlated with the prognosis of patients with DLBCL receiving rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisone therapy by adjusting serum cholesterol level.[3]

Second, although the authors have analyzed event-free survival in all patients with FL as a whole group, regardless of whether or not they received rituximab, there are still heterogeneous groups of patients as a result of the disparate approaches to the initial care. Because rituximab significantly improved the outcomes of patients with FL,[4-8] we think the authors should analyze outcomes separately according to statin use, with or without the addition of rituximab.

Third, previous studies have shown that statin use reduces the risk of cardiovascular disease[9] and cerebral vascular attack, and the influence of death from these diseases should be ruled out to evaluate the correlation between statin use and prognosis of lymphoma. Detailed information about death or the evaluation of progression-free survival is encouraged.

Fourth, in patients treated on phase III adjuvant colon clinical trials, disease-free survival (DFS) and overall survival are highly correlated, both within patients and across trials. Although the correlation between DFS and overall survival in patients with FL treated with rituximab-containing chemotherapy has still not been proven, these results suggest that DFS after 3 years of median follow-up is an appropriate end point for adjuvant colon cancer clinical trials of fluorouracil-based regimens.[10]

The influence of statin use on rituximab efficacy is a significant clinical problem. Further studies and follow-up are warranted to confirm the prognostic significance of statins for patients with DLBCL and FL receiving rituximab-containing chemotherapy.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST
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Multicenter phase II study of bendamustine for relapsed or refractory indolent B-cell non-Hodgkin lymphoma and mantle cell lymphoma

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Bendamustine is a unique cytotoxic agent that has demonstrated efficacy in the treatment of indolent B-cell non-Hodgkin lymphomas (B-NHLs). In this multicenter phase II trial, the efficacy and safety of bendamustine were evaluated in Japanese patients with relapsed or refractory indolent B-NHL or mantle-cell lymphoma (MCL). Patients received bendamustine (120 mg/m^2) on days 1–2 of a 21-day cycle, for up to six cycles. The primary endpoint was the overall response rate (ORR) as assessed by an extramural committee according to International Workshop Response Criteria (IWRC). Secondary endpoints included complete response (CR) rate, ORR according to Revised Response Criteria (revised RC), progression-free survival (PFS), and safety. Fifty-eight patients with indolent B-NHL and 11 with MCL were enrolled. By IWRC, bendamustine produced an ORR of 91% (95% confidence interval [CI], 82–97%; 90% and 100% in patients with indolent B-NHL and MCL, respectively), with a CR rate of 67% (95% CI, 54–78%). ORR and CR rates according to revised RC were 93% (95% CI, 84–98%) and 57% (95% CI, 44–68%), respectively. After a median follow-up of 12.6 months, median PFS had not been reached. Estimated PFS rates at 1 year were 70% and 90% among indolent B-NHL and MCL patients, respectively. Bendamustine was generally well tolerated. Reversible myelosuppression, including grade 3/4 leukopenia (65%) and neutropenia (72%), was the most clinically significant toxicity observed. Common non-hematologic toxicities included mild gastrointestinal events and fatigue. These results demonstrate the high efficacy and tolerability of single-agent bendamustine in relapsed patients with indolent B-NHL or MCL histologies. (ClinicalTrials.gov ID: NCT00612183). (*Cancer Sci* 2010)

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of lymphoid malignancies, comprising 3% to 5% of all malignancies in the USA, Japan, and worldwide.[1–3] A total of 65 980 new cases and 19 500 deaths due to NHL are estimated for 2009 in the USA,[2] with similar incidence and mortality rates in Japan.[3]

Indolent B-cell non-Hodgkin lymphomas (B-NHLs), including follicular lymphomas, are less aggressive than other NHL subtypes; however, they are generally considered incurable due to their relapsing nature.[4,5] Front-line therapy with a regimen of cyclophosphamide, doxorubicin, vincristine, and prednisolone in combination (CHOP) plus rituximab produces high response rates in these patients and has improved progression-free survival (PFS) and overall survival compared with chemotherapy alone.[6–9] These improvements notwithstanding, most patients relapse and many become refractory to chemotherapy and rituximab. Effective second-line therapies are needed, especially those that do not exhibit cross-resistance with chemotherapy or rituximab.

