Table 1. Baseline characteristics of patients who received LY2469298

Characteristic	100 mg/m^2 (n = 3)	375 mg/m^2 (n = 7)	All (n = 10)
Median age at study entry (range) (years)	72 (50–75)	60 (39–67)	60 (39–75)
Sex, *n*; male/female	2/1	3/4	5/5
Clinical stage at study entry, *n*; I/II/III/IV	1/2/0/0	0/2/1/4	1/4/1/4
FLIPI risk group, *n*; low/intermediate/high	2/1/0	2/1/4	4/2/4
Bone marrow involvement, *n*; negative/positive	3/0	3/4	6/4
Bulky disease, *n*; 0 to <6 cm/≥6 cm	3/0	5/2	8/2
Number of prior regimens, *n*; 0/1–2/≥3	0/3/0	0/4/3	0/7/3
Prior rituximab (R), *n*; none/R alone/R-chemo	0/0/3	0/2/5	0/2/8
Refractory to rituximab†, *n*; yes/no	1/2	2/5	3/7
FcγRIIIa genotype, *n*; FF/FV/VV	1/2/0	2/4/1	3/6/1

†Latest outcome of rituximab-containing regimen was partial response, stable disease or progressive disease. For complete response, patients relapsed ≤6 months after the last rituximab infusion.
F, phenylalanine; FL, follicular lymphoma; FLIPI, follicular lymphoma international prognostic index; *n*, number of patients; V, valine.

previously treated with chemotherapy and/or rituximab (but not progressing within 120 days following the last infusion of rituximab). Patients must have provided written informed consent for the study and for genetic testing for the polymorphism of the *FcγRIIIA* gene before enrolment. *FcγRIIIA* genotyping was performed by Cogenics Inc. (Morrisville, NC, USA) using polymerase chain reaction followed by allele-specific restriction enzyme digestion. DNA isolated from peripheral blood was used for the genotyping. Eligible patients were required to have the following: at least one measurable lesion ≥1.5 cm in the longest diameter (confirmed by computed tomography [CT] scanning); Eastern Cooperative Oncology Group performance status of 0 or 1; absolute neutrophil count (ANC) >1500/mm^3; platelet count >75 000/mm^3; hemoglobin ≥8 g/dL; serum creatinine ≤1.5 × upper limit of normal (ULN); total bilirubin ≤1.5 × ULN; alkaline phosphatase ≤1.5 × ULN; and alanine transaminase ≤1.5 × ULN.

Patients were not eligible if they had the following: evidence of hepatitis B or C virus infection; clinically significant transformation to diffuse large B-cell lymphoma; known allergy to antibody therapy or any of the study drug components; active concurrent malignancy; significant cardiac complications (e.g. New York Heart Association Congestive Heart Failure class III or higher); positive test for serum cardiac troponin; active infection; a history of blood transfusion or erythropoietin treatment within 10 days prior to enrolment; a history of growth factor administration within 28 days prior to enrolment; or were positive for human immunodeficiency virus (HIV-1) infection. Patients were required to discontinue all anti-lymphoma treatments at least 30 days prior to study enrolment

Study design and treatment. This open-label, multicenter, non-randomized, dose-escalation, phase I study was designed to investigate the safety and tolerability of weekly doses of LY2469298 in patients with relapsed or refractory CD20-positive FL. The study was conducted between October 2008 and December 2009 at two institutions (National Cancer Center Hospital, Tokyo and Nagoya Daini Red Cross Hospital, Nagoya, Japan). This study was approved by the institutional review boards of the two institutions and conducted in accordance with the ethical principles of the Declaration of Helsinki.

LY2469298 was administered intravenously, at a dose of either 100 or 375 mg/m^2, four times at weekly intervals. LY2469298, supplied in a glass vial containing 1 mL at a concentration of 20 mg/mL, was diluted in normal saline to a final concentration of 1 mg/mL and given through a 0.22-μm in-line filter. The first infusion of LY2469298 was administered slowly at a rate of ≤25 mg/h and increased by up to 50 mg/h every 30 min. Subsequent infusions could be administered at an initial rate of up to 100 mg/h with increments every 30 min until the 300 mg/h rate was reached. All patients were premedicated with antipyretic analgesic (e.g. acetaminophen) and anti-histamine (e.g. diphenhydramine) given 30 min before the infusion.

Table 2. Most common† and all grade 3 or 4 drug-related adverse events

Adverse events‡	100 mg/m^2				375 mg/m^2				Total
	Any grade	Grade 1–2	Grade 3	Grade 4	Any grade	Grade 1–2	Grade 3	Grade 4	
Hematological									
Lymphopenia	3	1	2	0	7	2	3	2	10
Leukopenia	1	1	0	0	6	6	0	0	7
Neutropenia	1	1	0	0	4	2	1	1	5
Thrombocytopenia	0	0	0	0	2	2	0	0	2
Non-hematological									
Pyrexia	1	1	0	0	7	7	0	0	8
Chills	2	2	0	0	5	5	0	0	7
Headache	1	1	0	0	2	2	0	0	3
Oropharyngeal discomfort	0	0	0	0	3	3	0	0	3
Epigastric discomfort	0	0	0	0	2	2	0	0	2
Fatigue	0	0	0	0	2	2	0	0	2
Respiratory tract infection	1	1	0	0	1	1	0	0	2
Increased LDH	0	0	0	0	2	2	0	0	2
Increased CRP	0	0	0	0	2	2	0	0	2
Rash	0	0	0	0	2	2	0	0	2

†Treatment-related adverse events reported in ≥20% of patients are listed. ‡All events that were possibly related to LY2469298 were reported.
CRP, C-reactive protein; LDH, lactic dehydrogenase.

Table 3. Number of patients developing infusion-related toxicities

Infusion	100 mg/m^2				375 mg/m^2			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
No. patients infused	3	3	3	3	7	6	6	6
Grade 1†	1	0	0	0	4	1	0	1
Grade 2†	2	0	0	0	3	0	0	0
Grade 3†	0	0	0	0	0	0	0	0
Grade 4†	0	0	0	0	0	0	0	0
Total	3	0	0	0	7	1	0	1

†Common Terminology Criteria for Adverse Events Version 3.0.

Table 4. Best overall response by *FcγRIIIA-158* genotype

FcγRIIIA-158 Genotype	100 mg/m^2			375 mg/m^2		
	FF	*VF*	*VV*	*FF*	*VF*	*VV*
No. patients	1	2	0	2	4	1
CR	0	1	0	0	1	1
CRu	0	0	0	0	1	0
PR	0	0	0	1	0	0
SD	1	1	0	1	1	0
PD	0	0	0	0	1	0

CR, complete response; CRu, complete response unconfirmed; PR, partial response; SD, stable disease; PD, progressive disease.

Infusion-related reactions were monitored continuously between the start of the infusion and 60 min after the infusion was completed. Infusions were to be slowed or suspended for any clinically significant infusion-related reaction.

The treatment plan was to enroll at least three patients at the 100 mg/m^2 dose level, and up to six patients at this level if one patient experienced a dose-limiting toxicity (DLT), before escalating to the higher dose level. If no more than one of six patients experienced a DLT at 100 mg/m^2, the dose was to be escalated to 375 mg/m^2, which was established as the recommended phase II dose in a phase I study conducted in the United States.[21] The protocol treatment was to be discontinued for a patient if any of the following occurred: disease progression; appearance of DLT; unacceptable toxicity; withdrawal of informed consent; serious deviation of study compliance; loss to follow up; or investigator's discretion.

Study evaluation. Safety and toxicity were evaluated by monitoring laboratory assessments and the incidence, severity and type of adverse events (AE). All AE were graded by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 3.0. Patients were monitored for DLT from the initial infusion to 2 weeks after the last infusion (5 weeks). The DLT was defined as any grade 3 or greater drug-related AE, with the following modifications: grade 3 hematological toxicity was defined as an ANC nadir of ≥500 to <1000/mm^3, or a decrease in platelet count or hemoglobin of 50–74% from the lower limit of normal or the baseline value, whichever was less; cardiac toxicity of grade 3 or greater that occurred during the DLT evaluation period. The following were defined *a priori* as not DLT: grade 3 infusion reactions (e.g. fever, rigors, bronchospasm, urticaria and hypotension) that were transient and resolved without sequelae; grade 3 tumor lysis syndrome that was transient and resolved without sequelae. An enzyme-linked immunosorbent assay (ELISA) was used to detect the level of human anti-human antibody (HACA) to LY2469298 in serum sampled before the first infusion and 5 weeks after the last infusion (performed by Millipore Corporation, St Charles, MO, USA).

Response assessment. Response assessments after treatment with LY2469298 were performed 9 and 21 weeks after the last infusion. The efficacy of LY2469298 was evaluated according to the International Workshop Response Criteria for Non-Hodgkin Lymphomas.[22] Objective responses included complete response (CR), unconfirmed CR (CRu) and partial response (PR). Baseline evaluation included disease-related symptoms (B symptoms), radiographic examination using CT and bone marrow biopsy.

B-lymphocyte monitoring and pharmacokinetic (PK) analysis. B-lymphocytes were monitored using FACS analysis (performed by Mitsubishi Chemical Medience Corporation, Tokyo, Japan) using an anti-CD19 monoclonal antibody conjugated to a fluorescent probe. Samples for both the PK and CD19 analyses were obtained before each infusion; 1 and 3–5 days after the first infusion; 1 and 3–5 days, 2, 5, 9 and 21 weeks after the last infusion; and at withdrawal from the study. Serum levels of LY2469298 were determined using an enzyme-linked immunosorbent assay (performed by the Charles River Laboratories Preclinical Services Montreal Inc, Senneville, QC, Canada).

Pharmacokinetic parameter estimates for LY2469298 were calculated by standard noncompartmental methods of analysis using WinNonLin Professional Version 5.0.1 (Pharsight, Cary, NC, USA). Noncompartmental parameters, such as the maximum concentration (C_{max}), the elimination half-life ($t_{1/2}$), area under the concentration–time curve (AUC), apparent clearance (CL), apparent volume of distribution (V_z), and mean residence time (MRT) of LY2469298 were reported following the fourth dose administration. The C_{max} was taken from the observed data. The apparent terminal rate constant (λ_z) was calculated from the regression of log concentration versus time over the terminal log-linear portion of the concentration–time profile. $T_{1/2}$ was calculated as $\ln 2/\lambda_z$. The AUC were calculated using the log-linear trapezoidal rule. Following the fourth dose, $AUC_{0\text{-tlast}}$ was calculated from 0 h post-dose to the last sampling point, and $AUC_{0\text{-}\tau}$ was calculated from 0 to 168 h post-dose. The CL was calculated as Dose/$AUC_{0\text{-}\tau}$, V_z was calculated as Dose/($\lambda_z \times AUC_{0\text{-}\tau}$) and MRT was calculated as ($AUMC_{0\text{-}\tau}$ + $\tau[AUC_{0\text{-}\infty} - AUC_{0\text{-}\tau}]$)/$AUC_{0\text{-}\tau}$ − infusion duration/2, where AUMC is the area under the moment curve.

Results

Patients. Ten Japanese patients with CD20-positive FL were enrolled in the present study (Table 1). Most patients had both clinical stage II or IV FL at study entry, and most of the advanced-stage patients (clinical stage III or IV) were enrolled in the 375 mg/m^2 cohort. The median age of the ten enrolled patients was 60 years. All patients had received one or more prior treatments of rituximab alone or rituximab-containing chemotherapy, and three of these were judged to be refractory to rituximab. The median number of prior regimens was two (range, 1–9). The Follicular Lymphoma International Prognostic Index (FLIPI)[23] identified four patients at low risk, two at intermediate risk and four at high risk. Among the ten enrolled patients, only one patient had *FcγRIIIA-158VV* alleles; the remaining nine patients were F-carriers (six with *FcγRIIIA-158VF* alleles and three with *FcγRIIIA-158FF* alleles). Six of the F-carriers (four with *FcγRIIIA-158VF* alleles and two with *FcγRIIIA-158FF* alleles) and one *FcγRIIIA-158VV* patient were enrolled in the 375 mg/m^2 cohort. All three patients in the 100 mg/m^2 cohort were F-carriers (two with *FcγRIIIA-158VF* alleles and one with *FcγRIIIA-158FF* alleles).