Mantle cell lymphoma (MCL) is a relatively aggressive subtype of B-NHL, associated with poor responsiveness to treatment and shortened survival, that occurs in 3% to 8% of NHL patients.[10–14] Limited effective therapies are available for this population, particularly for relapsed patients.

Bendamustine is a unique cytotoxic agent with a multifaceted mechanism of action.[15] Structurally, it includes both a mechlorethamine group and a benzimidazole ring, intended to confer properties of both alkylators and purine analogs.[16] Bendamustine acts by directly damaging DNA as well as inducing apoptosis and mitotic catastrophe.[15,17,18] Importantly, bendamustine shows a distinct pattern of activity and a lack of cross-resistance with other alkylating agents.[15,19]

Single-agent bendamustine has demonstrated efficacy in patients with relapsed, rituximab-refractory B-NHL in two North American trials. In a phase II trial by Friedberg *et al.*[20] bendamustine produced a response in 47 (80%) of 59 assessable patients with indolent B-NHL, with a median response duration of 9.0 months. Results from the subsequent single-arm pivotal trial indicated a response rate of 75% in rituximab-refractory indolent B-NHL patients with a median response duration of 9.2 months.[21] Based on the results of these trials, bendamustine was approved in the USA and Canada for the treatment of indolent B-NHL that has progressed during or within 6 months of treatment with rituximab or a rituximab-based regimen. The objective of the present trial was to determine the safety and efficacy of bendamustine in Japanese patients with previously treated indolent B-NHL or MCL.

Materials and Methods

Study design and objectives. This multicenter, open-label, single-arm, phase II clinical study was designed to determine the antitumor effect and safety of bendamustine in patients with relapsed or refractory indolent B-NHL or MCL. The primary endpoint was the overall response rate (ORR), defined as the

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proportion of patients achieving a partial remission (PR) or better, according to International Workshop Response Criteria (IWRC) for NHL.[24] Secondary endpoints included the complete response (CR) rate, the ORR according to the Revised Response Criteria for Malignant Lymphoma (revised RC),[25] PFS, and safety. This study was performed in compliance with the ethical principles provided by the Helsinki Declaration and the Japanese Pharmaceutical Affairs Act. The protocol was approved by the institutional review board of each participating institution.

Eligibility. Patients aged 20 to 75 years were eligible if they had previously treated, histopathologically confirmed indolent B-NHL or MCL[22] that failed to respond to, or relapsed after, prior therapy. There was no maximum number of allowed prior therapies. Patients were required to have a measurable lesion >1.5 cm in one dimension, an Eastern Cooperative Oncology Group performance status[23] of 0 or 1, and a life expectancy ≥3 months. Adequate hematologic (neutrophils ≥1500/μL and platelets ≥100 000/μL), renal (serum creatinine <1.5 × the upper limit of the normal [ULN] at each study institution), hepatic (aspartate aminotransferase [AST] and alanine aminotransferase [ALT] <2.5 × ULN; total bilirubin <1.5 × ULN), and respiratory and cardiovascular (arterial oxygen partial pressure ≥65 mmHg; no abnormal electrocardiogram findings in need of treatment) function were required. Patients could have no carryover effect of prior therapy, with a required 4-week wash-out period (at least 3 months for antibody therapy). Patients were excluded if they had an apparent infection (including viral infection), other serious medical disorder, infiltration of lymphoma to the central nervous system, or an active malignancy other than lymphoma. Patients were required to provide written informed consent.

Treatment. Bendamustine 120 mg/m^2 was administered by intravenous infusion over 60 min on days 1 and 2 every 21 days for up to six treatment cycles. Hospitalization was required during days 1–3 of cycle 1; subsequent treatment was allowed on an outpatient basis at the discretion of the investigator. Computed tomography (CT) examination was performed at enrollment, during the third and last cycles, and 3 months after the last cycle. When available, positron emission tomography (PET) examination was performed at enrollment and during the last cycle. Hematologic and biochemical laboratory tests were performed at study entry and at 1, 2, and 3 weeks after administration of each cycle. Before beginning each treatment cycle, recovery of neutrophil and platelet counts (to ≥1000/μL and ≥75 000/μL, respectively) and the absence of grade ≥3 toxicities was required. Dose reductions to 90 mg/m^2 were performed in patients with grade 4 neutropenia persisting for ≥1 week despite treatment with granulocyte colony-stimulating factor (G-CSF), febrile neutropenia (grade ≥3 neutropenia, accompanied by a fever of ≥38.5°C) persisting for 3 days or longer, platelet count <10 000/μL or hemorrhagic tendency requiring platelet transfusion, or other grade 3/4 toxicities at the discretion of the investigator. In the case of toxicity recurrence, the dose was reduced to 60 mg/m^2. Further recurrence of toxicity at 60 mg/m^2 resulted in discontinuation of treatment.

The use of prophylactic antiemetics and antibiotics for prevention of opportunistic infection was recommended. The use of G-CSF was permitted during cycles 2 through 6, as well as during cycle 1 when grade ≥3 neutropenia was confirmed. Prophylaxis for tumor lysis syndrome (e.g. allopurinol) was recommended in patients with high tumor burden.

Assessment. Response was assessed after the third and last cycles of treatment by an extramural committee according to the IWRC,[24] as well as by the revised RC.[25] Patients were classified by best tumor response: CR (disappearance of all evidence of disease), unconfirmed CR (CRu; a CR with indeterminate bone marrow histology or a more than 75% decrease from baseline in the sum of the products of the greatest perpendicular diameters [SPD] of all the measurable lesions but with a residual mass; used in IWRC only), partial response (PR; a more than 50% decrease from baseline in the SPD of all the measured lesions, no increase in size of any other lesions, and no new lesions), stable disease (failure to achieve a PR, but without disease progression), or progressive disease (any new lesions or an increase by ≥50% of a previously involved site from nadir). PFS was determined at 3 months after completion of the last cycle, with additional assessments performed every 3 months during the study. PFS was calculated as the time from study enrollment to either disease progression (including relapse and exacerbation) or death from any cause.

Adverse events were reported according to the Common Terminology Criteria for Adverse Events version 3.0.[26] Serious

Table 1. Patient demographics and baseline characteristics

	Indolent B-NHL (n = 58)	MCL (n = 11)
Age, years		
Median	58.5	70
Range	33–75	59–75
Sex, *n*		
Male	33	7
Female	25	4
ECOG performance status, *n*		
0	52	7
1	6	4
Diagnosis (WHO classification), *n*		
Small lymphocytic lymphoma	3	
Lymphoplasmacytic lymphoma	1	
Splenic marginal zone B-cell lymphoma	0	
MALT lymphoma	1	
Nodal marginal zone B-cell lymphoma	1	
Follicular lymphoma	52	
Mantle cell lymphoma		11
Stage at diagnosis, *n*		
Stage I or II	7	4
Stage III	21	1
Stage IV	29	6
Unknown	1	0
Risk category, *n*†		
Low risk	30	2
Intermediate risk	13	8
High risk	15	1
Prior treatment, *n*		
Chemotherapy	56	11
Purine analogs	20	4
Rituximab	55	11
Ibritumomab tiuxetan	2	0
Radiotherapy	7	2
Corticosteroids alone	3	0
Other	8	4
Number of prior regimens		
Median	2	4
Range	1–9	1–16
Time since last treatment, months		
Median	13.4	6.6
Range	1.1–29.5	1.1–35.8

†Risk categories were determined using the Follicular Lymphoma International Prognostic Index (FLIPI) for patients with indolent B-cell non-Hodgkin lymphoma (NHL) and International Prognostic Index (IPI) for patients with mantle cell lymphoma (MCL). ECOG, Eastern Cooperative Oncology Group; MALT, mucosa-associated lymphoid tissue; WHO, World Health Organization.