Safety. Nine patients completed all four infusions and were evaluable for DLT. One patient was suspended from study treatment after the first infusion and did not continue because the study was suspended to resolve an issue with preparation of the study drug. The patient had no significant safety problems

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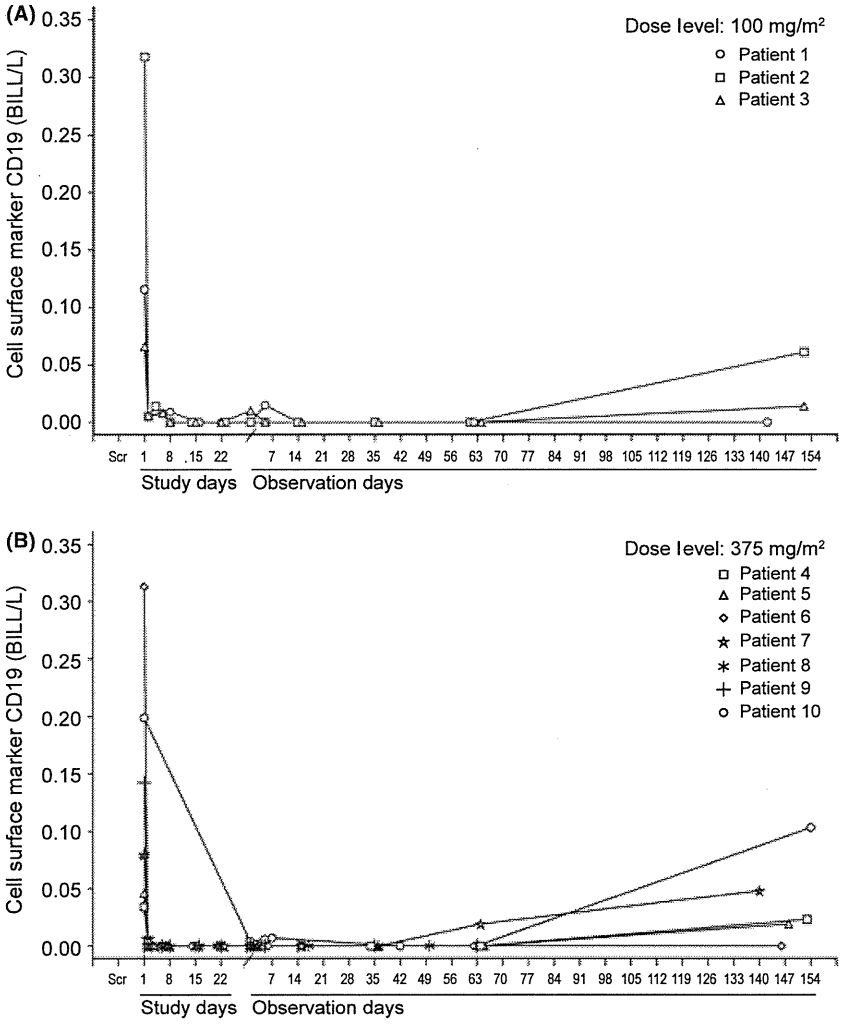

Fig. 1. Time course of the cell counts of CD19-positive B-lymphocytes in each patient. (A) The 100 mg/m^2 LY2469298 cohort. (B) The 375 mg/m^2 LY2469298 cohort. Study days = the number of days on the study starting with the first infusion. Observation days = the number of days after the last infusion. Scr, Screening.

Table 5. Pharmacokinetic parameters of LY2469298 after the last infusion

	Parameter								*FcγRIIIA-158* Genotype	Best overall response
	$t_{1/2}$ (h)	$AUC_{0-\tau}$ (μg·h/mL)	$AUC_{0-tlast}$ (μg·h/mL)	$AUC_{0-\infty}$ (μg·h/mL)	C_{max} (μg/mL)	MRT (h)	CL (L/h)	V_z (L)		
100 mg/m^2										
Patient 1	613	20300	106000	131000	157.55	987	0.00732	6.47	FF	SD
Patient 2	313	16400	50600	52600	105.45	452	0.0100	4.52	VF	CR
Patient 3	199	12400	37200	37400	121.53	413	0.0108	3.09	VF	SD
Mean†	337	16100	58400	63600	126	569	0.00924	4.49		
CV (%)†	62	25	58	72	21	51	21	38		
375 mg/m^2										
Patient 4	195	35100	88200	88700	389.47	329	0.0216	6.08	VF	SD
Patient 5	133	52400	138000	138000	400.56	352	0.00989	1.90	VF	CRu
Patient 6	451	57000	224000	249000	552.11	641	0.0101	6.55	VV	CR
Patient 7	28.8	9760	9950	9950	190.23	45.2	0.0665	2.76	FF	SD
Patient 8	130	52100	111000	111000	446.37	268	0.0112	2.10	VF	PD
Patient 9	499	49700	171000	192000	351.65	560	0.0125	9.02	VF	CR
Mean†‡	238	48600	139000	146000	423	407	0.0125	4.28		
CV (%)†‡	73	19	37	43	17	38	33	81		

†Geometric mean and CV%. ‡Excluding data from patients 7 (outlier) and 10 (early discontinuation of treatment). $AUC_{0-\tau}$, area under the concentration versus time curve during one dose interval; $AUC_{0-tlast}$, area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration; $AUC_{0-\infty}$, area under the concentration versus time curve extrapolated to infinity; CL, total body clearance; C_{max}, maximum serum concentration; CV, coefficient of variation; MRT, mean residence time; $t_{1/2}$, terminal elimination half-life; V, volume of distribution; CR, complete response; CRu, complete response unconfirmed; PR, partial response; SD, stable disease; PD, progressive disease.

and was excluded from DLT evaluation. No DLT were observed between the first infusion and 2 weeks after the last infusion.

Treatment-related AE were observed in all ten patients treated with at least one dose of LY2469298. Most of the AE were grade 1 or 2, and the most common AE were hematological (lymphopenia, leukopenia, neutropenia) or infusion-related (pyrexia, chills) (Table 2). The only encountered AE of grade 3 or greater were lymphopenia and neutropenia; neither of the patients with neutropenia required treatment with granulocyte colony-stimulating factor. There were no deaths, serious AE or discontinuations due to AE.

Infusion-related toxicities were observed in all patients; all of these were grade 2 or less, with the majority limited to the first infusion (Table 3). Most infusion-related toxicities were similar to those previously reported after rituximab treatment,[24–27] except for increased heart rate and somnolence, both of which were mild and manageable. In four patients (two in each cohort) the infusion rate was adjusted due to infusion-related toxicities. HAHA was not detected in the serum samples from all patients before and up to the 5 weeks after the last infusion.

Responses. Across both cohorts, objective responses were observed in the following five of ten patients: three patients (one in the 100 mg/m^2 cohort; two in the 375 mg/m^2 cohort) achieved CR; one patient (375 mg/m^2 cohort) achieved CRu; and one patient (375 mg/m^2 cohort) achieved PR (Table 4). Responses were observed at both dose levels: four of seven patients at 375 mg/m^2 and one of three at 100 mg/m^2. The patient with homozygous *FcγRIIIA-158VV* alleles achieved CR after receiving 375 mg/m^2. Among the F-carriers, four of nine patients achieved objective responses (three of six patients in the 375 mg/m^2 cohort). Among the heterozygous F-carriers, an objective response was observed in three of six patients (two CR and one CRu). Among the homozygous F-carriers, an objective response was observed in one of three patients (PR).

There was a quick and sustained reduction in the number of CD19+ B-lymphocytes in the peripheral blood following the first infusion, which began to recover during the 21-week observation period (Fig. 1).

Pharmacokinetics (PK). All ten patients were included in the PK analysis except patient 7 (dosed at 375 mg/m^2). The rate of LY2469298 disappearance from serum in this patient was very

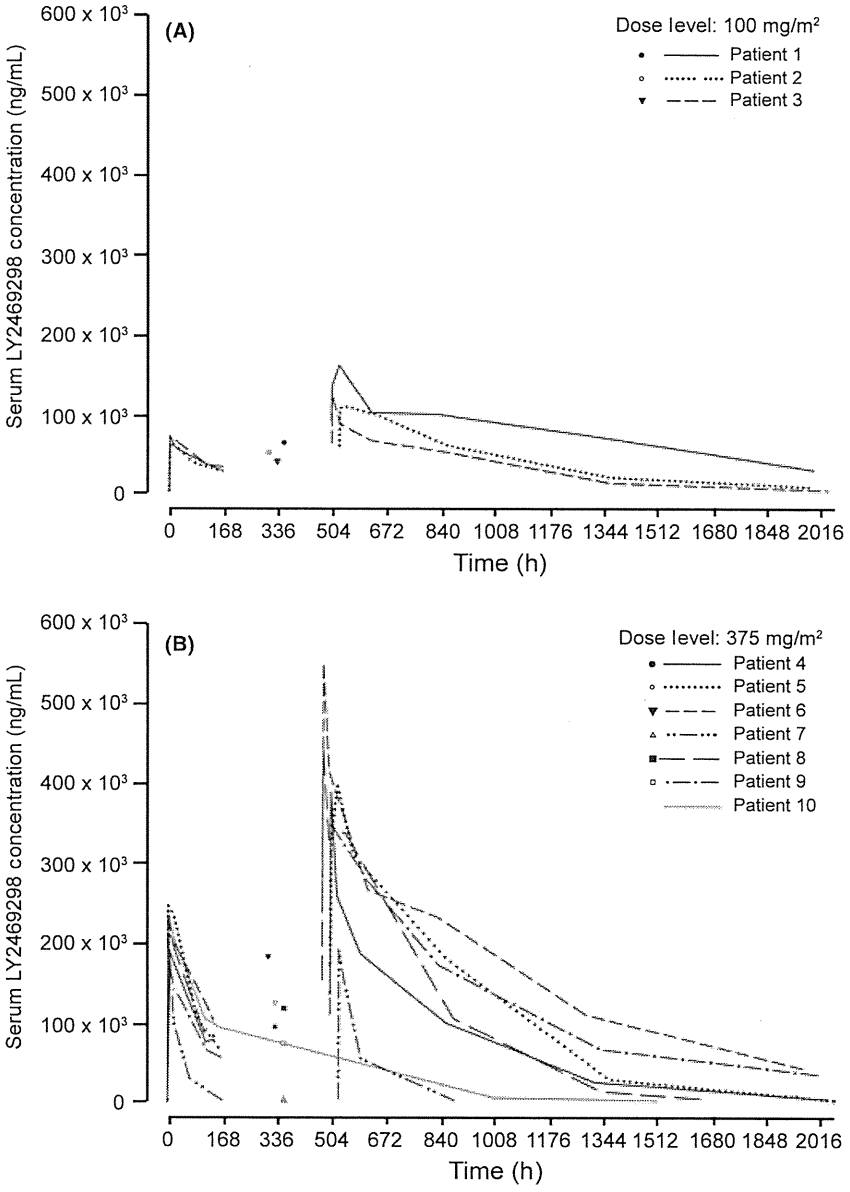

Fig. 2. Time course of serum concentrations of LY2469298 in each patient. (A) 100 mg/m^2 LY2469298 cohort. (B) 375 mg/m^2 LY2469298 cohort. Note that patient 10 discontinued from the study treatment after the first infusion, and patient 7 had a very short elimination half-life.

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fast and characterized by a very short elimination half-life. In the remaining nine patients, LY2469298 was detected in serum until 12 weeks after the last infusion. Observed individual and mean PK parameters for LY2469298 in serum after the last infusion are summarized in Table 5. Individual PK profiles are shown in Figure 2. The area under the concentration versus time curve (AUC) and maximum observed drug concentration (C_{max}) increased with the dose. The mean terminal elimination half-lives ($t_{1/2}$) of the antibody in the 100 and 375 mg/m^2 cohorts were 337 h (199–613) and 238 h (130–499), respectively. Other PK parameters were also similar between the two dose levels and were characterized by a moderate to high interpatient variability. No relationship between response and PK parameters was found.

Discussion

The primary aim of the present study was to investigate the safety and tolerability of LY2469298 administered in four weekly doses of 100 or 375 mg/m^2 to Japanese patients with CD20-positive FL who had received rituximab alone or rituximab-containing regimens. One patient with homozygous *FcγRIIIA-158VV* alleles, six with heterozygous *FcγRIIIA-158VF* alleles, and three with homozygous *FcγRIIIA-158FF* alleles were enrolled. Even though the sample number was small, the frequency of each polymorphism was within the range of previous reports.[15,17]

The safety profile of LY2469298 in Japanese patients was similar to that observed in a previous phase I study in non-Japanese patients.[21] The administration of LY2469298 was well tolerated at the higher dose of 375 mg/m^2 in all patients enrolled. No DLT were observed and the most frequent AE were hematological or infusion related, and all observed AE were manageable. Based on these results, a weekly dose of 375 mg/m^2 was recommended for further studies of LY2469298.

Among the three patients who achieved CR, two had heterozygous *FcγRIIIA-158VF* alleles and one had homozygous *FcγRIIIA-158VV* alleles. It is noteworthy that one patient with heterozygous *FcγRIIIA-158VF* alleles, who had received eight prior regimens, achieved CRu. Regarding the single patient who discontinued the study treatment after the first infusion, a PR was observed at day 148. As hypothesized, CD19-positive peripheral blood B-lymphocytes were depleted in all patients examined.

The PK parameters for LY2469298 when administered to Japanese patients were not remarkably different from those observed in a phase I study of LY2469298 in the United States[21] or those described in the literature for rituximab in Japanese patients.[26–29] For example, LY2469298 was eliminated from serum in a biphasic manner, and both the AUC and C_{max} increased with the dose. One patient with bone marrow involvement and hepatomegaly was noted to have a much shorter elimination half-life of LY2469298 despite treatment at the higher dose of 375 mg/m^2. While in a phase I study of rituximab reported by Maloney *et al.*,[24] one patient who had a large tumor burden, including splenomegaly, also had a shorter elimination half-life of rituximab, within 10 days after the infusion. However, given the limited sample size in the present study, it is difficult to determine the possible cause of the shorter elimination half-life of LY2469298 in this particular patient.

Recent studies suggest that *FcγRIIIA-158VF* polymorphism may have a predictive value for the efficacy of IgG1 antibodies in other tumor types.[30–32] Therefore, antibody engineering of the Fc-region, similar to that used in LY2469298, may be useful to enhance the efficacy of other therapeutic IgG1 antibodies. In the era of personalized medicine, further evaluation of *FcγRIIIA-158VF* polymorphism may be useful to predict and improve the efficacy of therapeutic IgG1 antibodies and ultimately improve the outcomes for patients with FL and other diseases treated with antibodies.

In conclusion, LY2469298, a humanized IgG1 monoclonal antibody with an increased affinity to CD20 and greater ability to mediate ADCC than rituximab *in vitro*, was well tolerated by Japanese patients with previously treated FL (who had received rituximab alone or rituximab-containing regimens). Objective responses were observed in 50% of the patients, mostly consisting of F-carriers. Further studies will determine the exact role of LY2469298 in the treatment of FL patients.

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Disclosure Statement

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References

1 Friedberg JW, Taylor MD, Cerhan JR *et al.* Follicular lymphoma in the United States: first report of the National Lympho Care study. *J Clin Oncol* 2009; **27**: 1202–8.
2 Gujral S, Polampalli SN, Badrinath Y *et al.* Immunophenotyping of mature B-cell non Hodgkin lymphoma involving bone marrow and peripheral blood: critical analysis and insights gained at a tertiary care cancer hospital. *Leuk Lymphoma* 2009; **50**: 1290–300.
3 Reff ME, Carner K, Chambers KS *et al.* Depletion of B cells *in vivo* by a chimeric mouse human monoclonal antibody to CD20. *Blood* 1994; **83**: 435–45.
4 Colombat P, Salles G, Brousse N *et al.* Rituximab (anti-CD20 monoclonal antibody) as single first-line therapy for patients with follicular lymphoma with a low tumor burden: clinical and molecular evaluation. *Blood* 2001; **97**: 101–6.
5 Hainsworth JD, Litchy S, Shaffer DW, Lackey VL, Grimaldi M, Greco FA. Maximizing therapeutic benefit of rituximab: maintenance therapy versus re-treatment at progression in patients with indolent non-Hodgkin's lymphoma–a randomized phase II trial of the Minnie Pearl Cancer Research Network. *J Clin Oncol* 2005; **23**: 1088–95.
6 Czuczman MS, Weaver R, Alkuzweny B, Berlfein J, Grillo-López AJ. Prolonged clinical and molecular remission in patients with low-grade or follicular non-Hodgkin's lymphoma treated with rituximab plus CHOP chemotherapy: 9-year follow-up. *J Clin Oncol* 2004; **22**: 4711–16.
7 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**: 3725–32.
8 Ogura M, Morishima Y, Kagami Y *et al.* Randomized phase II study of concurrent and sequential rituximab and CHOP chemotherapy in untreated indolent B-cell lymphoma. *Cancer Sci* 2006; **97**: 305–12.
9 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**: 1417–23.

10 Marcus R, Imrie K, Solal-Celigny P *et al.* Phase III study of R-CVP compared with cyclophosphamide, vincristine, and prednisone alone in patients with previously untreated advanced follicular lymphoma. *J Clin Oncol* 2008; **26**: 4579–86.
11 Fisher RI, LeBlanc M, Press OW, Maloney DG, Unger JM, Miller TP. New treatment options have changed the survival of patients with follicular lymphoma. *J Clin Oncol* 2005; **23**: 8447–52.
12 Swenson WT, Wooldridge JE, Lynch CF, Forman-Hoffman VL, Chrischilles E, Link BK. Improved survival of follicular lymphoma patients in the United States. *J Clin Oncol* 2005; **23**: 5019–26.
13 van Oers MH, Van Glabbeke M, Giurgea L *et al.* Rituximab maintenance treatment of relapsed/resistant follicular non-Hodgkin's lymphoma: long-term outcome of the EORTC 20981 phase III randomized intergroup study. *J Clin Oncol* 2010; **28**: 2853–8.
14 Tobinai K, Ogura M, Itoh K *et al.* Randomized phase II study of concurrent and sequential combinations of rituximab plus CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) chemotherapy in untreated indolent B-cell non-Hodgkin lymphoma: 7-year follow-up results. *Cancer Sci* 2010; **101**: 2579–85.
15 Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate *in vivo* cytoxicity against tumor targets. *Nat Med* 2000; **6**: 443–6.
16 Koene HR, Kleijer M, Algra J, Roos D, von dem Borne A, de Haas M. FcγRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell FcγRIIIa, independently of the FcγRIIIa-48L/R/H phenotype. *Blood* 1997; **90**: 1109–14.
17 Wu J, Edberg JC, Redecha PB *et al.* A novel polymorphism of FcγRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. *J Clin Invest* 1997; **100**: 1059–70.
18 Cartron G, Dacheux L, Salles G *et al.* Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcγRIIIa gene. *Blood* 2002; **99**: 754–8.
19 Ghielmini M, Rufibach K, Salles G *et al.* Single agent rituximab in patients with follicular or mantle cell lymphoma: clinical and biological factors that are predictive of response and event-free survival as well as the effect of rituximab on the immune system: a study of the Swiss Group for Clinical Cancer Research (SAKK). *Ann Oncol* 2005; **16**: 1675–82.
20 Weng W, Levy R. Two immunoglobulin G fragment C receptor polymorphisms independently predict response to rituximab in patients with follicular lymphoma. *J Clin Oncol* 2003; **21**: 3940–7.
21 Forero A, de Vos S, Pohlman B *et al.* Preliminary results of a phase I study of AME-133v, an Fc-engineered humanized monoclonal antibody, in low-affinity FcγRIIIa patients with previously-treated follicular lymphoma [abstract]. *American Association for Cancer Research 99th Annual Meeting;* April 12-16, 2008; San Diego, CA, USA. Abstract LB-70.
22 Cheson BD, Horning SJ, Coiffier B *et al.* Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. *J Clin Oncol* 1999; **17**: 1244–53.
23 Solal-Céligny P, Roy P, Colombat P *et al.* Follicular lymphoma international prognostic index. *Blood* 2004; **104**: 1258–65.
24 Maloney DG, Liles TM, Czerwinski D *et al.* Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B) in patients with recurrent B-cell lymphoma. *Blood* 1994; **84**: 2457–66.
25 McLaughlin P, Grillo-López AJ, Link BK *et al.* Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol* 1998; **16**: 2825–33.
26 Tobinai K, Kobayashi Y, Narabayashi M *et al.* Feasibility and pharmacokinetic study of a chimeric anti-CD20 monoclonal antibody (IDEC-C2B8, rituximab) in relapsed B-cell lymphoma. *Ann Oncol* 1998; **9**: 527–34.
27 Igarashi T, Kobayashi Y, Ogura M *et al.* Factors affecting toxicity, response and progression-free survival in relapsed patients with indolent B-cell lymphoma and mantle cell lymphoma treated with rituximab: a Japanese phase II study. *Ann Oncol* 2002; **13**: 928–43.
28 Tobinai K, Igarashi T, Itoh K *et al.* Japanese multicenter phase II and pharmacokinetic study of rituximab in relapsed or refractory patients with aggressive B-cell lymphoma. *Ann Oncol* 2004; **15**: 821–30.
29 Tobinai K. Monoclonal antibody therapy for B-cell lymphoma: clinical trials of an anti-CD20 monoclonal antibody for B-cell lymphoma in Japan. *Int J Hematol* 2002; **76**: 411–19.
30 Bibeau F, Lopez-Crapez E, Di Fiore F *et al.* Impact of FcγRIIa-FcγRIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. *J Clin Oncol* 2009; **27**: 1122–9.
31 López-Albaitero A, Lee SC, Morgan S *et al.* Role of polymorphic Fc gamma receptor IIIa and EGFR expression level in cetuximab mediated, NK cell dependent *in vitro* cytotoxicity of head and neck squamous cell carcinoma cells. *Cancer Immunol Immunother* 2009; **58**: 1853–64.
32 Musolino A, Naldi N, Bortesi B *et al.* Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of trastuzumab-based therapy in patients with HER-2/neu-positive metastatic breast cancer. *J Clin Oncol* 2008; **26**: 1789–96.