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adverse events were also recorded; these were defined as events that led to death or disability, were life-threatening, required hospitalization, caused congenital anomaly, or resulted in medically significant conditions.

Statistical analyses. The sample size was calculated based on an expected and threshold ORR of 55% and 35% in patients with indolent B-NHL and 40% and 15% in patients with MCL, respectively. The ORR was calculated as the proportion of treated patients who achieved a PR or better, along with a 95% confidence interval (CI). The CR rate was calculated as the proportion of treated patients who achieved a CR or CRu, along with a 95% CI. PFS was assessed by the Kaplan–Meier method, with a 95% CI based on Greenwood confidence bounds.

Results

Patients. Fifty-eight patients with indolent B-NHL and 11 patients with MCL were enrolled. Patient demographics and baseline characteristics are summarized in Table 1. Among patients with indolent B-NHL, histologies included follicular (n = 52), small lymphocytic (n = 3), and one each lymphoplasmacytic, nodal marginal zone, and mucosa-associated lymphoid tissue lymphomas. The median number of prior regimens administered was two (range, 1–9) in patients with indolent B-NHL and four (range, 1–16) in patients with MCL. Thirty-seven (64%) patients with indolent B-NHL and six (55%) patients with MCL were known to be responsive to their last prior therapy.

Disposition. All enrolled patients received treatment with bendamustine and were included in both efficacy and safety analyses (Table 2). Fifty (72%; 41 with B-cell NHL and nine with MCL) patients completed the planned treatment of three or more cycles of bendamustine. The median number of cycles administered was five (range, 1–6). All 19 early discontinuations were due to adverse events, mainly myelosuppression, including neutropenia (9), neutropenia/leukopenia (3), thrombocytopenia (2), leukopenia (1), pneumonia (1), fatigue/nausea (1), anorexia/nausea/vomiting (1), and fever/vomiting/increased ALT/AST (1).

Efficacy. Bendamustine produced an ORR of 91% according to the IWRC, with a CR rate (CR plus CRu) of 67% (Table 2). Among patients with indolent B-NHL, the ORR was 90%, including a CR/CRu rate of 66%. All 11 MCL patients responded to bendamustine (100%), with eight patients achieving a CR/CRu (73%). Results were similar using the revised RC, with an ORR of 93% and a CR rate of 57% (Table 3). Among 57 patients who underwent both CT and PET examinations, the evaluation of overall response according to IWRC and the revised RC did not agree in nine patients (seven of 47 with indolent B-NHL and two of 10 with MCL).

In patients with indolent B-NHL, ORR by Follicular Lymphoma International Prognostic Index (FLIPI)[27] risk category was 97% (29 of 30) in low-risk, 92% (12 of 13) in intermediate-risk, and 73% (11 of 15) in high-risk patients (P = 0.041). Response rates were similar by disease stage, number of prior therapy regimens, or response to most recent prior therapy. In patients who received fewer than three cycles of bendamustine, the ORR was 74% (95% CI, 49–91%), compared with 98% (95% CI, 89–100%) in patients who received three or more cycles (Table 4).

After a median follow-up of 12.6 months (range, 1.3–17.9 months), disease progression was observed in 21 patients, 19 with indolent B-NHL, and two with MCL; median PFS had not yet been reached (Fig. 1). Estimated PFS rates at 1 year were 74% in the overall population, 70% in patients with indolent B-NHL, and 90% in patients with MCL.