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Feasibility and pharmacokinetic study of bendamustine hydrochloride in combination with rituximab in relapsed or refractory aggressive B cell non-Hodgkin's lymphoma[6]

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Although bendamustine plus rituximab has demonstrated efficacy in indolent B cell non-Hodgkin's lymphoma (B-NHL), data for this combination in aggressive B-NHL are extremely limited. The present dose-escalation study evaluated the safety, efficacy, and pharmacokinetics of bendamustine hydrochloride in combination with rituximab in patients with relapsed/refractory, CD20-positive, aggressive B-NHL. Patients received rituximab 375 mg/m^2, i.v., on Day 1 and bendamustine at either 90 (Cohort 1) or 120 mg/m^2 (Cohort 2), i.v., on Days 2 and 3 of a 21-day cycle. The primary endpoint was the proportion of patients experiencing dose-limiting toxicity (DLT). Secondary endpoints were adverse events (AE), the overall response rate (ORR), and pharmacokinetic parameters. Nine patients received rituximab plus bendamustine: three in Cohort 1 and six in Cohort 2. Histologies included diffuse large B cell lymphoma (n = 5), mantle cell lymphoma (n = 2), and transformed lymphoma (n = 2). No DLT was observed at either dose level. Grade 3/4 hematologic AE included lymphocytopenia, leukocytopenia, and neutropenia (n = 9 each; 100%), and thrombocytopenia (n = 2; 22%). No Grade 3/4 gastrointestinal AE were reported. The ORR was 33% (one partial response) in Cohort 1 and 100% (five complete and one partial response) in Cohort 2. The maximum drug concentration and area under the blood concentration–time curve for bendamustine increased dose dependently, with time to maximum blood concentration = 1.0 h in both cohorts; these pharmacokinetic data were similar to those reported previously for single-agent bendamustine in patients with indolent B-NHL. In conclusion, bendamustine 120 mg/m^2 plus rituximab 375 mg/m^2 was feasible and generally well tolerated, with promising efficacy in relapsed or refractory aggressive B-NHL. (*Cancer Sci* 2011; 102: 1687–1692)

Bendamustine is a benzimidazole nitrogen mustard compound with structural similarities to alkylating agents and purine analogs. It has been shown to act as a bifunctional alkylator, forming both inter- and intrastrand DNA cross-links, which produce DNA damage that is more extensive and more durable than that caused by cyclophosphamide or bis-chloronitrosourea (BCNU; carmustine) but similar to that of melphalan.[1]

Bendamustine has a unique mechanism of action. In a study of 60 human tumor cell lines, bendamustine showed a pattern of antitumor activity that was distinct from other alkylating compounds tested.[2] Unlike other alkylators, bendamustine was able to induce cell death through both apoptosis and mitotic catastrophe.[2] Additional *in vitro* studies have shown a lack of cross-resistance between bendamustine and other chemotherapeutic agents, including other alkylators.[1]

Bendamustine demonstrates clinical activity against a variety of human cancers, including non-Hodgkin's lymphoma (NHL).[3–11] The efficacy of bendamustine (120 mg/m^2) in indolent B cell NHL (B-NHL) was shown in a US multicenter, single-arm study of 100 patients with rituximab-refractory disease, which resulted in an overall response rate (ORR) of 75% (17% complete responses [CR]) and a median duration of response of 9.2 months.[12] We also evaluated the efficacy and safety of bendamustine in Phase I and Phase II studies in Japanese patients with relapsed or refractory indolent B-NHL and mantle cell lymphoma (MCL) and reported ORR of 89% (8/9) and 91% (63/69), respectively.[9,10] In those two studies, objective responses were observed for all patients with MCL (n = 12).[9,10]

Two Phase II studies have demonstrated the efficacy of single-agent bendamustine in small numbers of patients with relapsed or refractory aggressive NHL. One study reported responses in eight of 18 (44%) patients with diffuse large B cell lymphoma (DLBCL; n = 12) or other aggressive lymphomas, including 10 patients who were refractory to prior chemotherapies.[7] The second study reported responses in 10 of 15 patients (67%) with rituximab-refractory, transformed B-NHL, with a median response duration of 2.3 months.[11]

Rituximab is a monoclonal antibody that binds specifically to surface CD20 on human B lymphocytes, leading to B cell depletion. *In vitro* combination therapy with rituximab and bendamustine induces apoptosis in CD20-positive follicular lymphoma and NHL cell lines and enhances the antitumor activity of rituximab.[13] The efficacy and tolerability of the bendamustine–rituximab combination has been demonstrated in two Phase II studies of patients with relapsed/refractory indolent B-NHL. In the first (German) study, 57 of 63 patients (90%) achieved an objective response, including CR in 60% of patients, and the median progression-free survival was 24 months.[8] In the second (US) study in 67 patients with relapsed and refractory MCL and low-grade NHL, an ORR of 92% was reported, including CR in 55% of patients.[14] In patients with MCL, the ORR ranged from 75% (50% CR)[8] to 92% (59% CR).[14]

The combination of cyclophosphamide, doxorubicin, vincristine, prednisone, and rituximab (R-CHOP) is the standard of care in newly diagnosed, aggressive B-NHL, including

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DLBCL.[15,16] Because bendamustine does not demonstrate cross-resistance with these drugs, it was hypothesized that the combination of bendamustine and rituximab would be effective in previously treated, aggressive B-NHL.

The primary objectives of the present study were to: (i) evaluate the safety of bendamustine in patients with relapsed or refractory aggressive B-NHL when administered via i.v. infusion for 2 consecutive days in combination with rituximab; and (ii) determine a bendamustine dose for subsequent Phase II studies. The secondary objectives of the present study were to investigate the antitumor effects of bendamustine plus rituximab and to describe the pharmacokinetic profile of unchanged bendamustine.

Materials and Methods

Trial design and endpoints. A multicenter, open-label, dose-escalation study was conducted between December 2008 and February 2010. The study was performed in compliance with the Declaration of Helsinki and the Good Clinical Practice Ministerial Ordinance and Guidelines for Clinical Evaluation of Anti-Tumor Drugs, and was approved by the institutional review board at each participating institution. All patients provided written informed consent prior to participating in the study.

The primary endpoint was the number of patients who experienced dose-limiting toxicity (DLT), defined as any adverse event (AE) reported during the first treatment cycle that had a possible causal relationship with the study drugs and met any of the following criteria: (i) Grade 4 neutropenia (neutrophil count <500 cells/mm^3) with a fever of 38°C or higher for more than 1 week; (ii) platelet count <10 000 cells/mm^3 or a hemorrhagic tendency requiring platelet transfusion; (iii) other Grade 4 hematologic toxicity, excluding decreased lymphocytes and changes in the leukocyte differential count; and (iv) other Grade ≥3 non-hematologic toxicity. Secondary endpoints included the frequency of AE, the ORR, the CR rate, and pharmacokinetic parameters.

Patient eligibility. Patients 20–75 years of age with measurable, histopathologically confirmed, CD20-positive, aggressive B-NHL (DLBCL, MCL, transformed lymphoma, or Grade 3 follicular lymphoma) were eligible for inclusion in the study if they had failed to achieve a CR or had relapsed after achieving a CR or partial response (PR) with one to two prior chemotherapies or antibody therapies. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0–1, a life expectancy ≥3 months, and functional major organs (defined as neutrophils ≥1500 cells/mm^3, platelets ≥100 000 cells/mm^3, alanine aminotransferase [ALT] and aspartate aminotransferase [AST] <2.5-fold the upper limit of normal [×ULN], total bilirubin and serum creatinine <1.5 × ULN, arterial oxygen partial pressure ≥65 mmHg, and no abnormal electrocardiogram findings requiring treatment).

Patients were excluded from the study for any of the following reasons: cancer treatment within the 3 weeks prior to the study; primary central nervous system lymphoma; serious active infection; serious hepatic, renal, cardiac, gastrointestinal, or pulmonary disorders; positive status for hepatitis B surface antigen, hepatitis C virus antibody, or human immunodeficiency virus antibody; other active cancer or a history of another malignancy in the preceding 5 years (except for basal cell carcinoma of the skin, squamous cell carcinoma, and carcinoma *in situ* of the cervix); autoimmune hemolytic anemia; serious hemorrhagic tendency; pregnancy or lactation; or unwillingness to use birth control. Patients were also excluded if they had ever received bendamustine or radioimmunotherapy; had received a cytokine preparation or blood transfusion within 2 weeks prior to screening, or an investigational drug within 3 months of enrollment; displayed symptoms of allergy or intolerance to rituximab, bendamustine, or analogous drugs, or premedications; or were receiving sulfamethoxazole–trimethoprim combination therapy or acyclovir to prevent opportunistic infections.

Treatment and dose escalation. Each 21-day treatment cycle consisted of rituximab (375 mg/m^2, i.v.) administered on Day 1 and bendamustine (90 or 120 mg/m^2, i.v.; Cohorts 1 and 2, respectively) administered over 60 min on Days 2 and 3, followed by an 18-day observation period. Patients received a maximum of six cycles. For safety reasons, hospitalization was required during the first cycle.

Three patients were to be enrolled in Cohort 1. If any DLT were observed in one or two patients, an additional three patients were enrolled into this cohort. If a DLT was observed in none of three or in two or fewer of six patients in Cohort 1, enrollment in Cohort 2 was initiated. An independent safety and data monitoring committee reviewed the data recorded from Cohort 1 following completion of the first cycle and provided guidance regarding advancement to Cohort 2. In Cohort 2, enrollment was suspended when the third patient was enrolled. Following confirmation of two or fewer DLT in the first cycle, three additional patients were enrolled in this cohort.

Rationale for doses and treatment schedule. The regimens used in the present study were based on the efficacy and safety results of previous studies in patients with indolent B-NHL and MCL[8–10,14] and transformed[11] or aggressive B-NHL.[7]

Supportive therapy. During each cycle, oral acetaminophen (400 mg) and chlorpheniramine maleate (2 mg) were administered prior to rituximab to prevent or alleviate infusion reactions. In addition, dexamethasone (20 mg, i.v.) was administered before bendamustine or rituximab (Days 1–3), followed by oral dexamethasone (10 mg) once daily on Days 4 and 5 of each cycle. Granisetron hydrochloride (3 mg) was administered i.v. once daily before bendamustine administration (Days 2 and 3) and followed by oral granisetron (2 mg) once daily on Days 4 and 5 of each cycle. Hydration and/or alkalization were recommended for patients at risk of tumor lysis syndrome. Granulocyte colony-stimulating factor was allowed during the first treatment cycle for patients with confirmed Grade 3 or greater neutropenia, and on Day 4 or later of Cycles 2–6. Opportunistic infection prophylaxis with oral sulfamethoxazole–trimethoprim and oral acyclovir 200 mg daily was allowed in Cycles 2–6.

Criteria for study withdrawal. Patient participation in the study was discontinued for any of the following reasons: failure to meet criteria for proceeding to the next treatment cycle (defined as neutrophils ≥1000 cells/mm^3, platelets ≥75 000 cells/mm^3, ALT and AST <5 × ULN, total bilirubin <2.0 mg/dL, serum creatinine <2.0 mg/dL, and no persistent Grade ≥ 3 AE except leukocytopenia or lymphocytopenia) within 36 days after the start of the last treatment cycle; withdrawal of patient consent; deviation from the study protocol; inability to receive study drug due to AE or disease progression; death; loss to follow-up; study termination; pregnancy; or other reasons at the discretion of the investigator.

Safety and efficacy assessment. Physical examination and laboratory testing were conducted on study drug administration days and weekly during the observation period. Any AE observed were graded according to Common Terminology Criteria for Adverse Events version 3.0.[17] Computed tomography (CT) and positron emission tomography (PET) scans were performed at the time of screening, third cycle, last cycle, and discontinuation. Tumor response was assessed based on CT/PET data by the investigator as well as by an extramural central review committee. Patients' best responses were categorized as CR, PR, stable disease, or progressive disease according to the Revised Response Criteria for Malignant Lymphoma.[18]

Pharmacokinetic analysis. Blood samples were collected on Day 2 of the first cycle prior to the start of the infusion, 30 min after the start of the infusion, at completion of the infusion, and

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then 30 min and 1 and 2 h thereafter. Plasma concentrations of bendamustine were measured by high-performance liquid chromatography tandem mass spectrometry. The maximum drug concentration (C_{max}), time to maximum blood concentration (t_{max}), area under the blood concentration–time curve (AUC), and half-life ($t_{1/2}$) of unchanged bendamustine were calculated by non-compartmental analysis (Model 2) using WinNonlin version 5.0.1 software (Pharsight, Mountain View, CA, USA).

Statistical analysis. Baseline patient and disease characteristics were summarized using descriptive statistics. The incidence of DLT and 90% confidence intervals were calculated based on binomial probability for each treatment group. Summary statistics were calculated for hematologic toxicities and non-hematologic toxicities. The ORR was calculated as the proportion of patients who achieved a CR or PR.