Safety. The main toxicity observed with bendamustine treatment was reversible myelosuppression, including grade 3/4 neutropenia (72%), leukopenia (65%), and thrombocytopenia (16%) (Table 5). Twenty-seven (39%) patients received growth factor support, two patients received platelet transfusions, and one patient received a transfusion of packed red blood cells.

Common non-hematologic adverse events included nausea (86%), fatigue (62%), and anorexia (61%), and were mainly grade 1/2 in severity (Table 6). Seventeen infections of any grade were observed in 11 patients (16%); grade 3 infection was observed in five patients (7%; one each febrile neutropenia, pneumonia, upper airway infection, varicella/herpes zoster, and

Table 2. Objective response to bendamustine treatment, by IWRC

Disease type	n	Response by IWRC, n (%)†				CR/CRu,% (95% CI)	ORR, % (95% CI)
		CR	CRu	PR	SD		
All	69	27 (39)	19 (28)	17 (25)	6 (9)	67 (54–78)	91 (82–97)
Indolent B-NHL	58	20 (34)	18 (31)	14 (24)	6 (10)	66 (52–78)	90 (79–96)
Follicular	52	19 (37)	17 (33)	11 (21)	5 (10)	69	90
Small lymphocytic	3	0	1 (33)	1 (33)	1 (33)	33	67
Lymphoplasmacytic	1	0	0	1 (100)	0	0	100
MALT	1	0	0	1 (100)	0	0	100
Nodal marginal zone	1	1 (100)	0	0	0	100	100
MCL	11	7 (64)	1 (9)	3 (27)	0	73 (39–94)	100 (72–100)

†International Workshop Response Criteria (IWRC) for NHL.[24] CI, confidence interval; CR, complete response; CRu, unconfirmed CR; MALT, mucosa-associated lymphoid tissue; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; PR, partial response; SD, stable disease.

Table 3. Objective response to bendamustine treatment, by revised RC

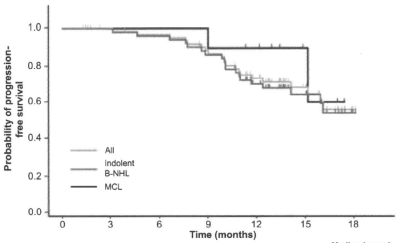
Disease type	n	Response by revised RC, n (%)†				CR, % (95% CI)	ORR, % (95% CI)
		CR	PR	SD	PD		
All	69	39 (57)	25 (36)	4 (6)	1 (1)	57 (44–68)	93 (84–98)
Indolent B-NHL	58	31 (53)	22 (38)	4 (7)	1 (2)	53 (40–67)	91 (81–97)
MCL	11	8 (73)	3 (27)	0	0	73 (39–94)	100 (72–100)

†Revised Response Criteria for Malignant Lymphomas (revised RC).[25] CI, confidence interval; CR, complete response, MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; PD, progressive disease; PR, partial response; SD, stable disease.

Table 4. Objective response to bendamustine treatment, by number of treatment cycles

Cycles of bendamustine	*n*	Response by IWRC, *n* (%)†				CR/CRu, % (95% CI)	ORR, % (95% CI)
		CR	CRu	PR	SD		
≥3 cycles							
All	50	25 (50)	13 (26)	11 (22)	1 (2)	76 (62–87)	98 (89–100)
Indolent B-NHL	41	19 (46)	12 (29)	9 (22)	1 (2)	76 (60–88)	98 (87–100)
MCL	9	6 (67)	1 (11)	2 (22)	0	78 (40–97)	100 (66–100)
<3 cycles							
All	19	2 (11)	6 (32)	6 (32)	5 (26)	42 (20–67)	74 (49–91)
Indolent B-NHL	17	1 (6)	6 (35)	5 (29)	5 (29)	41 (18–67)	71 (44–90)
MCL	2	1 (50)	0	1 (50)	0	50 (1–99)	100 (16–100)

†International Workshop Response Criteria (IWRC) for NHL.[24] CI, confidence interval; CR, complete response, CRu, unconfirmed CR; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; ORR, overall response rate; PR, partial response; SD, stable disease.



	n	Events, *n* (%)	Censored, *n* (%)	PFS probability at 1-year,%	Median (range) follow-up time, months
All	69	21 (30)	48 (70)	73.6	12.6 (1.3–17.9)
Indolent B-NHL	58	19 (33)	39 (67)	70.4	12.3 (1.3–17.9)
MCL	11	2 (18)	9 (82)	90.0	12.8 (1.7–17.2)

Fig. 1. Kaplan–Meier analysis of progression-free survival (PFS) in patients with relapsed indolent B-cell non-Hodgkin lymphoma (NHL) and mantle cell lymphoma (MCL) who were treated with bendamustine.

viral pharyngitis), and no grade 4 infection was observed. Twenty adverse events in 11 patients were considered serious and required hospitalization. All serious adverse events were resolved with or without treatment. There were no deaths attributed to bendamustine treatment.

Dose reductions or delays were not necessary for most patients. In 11 patients (19%), the bendamustine dose was reduced from 120 mg/m^2 to 90 mg/m^2; in two additional patients (3%), the dose was reduced from 120 mg/m^2 to 90 mg/m^2 and then reduced again to 60 mg/m^2. The mean dose intensity administered was 70.14 ± 8.78 mg/m^2/week, which is equivalent to a mean relative dose intensity of 88% ± 11%. During cycles 2 through 5, treatment was delayed in 47 (21%) of 219 cycles administered. No increase in the length of treatment delays was observed at later cycles; mean intervals between the start of treatment cycles ranged from 22.8 ± 3.9 days (between the first and second cycles) and 25.0 ± 5.3 days (between the fifth and sixth cycles).

Discussion

Bendamustine produced high response rates and durable responses in both indolent B-NHL and MCL patients. Our results in patients with indolent B-NHL complement previous studies of bendamustine in relapsed indolent B-NHL. The 91%

Table 5. Hematologic toxicity in bendamustine-treated patients, by grade† (*n* = 69)

Event	Patients affected, *n*					
	Grade, *n*				All grades *n* (%)	Grade 3/4 *n* (%)
	1	2	3	4		
Leukopenia	5	17	37	8	67 (97)	45 (65)
Neutropenia	2	10	17	33	62 (90)	50 (72)
Thrombocytopenia	31	10	7	4	52 (75)	11 (16)
Anemia	25	17	2	2	46 (67)	4 (6)

†As graded by Common Terminology Criteria for Adverse Events, version 3.0.[26]

ORR (by IWRC) observed in this study compares favorably with the 75–80% ORR observed in the two North American trials.[20,21] Differences in patient population may account for the higher response rates observed in our study because patients were required to be rituximab refractory or intolerant in the North American studies. In patients with MCL, the observed response rate of 100% in our trial was particularly encouraging. A high response rate in MCL patients was also observed with bendamustine plus rituximab in a North American phase II trial.[28] Although the number of MCL patients treated in both trials was small, bendamustine appears to be at least as effective in these patients as in those with indolent B-NHL, providing a valuable treatment option in a population that typically demonstrates poor response rates.

The responses were durable in both subtypes, and the median PFS was not reached after a median follow-up of 12.6 months. The proportion of MCL patients estimated to be progression free at 1 year was similar to that in patients with indolent B-NHL, even though the MCL population was more heavily pretreated.

We assessed patient response using both the IWRC and the revised RC in order to incorporate PET findings into our assessment while allowing for accurate comparison of our data with historical data. The ORRs assessed using the two sets of criteria were very similar, although the CR rate tended to be lower with the revised RC, possibly due to the elimination of the CRu category in the revised criteria. Overall, the results from either method were in general agreement, and the discrepancies did not affect efficacy conclusions.