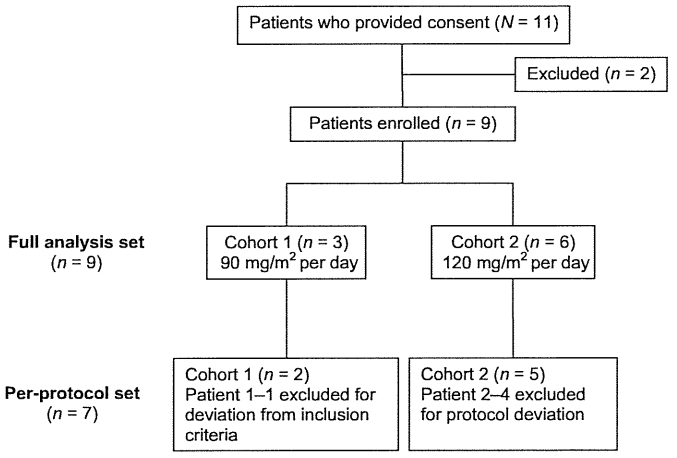

Fig. 1. Patient disposition in the study arms.

Results

Patient disposition and baseline characteristics. Nine patients were enrolled at four study centers: three received bendamustine at 90 mg/m^2 (Cohort 1) and six received bendamustine at 120 mg/m^2 (Cohort 2). These patients constituted the full analysis set (FAS; Fig. 1). There were two protocol violations. One patient in Cohort 1 (Patient 1-1) deviated from the inclusion criteria: this patient was enrolled based on a clinical diagnosis of DLBCL, but upon rebiopsy after completion of study treatment, the patient was found to have adenocarcinoma. One patient in Cohort 2 (Patient 2-4) did not meet the criteria to begin the next treatment cycle: this patient had persistent Grade 3 hypokalemia after Cycle 3, but proceeded to start the fourth cycle. Treatment in this patient was discontinued during the fourth cycle. These two patients were excluded from the per-protocol set.

The median age of enrolled patients was 65 years (range 56–74 years); five patients were male and four were female. Baseline characteristics are presented in Table 1. Disease histologies included DLBCL, MCL, and transformed lymphoma. Eight patients (89%) had received prior treatment with R-CHOP and all patients had received prior rituximab.

Safety. Safety analyses were conducted on the FAS. A total of 38 treatment cycles was administered (10 in Cohort 1 and 28 in Cohort 2); all patients completed three or more treatment

Table 1. Baseline characteristics of patients with aggressive B cell non-Hodgkin's lymphoma treated with bendamustine plus rituximab

Patient no.	Histology†	Disease stage‡	IPI risk group	Prior treatment
Dose level 1: 90 mg/m^2, i.v., bendamustine plus 375 mg/m^2, i.v., rituximab				
1-1	DLBCL	III	High-intermediate	R-CHOP × 8 CHASER × 2
1-2	DLBCL	IV	Low-intermediate	R-CHOP × 8 CHASE × 2
1-3	DLBCL	I	Low-intermediate	R-CHOP × 6
Dose level 2: 120 mg/m^2, i.v., bendamustine plus 375 mg/m^2, i.v., rituximab				
2-1	DLBCL	II	Low	R-CHOP × 8
2-2	DLBCL	I	Low	R-CHOP × 8
2-3	TL	II	Low-intermediate	R-CHOP × 3 R-CMOPP × 8
2-4	MCL	II	Low	R-CHOP × 6
2-5	TL	III	Low-intermediate	R-CHOP × 8 R-F × 3
2-6	MCL	IV	Low-intermediate	R-HyperCVAD/MA × 4

†World Health Organization classification.[19] ‡Ann Arbor classification.[20] CHASE, cyclophosphamide, cytarabine, etoposide, and dexamethasone; CHASER, cyclophosphamide, cytarabine, etoposide, dexamethasone, and rituximab; DLBCL, diffuse large B cell lymphoma; IPI, International Prognostic Index; MCL, mantle cell lymphoma; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CMOPP, rituximab plus cyclophosphamide, vincristine, procarbazine, and prednisone; R-F, rituximab plus fludarabine; R-HyperCVAD/MA, rituximab plus fractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, alternating with methotrexate and cytarabine; TL, transformed lymphoma.

Table 2. Dose-limiting toxicity and Grade 3/4 toxicity associated with bendamustine plus rituximab in patients with aggressive B cell non-Hodgkin's lymphoma

	No. events	
	90 mg/m^2 bendamustine (n = 3)	120 mg/m^2 bendamustine (n = 6)
DLT (Cycle 1†)		
Grade 4 neutropenia‡	0	0
Platelets <10 000 cells/mm^3§	0	0
Other Grade 4 hematologic toxicity¶	0	0
Grade ≥3 non-hematologic toxicity	0	0
Grade 3/4 events in all cycles		
Lymphocytopenia, Grade 4	3	6
Leukocytopenia, Grade 3/4	3/0	5/1
Neutropenia, Grade 3/4	2/1	3/3
Decrease in CD4, Grade 3/4	2/1	2/3
Thrombocytopenia, Grade 3	0	2
Febrile neutropenia, Grade 3	1	0
Decreased IgA, Grade 3	1	0
Decreased IgG, Grade 3	1	0
Increased LDH, Grade 3	1	0
Hypokalemia, Grade 3	0	1
Hyperuricemia, Grade 3	0	1
Lower back pain, Grade 3	0	1

†Dose-limiting toxicity (DLT) was assessed during Cycle 1 only. ‡Grade 4 neutropenia lasting >1 week and accompanied by fever (38°C or higher). §Grade 4 thrombocytopenia or hemorrhage requiring platelet transfusion. ¶Any other Grade 4 hematologic toxicity excluding lymphocytopenia and differential white blood cell count (%). LDH, lactate dehydrogenase.

cycles and two patients (both in Cohort 2) completed six cycles. All patients experienced at least one delay of treatment to allow for recovery from AE (most commonly neutrophil counts <1000 cells/mm^3), in compliance with the protocol. In Cohort 1, the median cycle duration was 22 days (range 21–28 days) for Cycle 1, 21 days (range 21–35) for Cycle 2, and 28 days for Cycle 3 (n = 1). In Cohort 2, the median cycle duration was 21 days (range 21–28 days) for Cycles 1, 2, and 4, and 28 days (range 21–41 days) for Cycles 3 and 5.

Although all nine patients experienced AE, bendamustine was generally well tolerated. No DLT was observed with either dose of bendamustine (Table 2). No deaths were reported. Grade 3/4 hematologic AE included lymphocytopenia, leukocytopenia, neutropenia (nine of nine patients each), and thrombocytopenia (two of nine patients). No Grade 3/4 gastrointestinal events or fatigue were reported.

One case of Grade 1 oral candidiasis was recorded in Cohort 2. Incidences of nausea and vomiting were low with granisetron and dexamethasone prophylaxis (in Cohort 1, one case of Grade 2 nausea; in Cohort 2, three cases of Grade 1 nausea and one case of Grade 1 vomiting). Two serious AE were reported in a 65-year-old male patient with MCL in Cohort 2, consisting of Grade 1 fatigue and Grade 2 fever without neutropenia. These events were thought to be caused by incidental infection and were considered serious because the patient required hospitalization for i.v. hydration and antibiotic therapy. Both events resolved with treatment and were considered by the investigator to be unrelated to the study drugs.

Efficacy. Efficacy analyses were conducted on the FAS. The ORR, as determined by central review, were 33% and 100% in Cohorts 1 and 2, respectively (Table 3). A CR was achieved in five patients in Cohort 2 (two DLBCL, two MCL, one transformed lymphoma) and a PR was achieved in one patient with DLBCL in Cohort 1 and in one patient with transformed lymphoma in Cohort 2. Of the four patients with pathologically confirmed *de novo* DLBCL, 3 (75%) achieved an objective response, including two CR and one PR.

Pharmacokinetics. Pharmacokinetic analyses were conducted on the FAS (Table 4). Plasma levels of bendamustine peaked upon completion of infusion (t_{max} = 1 h; Fig. 2). The C_{max} and AUC increased in a dose-dependent manner. Unchanged bendamustine was rapidly eliminated from the circulation, and the mean elimination $t_{½}$ was similar in Cohorts 1 and 2 (0.36 and 0.32 h, respectively).

Table 3. Treatment response according to bendamustine dose and histology in patients (N = 9; full analysis set) treated with bendamustine plus rituximab for aggressive B cell non-Hodgkin's lymphoma

Dose (mg/m^2)	Histology	Best response† (n)					ORR (%)	CR (%)
		N	CR	PR	SD	PD		
90	DLBCL	3	0	1	1‡	1	33	0
120	DLBCL	2	2	0	0	0	100	100
	MCL	2	2	0	0	0	100	100
	TL	2	1	1	0	0	100	50
	Cohort total	6	5	1	0	0	100	83
All patients		9	5	2	1	1	78	56

†As determined by an extramural central review committee. ‡This patient was determined to be pathologically ineligible after completing the study. Among the eight patients with pathologically eligible disease, the overall response rate (ORR) and complete response (CR) rate were 88% and 63%, respectively. DLBCL, diffuse large B cell lymphoma; MCL, mantle cell lymphoma; PD, progressive disease; PR, partial response; SD, stable disease; TL, transformed lymphoma.

Discussion

The present study is the first clinical trial to evaluate the combination of bendamustine and rituximab in patients with relapsed/refractory aggressive B-NHL including DLBCL. Our results support the safety and tolerability of both bendamustine doses tested in combination with rituximab. All nine patients experienced AE; however, no DLT was observed and the two serious AE (Grade 1 fatigue and Grade 2 fever without neutropenia) were considered unrelated to study treatment.

Our earlier Phase I dose-escalation and pharmacokinetic study[9] of bendamustine monotherapy using the same doses (90 and 120 mg/m^2) in refractory/relapsed indolent B-NHL and MCL also did not identify a maximum tolerated dose. Based on the findings from this earlier Phase I study and other studies,[9–12] the 120 mg/m^2 dose of bendamustine was selected for further evaluation in Phase II trials. A multicenter Phase II study using this dose of bendamustine showed that it was effective with an acceptable safety profile.[10]

The safety and tolerability of bendamustine plus rituximab observed in the present study were consistent with observations reported in larger Phase II trials evaluating this drug combination in patients with MCL and indolent B-NHL.[8,14] Most common non-hematologic AE were gastrointestinal in nature and were generally mild in both cohorts. The use of granisetron and dexamethasone minimized the incidence of nausea and vomiting in the present study.

Table 4. Pharmacokinetic parameters of unchanged bendamustine in patients treated with bendamustine plus rituximab for aggressive B cell non-Hodgkin's lymphoma

Bendamustine dose	*N*	Patient no.	Response	C_{max} (μg/mL)	t_{max} (h)	$t_{½}$ (h)	AUC (μg h/mL)
90 mg/m^2	3	1-1	SD	4.9	1.0	0.42	5.9
		1-2	PD	4.1	1.0	0.32	4.9
		1-3	PR	2.4	1.0	0.33	2.8
Mean ± SD				3.8 ± 1.3	1 ± 0	0.36 ± 0.06	4.5 ± 1.6
120 mg/m^2	6	2-1	CR	4.8	1.0	0.34	5.7
		2-2	CR	7.8	1.0	0.29	8.5
		2-3	PR	5.7	1.0	0.39	7.6
		2-4	CR	6.2	1.0	0.26	6.5
		2-5	CR	3.9	1.0	0.42	4.4
		2-6	CR	4.1	1.0	0.23	4.2
Mean ± SD				5.4 ± 1.5	1 ± 0	0.32 ± 0.07	6.1 ± 1.7

AUC, area under the curve; B-NHL, B cell non-Hodgkin lymphoma; C_{max}, maximum concentration; CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease; t_{max}, time to maximum concentration; $t_{½}$, half-life.