In subgroup analyses, we found that patients who completed three or more cycles of treatment had higher response rates than patients who received fewer than three treatment cycles. Conversely, dose reductions did not appear to adversely affect treatment outcome because all 11 patients who underwent dose reductions responded to treatment. Based on these results, it

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Table 6. Non-hematologic toxicity in bendamustine-treated patients, by grade† (*n* = 69)

Event‡	Patients affected, *n*					
	Grade, *n*				All grades	Grade 3/4
	1	2	3	4	*n* (%)	*n* (%)
Nausea	37	22	0	0	59 (86)	0
Fatigue	35	8	0	0	43 (62)	0
Anorexia	27	13	2	0	42 (61)	2 (3)
Constipation	26	6	0	0	32 (46)	0
Rash	10	21	1	0	32 (46)	1 (1)
Vomiting	14	12	3	0	29 (42)	3 (4)
Weight loss	17	6	1	0	24 (35)	1 (1)
Fever	15	6	0	0	21 (30)	0
Phlebitis	12	7	2	0	21 (30)	2 (3)
Vascular pain	20	0	0	0	20 (29)	0
Injection site reaction	15	3	0	0	18 (26)	0
Dysgeusia	14	3	0	0	17 (25)	0
Diarrhea	11	5	0	0	16 (23)	0
Headache	14	2	0	0	16 (23)	0
Other skin reaction (redness)	12	1	0	0	13 (19)	0
Oral mucositis	10	2	0	0	12 (17)	0
Gastric discomfort	8	3	0	0	11 (16)	0
Infection§	5	1	5	0	11 (16)	5 (7)
Candida stomatitis	1	1	0	0	2 (3)	0
Febrile neutropenia	0	0	1	0	1 (1)	1 (1)
Herpes	3	1	0	0	4 (6)	0
Pneumonia	0	0	1	0	1 (1)	1 (1)
Upper airway infection	0	0	1	0	1 (1)	1 (1)
Varicella/herpes zoster	1	1	1	0	3 (4)	1 (1)
Viral pharyngitis	0	0	1	0	1 (1)	1 (1)
Other infection	1	0	0	0	1 (1)	0

†As graded by Common Terminology Criteria for Adverse Events, version 3.0.[26] ‡Events occurring in ≥15% of patients. §Indicates the number of patients developing any infection, at the greatest severity; totals do not sum because more than one type of infection could occur per patient.

might be reasonable that dose reductions be performed when necessary to allow continuation of treatment. Neither the extent of previous therapy (<3 or ≥3 prior therapies, or 1, 2, or 3 + prior therapies) nor the refractoriness of patients to prior therapy affected response rates. The distinct mechanism of action of bendamustine may partly explain its low cross-resistance in heavily pretreated or refractory patients.

Reversible myelosuppression was the primary adverse event associated with bendamustine and was the reason given most frequently for discontinuation of treatment. Tumor lysis syndrome was not observed in this study. Few serious adverse events occurred, and all were resolved with or without treatment. No secondary malignancies were observed during the study period (median follow-up of 12.6 months). Gastrointestinal events were common, but were typically mild in severity. The majority of adverse events that occurred were manageable with supportive care and dose reductions, and most patients were able to continue to receive bendamustine without delays or dose reductions. The toxicity of bendamustine did not appear to be additive, with no increase in treatment delays noted with subsequent treatment cycles.

In conclusion, the results of this study support the use of bendamustine in relapsed indolent B-NHL and show promising results for single-agent bendamustine in relapsed MCL. Ongoing studies of bendamustine in combination with rituximab will further clarify the role of this distinct alkylator in both relapsed and newly diagnosed patients.

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Disclosure Statement

The authors have no conflict of interest.

Abbreviations

CHOP	cyclophosphamide, doxorubicin, vincristine, and prednisolone
CI	confidence interval
CR	complete response
CRu	unconfirmed CR
CT	Computed tomography
ECOG	Eastern Cooperative Oncology Group
FLIPI	Follicular Lymphoma International Prognostic Index
G-CSF	granulocyte colony-stimulating factor
IPI	International Prognostic Index
IWRC	International Workshop Response Criteria
MALT	mucosa-associated lymphoid tissue
MCL	mantle cell lymphoma
NHL	non-Hodgkin lymphoma
ORR	overall response rate
PD	progressive disease
PET	positron emission tomography
PFS	progression-free survival
PR	partial response
SD	stable disease
SPD	sum of the products of the greatest perpendicular diameters
ULN	upper limit of the normal
WHO	World Health Organization.

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Medical Oncology

Tumor Size Is a Potential Predictor of Response to Tyrosine Kinase Inhibitors in Renal Cell Cancer

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OBJECTIVES To investigate the correlations between the initial tumor size and size reduction rate in patients treated with targeted agents. To select the patients who can benefit the most from treatment with targeted agents, it will be necessary to find a tumor characteristic that predicts their effectiveness.

METHODS The data from 139 metastatic and 16 primary lesions treated with the targeted agents were retrospectively analyzed. They consisted of 86 sunitinib-treated and 69 sorafenib-treated lesions in 54 patients with metastatic renal cell carcinoma who had undergone treatment from April 2008 to July 2010. The relationship between the longest tumor diameter at baseline and the rate of reduction in tumor size was assessed using the Spearmancorrelation test.

RESULTS A linear, moderate to strong association between the initial tumor size and tumor size reduction rate was shown (correlation coefficient -0.441, $P < .001$). When these tumors were divided into 2 groups at the threshold value (23.95 mm), which was decided by the receiver operating characteristic curve analysis, the smaller tumors demonstrated a significantly greater size reduction than the larger tumors according to the Mann-Whitney U test ($P < .001$). Both univariate and multivariate linear regression analyses revealed that only the initial tumor size was associated with the rate of reduction in individual tumors ($P < .001$).

CONCLUSIONS The initial tumor size was a good predictor of the tumor size reduction. This simple observation could be useful for physicians who treat patients with metastatic renal cell carcinoma. In addition, in assessing clinical trials of targeted agents for metastatic renal cell carcinoma using the ResponseEvaluation Criteria in Solid Tumors, perhaps this association should be considered. UROLOGY xx: xxx, xxxx. © 2011 Elsevier Inc.

Surgical excision remains the standard and, indeed, the only curative therapy for patients with localized renal cell carcinoma (RCC). However, at the initial diagnosis, one third of patients with RCC will have visceral metastasis, and one half of the remainder will eventually develop distant metastases.[1] Previously, despite its limited clinical activity and significant toxicity, cytokine-based therapy was the mainstay treatment of metastatic RCC (mRCC).[1,2] A better understanding of the molecular biology of RCC has identified signaling pathways related to a hypoxia-inducible factor as rational targets, including the receptors of vascular endothelial growth factor and the mammalian target of rapamycin kinase for anticancer therapy for patients with mRCC.[3]

Because the agents aimed at these molecular targets have demonstrated significant objective responses with moderate and easily manageable toxic effects, a major breakthrough in the treatment paradigm for mRCC has occurred. Among them, sorafenib (Nexavar, Bayer Pharmaceuticals Corporation, West Haven, CT) and sunitinib (Sutent, Pfizer Inc., New York, NY) are tyrosine kinase inhibitors (TKIs), and target vascular endothelial growth factor receptors and platelet-derived growth factor receptors.[4,5]

As other new agents with alternative molecular targets emerge in RCC therapy, to select the patients who can benefit the most from these vascular endothelial growth factor receptor-targeted agents, it is necessary to find a biomarker or tumor characteristic that can predict their effectiveness. Because these agents function as angiogenesis inhibitors,[4,5] the initial tumor size and volume might be important in whether tumors can be expected to shrink using these treatments. Initially, we hypothesized

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