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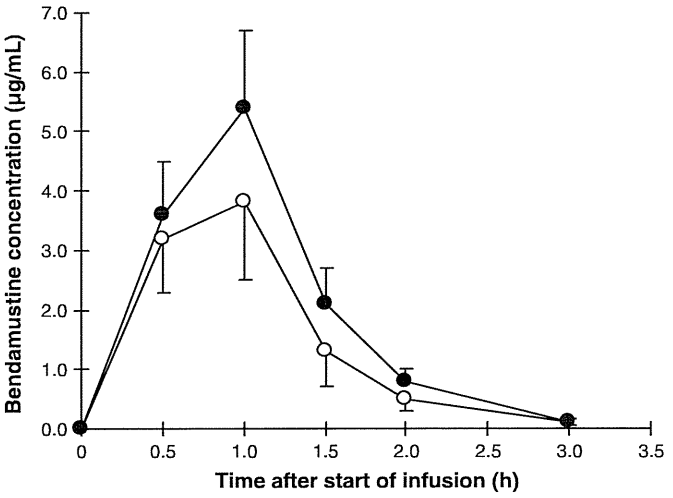

Fig. 2. Mean (±SD) plasma bendamustine concentrations following administration of either 90 (○) or 120 mg/m^2 (●) bendamustine (n = 3 and 6, respectively) to patients with aggressive B cell non-Hodgkin's lymphoma. Each i.v. infusion was administered over 1 h. Peak plasma concentrations were 3.8 ± 1.3 μg/mL following infusion of 90 mg/m^2 bendamustine and 5.4 ± 1.5 μg/mL following infusion of 120 mg/m^2 bendamustine. Concentrations <0.0005 μg/mL were recorded as 0 μg/mL.

The pharmacokinetic profile of unchanged bendamustine was comparable to that reported previously in Japanese patients with indolent B-NHL and MCL.[9] In that study, 120 mg/m^2 bendamustine produced a mean C_{max} of 8.6 ± 4.5 μg/mL, compared with a mean value of 5.4 ± 1.5 μg/mL in the present study. In both studies, there was considerable variation in C_{max} between patients; although mean C_{max} values differed slightly, the ranges observed overlapped. Furthermore, the pharmacokinetic profile observed in Japanese patients is similar to that reported for patients in studies conducted in Europe and the US, suggesting little or no effect of ethnicity on the pharmacokinetics of bendamustine.[21–24]

In an *in vitro* study in lymphoma cell lines, the IC_{50} of bendamustine was identified as 20 μM or 7.2 μg/mL.[25] In the present clinical study, the maximum plasma concentration ranged from 54% to 108% of this inhibitory concentration in Cohort 2; the observed ORR of 100% in this group suggests that such plasma concentrations are associated with antitumor activity in combination with rituximab.

Although no definitive conclusions can be drawn from the findings of the present study owing to the limitations of a small sample size and a variety of histologic subtypes, the preliminary efficacy findings are promising. Bendamustine plus rituximab elicited objective responses in both cohorts, with 100% of patients responding at the higher (120 mg/m^2) dose level. In addition, a CR was observed in 83% of patients and occurred across all disease histologies included.

Among patients with pathologically confirmed *de novo* DLBCL, the ORR was 75% (3/4), including a CR rate of 50%. In patients with relapsed DLBCL, autologous stem cell transplant (ASCT) following high-dose chemotherapy is the standard of care for patients responding to salvage therapy; however, no chemotherapy regimen has emerged as a preferred salvage regimen.[26,27] The identification of salvage regimens with increased response rates is of significant clinical interest, particularly in patients who have received prior rituximab, among whom response rates are approximately 50%.[27] Furthermore, patients >65 years or those with major organ dysfunction are considered ineligible for ASCT and no standard of care exists for these patients. Among the five patients with *de novo* DLBCL enrolled in the present study, four were considered ineligible for and one declined ASCT. The response rate observed with bendamustine plus rituximab in these patients supports continued investigation of this combination therapy in this patient group.

Bendamustine plus rituximab appeared safe and well tolerated at both dose levels evaluated. Furthermore, although no conclusions can be drawn about efficacy, this combination demonstrated preliminary activity warranting further exploration in patients with aggressive disease. In consideration of the tolerability of this regimen, administration of the highest dose level evaluated (i.e. 120 mg/m^2 bendamustine plus 375 mg/m^2 rituximab) appears feasible in this patient population and should be evaluated further in subsequent Phase II trials. Based on the results of the present study, a multicenter, international Phase II study of bendamustine plus rituximab in patients with relapsed or refractory DLBCL is currently underway (clinicaltrials.gov ID no. NCT01118845).

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Disclosure Statement

The authors have no conflicts of interest to report.

References

1 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**: 415–21.
2 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**: 309–17.
3 Schmittel A, Knodler M, Hortig P, Schulze K, Thiel E, Keilholz U. Phase II trial of second-line bendamustine chemotherapy in relapsed small cell lung cancer patients. *Lung Cancer* 2007; **55**: 109–13.
4 Pönisch W, Mitrou PS, Merkle K *et al.* Treatment of bendamustine and prednisone in patients with newly diagnosed multiple myeloma results in superior complete response rate, prolonged time to treatment failure and improved quality of life compared to treatment with melphalan and prednisone: a randomized phase III study of the East German Study Group of Hematology and Oncology (OSHO). *J Cancer Res Clin Oncol* 2006; **132**: 205–12.
5 von Minckwitz G, Chernozemsky I, Sirakova L *et al.* Bendamustine prolongs progression-free survival in metastatic breast cancer (MBC): a phase III prospective, randomized, multicenter trial of bendamustine hydrochloride, methotrexate and 5-fluorouracil (BMF) versus cyclophosphamide, methotrexate and 5-fluorouracil (CMF) as first-line treatment of MBC. *Anticancer Drugs* 2005; **16**: 871–7.
6 Knop S, Straka C, Haen M, Schwedes R, Hebart H, Einsele H. The efficacy and toxicity of bendamustine in recurrent multiple myeloma after high-dose chemotherapy. *Haematologica* 2005; **90**: 1287–8.
7 Weidmann E, Kim SZ, Rost A, Schuppert H, Seipelt G, Hoelzer D, Mitrou PS. Bendamustine is effective in relapsed or refractory aggressive non-Hodgkin's lymphoma. *Ann Oncol* 2002; **13**: 1285–9.
8 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**: 3383–9.
9 Ogura M, Uchida T, Taniwaki M *et al.* Phase I and pharmacokinetic study of bendamustine hydrochloride in relapsed or refractory indolent B-cell non-Hodgkin lymphoma and mantle cell lymphoma. *Cancer Sci* 2010; **101**: 2054–8.
10 Ohmachi K, Ando K, Ogura M *et al.* Multicenter phase II study of bendamustine for relapsed or refractory indolent B-cell non-Hodgkin lymphoma and mantle cell lymphoma. *Cancer Sci* 2010; **101**: 2059–64.

11 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**: 204–10.
12 Kahl B, Carbone PP, Bartlett N, Leonard J, Chen L. Bendamustine is effective therapy in patients with rituximab-refractory indolent B-cell non-Hodgkin's lymphoma: results from a multicenter study. *Cancer* 2010; **116**: 106–14.
13 Chow KU, Sommerlad WD, Boehrer S *et al.* Anti-CD20 antibody (IDEC-C2B8, rituximab) enhances efficacy of cytotoxic drugs on neoplastic lymphocytes *in vitro*: role of cytokines, complement, and caspases. *Haematologica* 2002; **87**: 33–43.
14 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**: 4473–9.
15 Coiffier B, Lepage E, Briere J *et al.* CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med* 2002; **346**: 235–42.
16 Pfreundschuh M, Trumper L, Osterborg A *et al.* CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. *Lancet Oncol* 2006; **7**: 379–91.
17 National Cancer Institute, US National Institutes of Health. *Cancer Therapy Evaluation Program: Common Terminology Criteria for Adverse Events v3.0 (CTCAE)*. Bethesda: National Cancer Institute, US National Institutes of Health, 2006. [Cited 25 Jul 2010.] Available from URL: http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf.
18 Cheson BD, Pfistner B, Juweid ME *et al.* Revised response criteria for malignant lymphoma. *J Clin Oncol* 2007; **25**: 579–86.
19 Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *World Health Organization Classification of Tumors, Pathology and Genetics of Tumors of Hematopoietic and Lymphoid Tissues*, 3rd edn. Geneva, Switzerland: International Agency for Research on Cancer (IARC) press; 2001: 119–87.
20 Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the Committee on Hodgkin's Disease Staging Classification. *Cancer Res* 1971; **31**: 1860–1.
21 Owen JS, Melhem M, D'Andrea D, Darwish M. Bendamustine pharmacokinetic profile and exposure–response relationships in patients with indolent non-Hodgkin's lymphoma. *Cancer Chemother Pharmacol* 2010; **66**: 1039–49.
22 Matthias M, Preiss R, Sohr R, Possinger K. Pharmacokinetics of bendamustin in patients with malignant tumors (Abstract). *Proc Am Soc Clin Oncol* 1995; **14**: 458.
23 Rasschaert M, Schrijvers D, Van den Brande J, Dyck J, Bosmans J, Merkle K, Vermorken JB. A phase I study of bendamustine hydrochloride administered day 1 + 2 every 3 weeks in patients with solid tumours. *Br J Cancer* 2007; **96**: 1692–8.
24 Rasschaert M, Schrijvers D, Van den Brande J, Dyck J, Bosmans J, Merkle K, Vermorken JB. A phase I study of bendamustine hydrochloride administered once every 3 weeks in patients with solid tumors. *Anticancer Drugs* 2007; **18**: 587–95.
25 Leoni LM, Bailey B, Niemeyer CC *et al. In vitro* and *ex vivo* activity of SDX-105 (bendamustine) in drug-resistant lymphoma cells (Abstract). *AACR Meeting Abstracts* 2004; **45**: Abstract 1215.
26 Philip T, Guglielmi C, Hagenbeek A *et al.* Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med* 1995; **333**: 1540–5.
27 Gisselbrecht C, Glass B, Mounier N *et al.* Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol* 2010; **28**: 4184–90.

Appendix

Institutions participating in the Japanese Bendamustine Lymphoma Study Group: National Cancer Center Hospital, Tokai University School of Medicine, Nagoya Daini Red Cross Hospital, and Kyoto Prefectural University of Medicine; protocol committee members: Drs Kenichi Ishizawa (Tohoku University Hospital), Kensei Tobinai, Takashi Watanabe, Kiyoshi Ando, Michinori Ogura, and Masafumi Taniwaki; independent data and safety monitoring committee members: Drs Toshiyuki Takagi (Kimitsu Chuo Hospital), Hirokazu Murakami (Gunma University), and Noriko Usui (Jikei University School of Medicine Dai-san Hospital); and CT/PET central review committee members: Drs Takashi Terauchi (National Cancer Center Hospital), Ukihide Tateishi (Yokohama City University), and Mitsuaki Tatsumi (Osaka University).

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ORIGINAL ARTICLE

Bulky disease has an impact on outcomes in primary diffuse large B-cell lymphoma of the breast: a retrospective analysis at a single institution

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Abstract

Objectives: Primary breast lymphoma (PBL) is rare, and its clinical behavior and standard initial treatment are not yet established. *Methods:* We retrospectively analyzed the clinicopathological features and treatment outcomes of 14 patients with primary breast diffuse large B-cell lymphoma. *Results:* There were nine patients with stage IE and five with stage IIE disease. The median largest tumor diameter was 4.5 cm, and five patients had bulky disease >5 cm. The complete response rate was 94%. However, the 5-year progression-free survival rate was 52% with a median follow-up of 5.2 years. Patients with bulky disease had an unfavorable prognosis. All five patients with bulky disease progressed or relapsed. Of the four patients that recurred in the central nervous system (CNS), three had bulky disease although some received rituximab. There were no CNS recurrences in the three patients who received CNS prophylaxis. All eight patients who responded to radiotherapy (RT) did not have recurrences in the ipsilateral breast, although one patient with bulky disease relapsed in the adjacent regional lymph nodes within the RT field despite immunochemotherapy. *Conclusions:* Patients with bulky disease had a poorer prognosis and recurred frequently in the CNS. CNS prophylaxis might yield better outcomes, but a larger, prospective trial is needed to elucidate the optimal initial treatment of PBL in the rituximab era.

Key words diffuse large B-cell lymphoma; primary breast lymphoma; CNS prophylaxis

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Primary breast lymphoma (PBL) is a rare subtype of non-Hodgkin' lymphoma (NHL), comprising <1% of all NHLs (1–3) and approximately 2% of extranodal presentations (1, 3–6). In addition, <1% of all breast malignancies are lymphomas (2–4, 7). Diffuse large B-cell lymphoma (DLBCL) is the most common histologic subtype of PBL, accounting for 40–80% of cases (4, 6). Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy are considered the standard regimen for DLBCL. Recently, the addition of the anti-CD20 antibody rituximab to the CHOP regimen has improved the outcome of patients with B-cell lymphoma (8–10).

There are many retrospective series (2, 3, 5, 11–18) but only two small prospective clinical trials (19, 20) which have reported the clinicopathological features of PBL. Most studies recommend a chemotherapy regimen containing anthracycline followed by radiotherapy (RT) (12–14). The 5-year survival rate in the recent larger series ranges from 61% (11) to 73% (12) with anthracycline-containing regimens with or without RT. Some series have suggested that PBL has a poorer prognosis than

aggressive NHLs with mainly nodal involvement; moreover, PBL tends to recur predominantly in extranodal sites, with especially significant risk in both the ipsilateral and contralateral breast and in the central nervous system (CNS) with a recurrence rate ranging from 7% to 29% (5, 11, 13, 16, 17, 19, 21, 22). However, other series have reported that the prognosis and incidence of CNS involvement of PBL were similar to those of aggressive nodal NHLs of the same stage (12, 18, 20). Thus, the clinical course and pathological features of PBL, and what constitutes the optimal initial treatment, remain to be elucidated. Therefore, we conducted a retrospective, single-institution study to analyze the clinicopathological features, treatment efficacy, and prognostic factors in patients with PBL.

Patients and methods

Patients

Data on 20 patients with PBL who were treated at the National Cancer Center Hospital from January 1999 to December 2008 were analyzed retrospectively. Patients with recurrent lymphoma in the breast and those initially presenting with systemic disease including breast involvement were excluded according to the definition previously reported (1). Patients were considered to have bulky disease if the largest tumor was >5 cm in diameter. The study protocol was approved by the institutional review board of the National Cancer Center Hospital, Tokyo, Japan.

Staging

The initial staging in all patients included history and physical examination, blood tests, computed tomography (CT) of the neck, chest, abdomen, and pelvis, bone marrow aspiration, and upper gastrointestinal endoscopy. Patients were staged according to the Ann Arbor classification system (23). Staging of extranodal NHLs within bilateral paired organs remains controversial, but in this study, patients with bilateral presentation were categorized as stage IE. Evaluation of the CNS at diagnosis by CT, magnetic resonance imaging (MRI), or lumbar puncture with cerebrospinal fluid analysis was performed only if clinically indicated. The International Prognostic Index (IPI) (24) was used to assess prognosis.

Treatment

Patients received 3 or 4 courses of CHOP with or without RT, whereas patients with bulky disease received 6 or more courses of CHOP. RT was scheduled before the commencement of chemotherapy because the disease was local, and the radiation field included the involved breast and the regional lymph nodes (the axillary and the supraclavicular region). Rituximab has been available for DLBCL through the Japanese National Health Insurance system since September 2003, and patients also received rituximab since then. CNS prophylaxis, consisting of intrathecal methotrexate (IT-MTX, four doses of 15 mg each), was administered at the treating physician's discretion.

Statistical analysis

Response was assessed after completion of the initial therapy according to the response criteria for NHLs (25). Overall survival (OS) was calculated from the date of diagnosis to the date of last follow-up or death from any cause. Progression-free survival (PFS) was calculated from the date of diagnosis to the date of disease progression, death from any cause, or last follow-up. OS and PFS were estimated by the Kaplan–Meier method (26). The following variables were analyzed for prognostic significance for OS and PFS: Ann Arbor clinical stage (stage IE vs. stage IIE), the largest tumor size (≤ 5 cm vs. >5 cm), and age (<60 vs. ≥ 60). Because the proportion of patients with elevated lactate dehydrogenase (LDH) levels (2 of 14 patients) or the patients classified as the low-intermediate or high-risk group according to the IPI (1 each of 14 patients) was very low (Table 1), these variables were not analyzed. The log-rank test was used to compare survival curves. A P value <0.05 for a two-sided test was considered statistically significant. All statistical analyses were performed using Dr SPSS II software, release 11.0.1J (SPSS Japan, Tokyo, Japan).

Results

Patient characteristics

Using the World Health Organization (WHO) classification, 4th edition (27), there were 17 patients with DLBCL, two patients with follicular lymphoma, one patient with mucosa-associated lymphoid tissue (MALT) lymphoma, and no patients with T/NK-cell lymphoma. Because the proportion of the patients with primary breast low-grade B-cell NHLs was very low (15%, 3/20), the patient population in this study was limited to the DLBCL patients. As three patients were excluded because of Stage IV disease, 14 DLBCL patients were analyzed in this study.

The diagnosis of PBL was established with a core needle biopsy in eight patients, excisional biopsy in four patients, and mastectomy with regional lymph node resection in two patients. Patient characteristics are summarized in Table 1. There were nine patients with stage

Table 1 Patient characteristics

	No. (%)
Age	
Median 57.5 years	
Range 24–69 years	
<60 years	10 (71)
≥60 years	4 (29)
Gender	
Male	0 (0)
Female	14 (100)
Primary site of lymphoma	
Unilateral breast	12 (86)
Bilateral breast	2 (14)
Tumor size[1]	
<5cm	9 (64)
>5 cm	5 (36)
Nodal involvement	
None	9 (64)
Axillary only	4 (29)
Supraclavicular + axillary	1 (7)
ECOG Performance Status	
0	13 (93)
1	1 (7)
LDH	
Normal	12 (86)
Elevated	2 (12)
Ann Arbor stage	
IE	9 (64)
IIE	5 (36)
B symptoms	
Absent	13 (93)
Present	1 (7)
IPI	
Low	12 (86)
Low-intermediate	1 (7)
High	1 (7)

ECOG PS, Eastern Cooperative Oncology Group Performance status; LDH, lactate dehydrogenase; IPI, International Prognostic Index.
[1]For bilateral cases, the diameter of the larger tumor is indicated.

IE and five with stage IIE disease. The median age of all 14 female patients was 57.5 years (range 24–69 years). There were two patients who presented with bilateral breast involvement at diagnosis. The median diameter of the largest tumor was 4.5 cm, with five patients exhibiting bulky disease. Regional nodal involvement was observed in 36% of the patients.

Treatment

Table 2 shows the initial treatment regimens. There was one patient who had undergone mastectomy and a regimen of cyclophosphamide, methotrexate, and fluorouracil under the clinical diagnosis of breast cancer but was later histopathologically diagnosed as having DLBCL. The remaining 13 patients received CHOP with or without RT. Rituximab was administered in seven patients.

Table 2 Initial treatment

			No. (%)
Treatment	Chemotherapy only		3 (21)
	Surgery + Chemotherapy		2 (14)
	Chemotherapy + RT		9 (64)
Surgery	Mastectomy + axillary dissection		2
Chemotherapy	CMF regimen		1
	CHOP regimen		13
	No. of cycles	2–4	3
		6	6
		8	4
	With Rituximab	3–8	7 (50)
	With IT-MTX	4	3 (21)
RT			
Field	Involved breast + regional LNs		9 (64)
Dose (breast)	Median (Range)		40 (40–46)
Dose (regional)			30 (30–32)

CMF, cyclophosphamide, methotrexate, and fluorouracil; CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; RT, radiotherapy; IT-MTX, intrathecal administration of methotrexate; LNs, lymph nodes.

RT to the involved breast was administered in nine patients (8 of 9 patients with stage IE disease and 1 of 4 with stage IIE disease) with total doses ranging between 40 and 46 Gray (Gy) and to the regional lymph nodes (the axillary and the supraclavicular regions) with total doses ranging between 30 and 32 Gy. Intrathecal CNS prophylaxis was given in three patients.

Outcomes

After the initial treatment, 13 patients (93%) showed a complete response (CR) and one patient (7%) showed a partial response (PR). With a median follow-up period of 5.2 years, the estimated OS and PFS rates at 5 years were 76% and 52%, respectively (Fig. 1). The median PFS and OS were not reached at the time of analysis.

Prognostic factors

The presence of bulky disease adversely affected both rates of OS and PFS (Fig. 2A and B, respectively). The Ann Arbor stage and age were not predictive of either OS or PFS (data not shown).

Relapse or progression

The details of patients with relapse or progression are shown in Table 3. Among the 13 patients who achieved CR after the initial treatment, six patients (43%) relapsed. Patient 3 achieved PR but progressed within 3 months of completion of initial therapy. In total, 7 of the 14 patients relapsed or progressed.

All five patients with bulky disease relapsed or progressed, whereas only 2 of 9 patients with tumors ≤5 cm experienced relapse. Of the two patients with bilateral breast involvement, one patient relapsed. The median interval between the completion of the initial therapy and relapse was 12 months. Relapse occurred more than 2 years after the completion of the initial therapy in two patients, occurring in the axillary lymph nodes and the CNS, respectively (Patients 5 and 6).

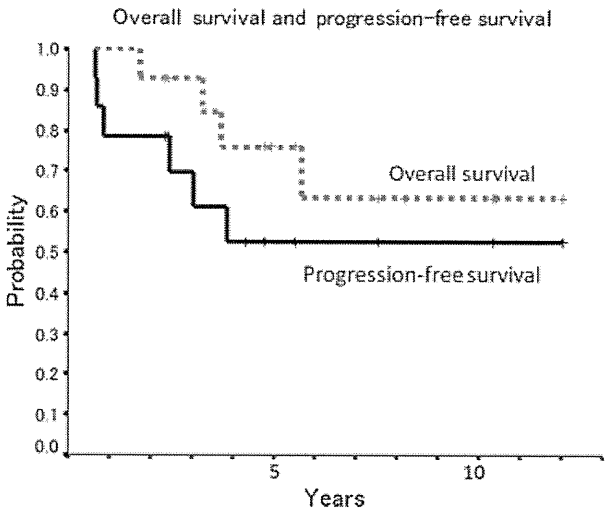

Figure 1 Overall and progression-free survival for all 14 patients.

CNS involvement and CNS prophylaxis

There were four patients (29%) who had recurrence in the CNS, including three mortalities because of progressive disease. The CNS was the first site of relapse or progression in three patients (21%); it was the third relapse site in the remaining patient. Two patients had CNS relapse within 3 months after the initial therapy, and the other two patients had CNS relapse more than 3 years after the initial therapy. Half of the patients had parenchymal brain metastases, and half had leptomeningeal involvement. In the five patients with bulky disease, 3 (60%) had CNS relapses. In patients with disease ≤ 5 cm, one patient had (11%) progression into the CNS as the third relapse site (Patient 1 in Table 3). There were

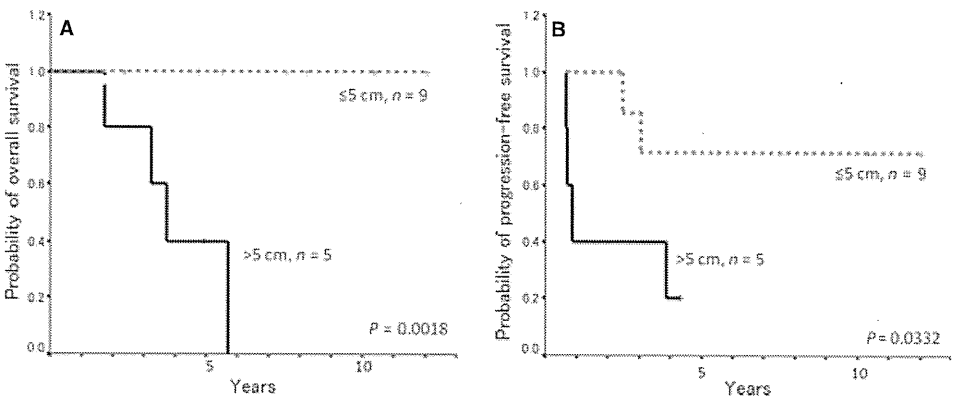

Figure 2 Overall (A) and progression-free survival (B) by tumor size.

Table 3 Details of relapse or progression

Patient No.	Stage	Tumor size (cm)	Initial treatment	Response to initial treatment	Interval to relapse or progression (months)[2]	First site of relapse or progression	Subsequent site(s) of relapse or progression	Outcome
1	I	≤5	CHOP → RT	CR	12	Hypodermis	Hypodermis, CNS (parenchyma)	Alive
2	I	≤5	CHOP → RT	CR	18	Cervical LNs, BM	Multiple LNs	Alive
3	I	>5	CHOP → RT	PR	3	Initial breast	–	Dead
4	I	>5	R-CHOP → RT	CR	3	CNS (parenchyma)	–	Dead
5	I	>5	R-CHOP → RT	CR	44	Axillary LNs	–	Alive
6	II	>5	CHOP → RT	CR	40	CNS (leptomeninges)	–	Dead
7	II[1]	>5	R-CHOP	CR	2	CNS (leptomeninges)	Rectum, adrenal grand	Dead

CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; R, rituximab; RT, radiotherapy; CR, complete response; PR, partial response; PD, progressive disease; CNS, central nervous system; LNs, lymph nodes; BM, bone marrow.

[1]Bilateral breast.

[2]Interval to relapse or progression after the completion of therapy.

no CNS recurrences in the three patients who received CNS prophylaxis. However, 4 of 11 patients (36%) who had not received CNS prophylaxis had CNS involvement.

Relapse/Progression in the breast site and IF-RT

There was one progression in the ipsilateral breast. The progression occurred in the initial breast site after PR induced by the initial treatment. Radiotherapy was administered after chemotherapy in nine patients. Within the RT field, there was 1 progression after PR in the initially involved breast and 1 recurrence after CR in the axillary lymph nodes in 30 Gy irradiated region 3.7 years after the completion of RT (Patients 3 and 5 in Table 3); both patients had bulky disease. In the remaining seven patients who received RT, no relapses occurred within the RT field or in the contralateral breast. On the other hand, none of the eight patients who had not received RT relapsed in the ipsilateral breast.

Immunochemotherapy

All the seven patients who received rituximab showed CR, among them three relapses occurred: 2 in the CNS and 1 in the axillary lymph nodes within the RT field. All three patients with bulky disease who received rituximab relapsed. On the other hand, all four patients with ≤5 cm bulk who received rituximab maintained a CR. Addition of rituximab to chemotherapy did not have any prognostic significance on either OS or PFS (data not shown).

Discussion

Some studies have suggested that PBL portends a poorer prognosis than aggressive nodal NHL, with a 5-year survival rate ranging from 61% to 73% in recent larger series (11, 12). In the present study, the estimated 5-year OS and PFS rates were 69% and 36%, respectively. Our results were similar to those previously reported (11, 12). Furthermore, most patients (13/14 patients, 93%) had a CR, although seven patients (50%) relapsed. The median interval between the completion of the initial therapy and relapse was 12 months, with two patients relapsing more than 2 years later. A high proportion of relapses despite the high CR rate might contribute to poor prognosis in PBL patients. Therefore, improvement in risk stratification of patients and tailoring initial treatment regimens to such may lead to better outcomes in PBL by reducing the rate of relapse.

We found that if the maximum tumor diameter at diagnosis was above 5 cm, there was a negative prognostic impact on OS and PFS. It is noteworthy that all of the patients with bulky disease relapsed or progressed. Patients with bulky disease comprised 3 of the 4 patients with CNS involvement and one patient with ipsilateral breast progression. Prognostic factors such as age (7, 28), IPI (2, 18, 21), PS (11, 21, 29), stage (2, 7, 13, 21), LDH (21, 29), tumor size (2, 18, 30), and the number of extranodal sites (11) have been shown to predict outcomes in patients with PBL.

We observed four CNS relapses in 14 patients. The higher incidence of CNS involvement (4/14 patients, 29%) in our study when compared to previous studies (5, 11–13, 16–22) may reflect bias because of the small sample size. Some retrospective studies have reported a high incidence (ranging from 12% to 39%) of CNS relapse in patients with DLBCL of the breast (5, 13, 17, 19, 21, 22). In contrast, The International Extranodal Lymphoma Study Group (IELSG) and Stanford University reported only 5% and 3% rates of CNS relapse, respectively (12, 29); however, 38% of the patients in the latter study were diagnosed as having low-grade B-cell lymphomas. Therefore, they suggested that CNS prophylaxis did not appear to be routinely indicated (12, 29). However, the risk of CNS relapse and the efficacy of CNS prophylaxis have not yet been clearly defined. Although the eligibility criteria of our study was limited to patients with localized disease, our study showed a relatively high incidence of CNS relapse (4/14 patients, 29%), which was similar to as that in the previous report; that is 30% of patients with early stage PBL (22). In DLBCL patients, the high levels of LDH and the high-risk group according to the IPI were the predictors for CNS involvement (31). However, these populations were very small in our study. On the other hand, when viewed in the tumor diameter, there was a higher risk of CNS relapse in patients with bulky disease (>5 cm) (3/5 patients, 60%), whereas only 1 of the 9 patients with ≤5 cm disease had CNS involvement, and as the third site of relapse. Therefore, this may imply that tumor size is an important risk factor for CNS involvement. Additionally, the higher rate of CNS relapse (4/11 patients, 36%) in patients who did not receive CNS prophylaxis, in contrast to no CNS relapses in patients who did receive CNS prophylaxis, suggests that CNS prophylaxis is beneficial although the number of patients was small.

In our study, two patients had CNS relapse within 3 months after the initial therapy. We cannot deny the possibility of initial CNS involvement at the time of diagnosis because the patients who were analyzed in the current study had not undergone the examination of the cerebrospinal fluid before the treatment was instituted. On the contrary, the other two patients had CNS relapse more than 3 years after the initial therapy. There is another possibility that CNS relapse after durable CR resulted from a secondary CNS lymphoma because the

recent analysis of the complementarity-determining region 3 of the immunoglobulin heavy chain revealed that the lymphoma clone in CNS was different from that of the original breast lymphoma in one patient (32).

Although a high relapse rate in the ipsilateral breast has been reported in a number of retrospective studies (13, 21), the IELSG study showed that the rate of ipsilateral progression was substantially reduced by the use of RT, which might have contributed to an improvement in outcomes in patients with localized high-grade PBL (12). In our study, among the nine patients who received RT, there was one progression and one relapse observed within the RT field. The patient who progressed had only a PR after the initial course of chemotherapy followed by radiotherapy, and the tumor that relapsed in the axilla was in 30 Gy irradiated region. The remaining seven patients had no relapses within the RT field. In addition, patients who either progressed or relapsed within the RT field had bulky disease. Although a high rate of relapse in the contralateral breast has been previously reported (5, 12, 17, 22), there were no instances of contralateral breast relapse in this study.

Finally, rituximab plus CHOP (R-CHOP) has become the standard chemotherapy regimen for DLBCL. The studies that analyzed outcomes in PBL showed that there was no improvement even when rituximab was added to CHOP (18, 20). In the present study, we administered R-CHOP to seven patients. All the patients with bulky disease treated with rituximab relapsed, but their counterparts with tumors ≤5 cm did not. This might suggest that the patients with bulky disease have a poorer prognosis even in the rituximab era. Additionally, 2 of the 7 patients (29%) treated with rituximab experienced CNS relapse. These results may indicate that CNS prophylaxis reduces the probability of relapse and improves outcomes in the rituximab era.

In conclusion, our study delineated the clinicopathologic features of primary DLBCL of the breast. Most patients achieved a CR, although they relapsed at a high rate with some patients experiencing late relapses. Patients with bulky disease demonstrated a poor prognosis. A high rate of CNS relapses in patients with PBL suggests that CNS prophylaxis might yield better outcomes, especially in patients with bulky tumors > 5 cm. However, given the small number of patients in this study, a larger scale, prospective trial is needed to elucidate the optimal treatment strategy for PBL, especially in the rituximab era.

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Conflicts of interest

None declared.

References

1. Wiseman C, Liao KT. Primary lymphoma of the breast. *Cancer* 1972;**29**:1705–12.
2. Ha CS, Dubey P, Goyal LK, Hess M, Cabanillas F, Cox JD. Localized primary non-Hodgkin's lymphoma of the breast. *Am J Clin Oncol* 1998;**21**:376–80.
3. Domchek SM, Hecht JL, Fleming MD, Pinkus GS, Canellos GP. Lymphomas of the breast. Primary and secondary involvement. *Cancer* 2002;**94**:6–13.
4. Brogi E, Harris NL. Lymphomas of the breast: pathology and clinical behavior. *Semin Oncol* 1999;**26**:357–64.
5. Gholam D, Bibeau F, El Weshi A, Bosq J, Ribrag V. Primary breast lymphoma. *Leukemia Lymphoma* 2003;**44**:1173–8.
6. López-Guillermo A, Colomo L, Jiménez M, *et al.* Diffuse large B cell lymphoma: clinical and biological characterization and outcome according to the nodal or extranodal primary origin. *J Clin Oncol* 2005;**23**:2797–804.
7. Jeon HJ, Akagi T, Hoshida Y, Hayashi K, Yoshino T, Tanaka T, Ito J, Kamei T, Kawabata K. Primary non-Hodgkin malignant lymphoma of the breast. An immunohistochemical study of seven patients and literature review of 152 patients with breast lymphoma in Japan. *Cancer* 1992;**70**:2451–9.
8. Coiffier B, Lepage E, Briere J, *et al.* CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large B cell lymphoma. *N Engl J Med* 2002;**346**:235–42.
9. Pfreundschuh M, Trümper L, Osterborg A, *et al.* CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. *Lancet Oncol* 2006;**7**:379–91.
10. Habermann TM, Weller EA, Morrison VA, *et al.* Rituximab-CHOP versus CHOP with or without maintenance rituximab in patients 60 years of age or older with diffuse large B-cell lymphoma: an update. *J Clin Oncol* 2006; **24**:3121–7.
11. Validire P, Capovilla M, Asselain B, *et al.* Primary breast non-Hodgkin's lymphoma: a large single center study of initial characteristics, natural history, and prognostic factors. *Am J Hematol* 2009;**84**:133–9.
12. Ryan G, Martinelli G, Kuper-Hommel M, *et al.* Primary diffuse large B-cell lymphoma of the breast: prognostic factors and outcomes of a study by the International Extranodal Lymphoma Study Group. *Ann Oncol* 2008;**19**:233–41.
13. Wong WW, Schild SE, Halyard MY, Schomberg PJ. Primary non-hodgkin lymphoma of the breast: the Mayo clinic experience. *J Surg Oncol* 2002;**80**:19–25.

14. Kuper-Hommel MJ, Snijder S, Janssen-Heijnen ML, Vrints LW, Kluin-Nelemans JC, Coebergh JW, Noordijk EM, Vreugdenhil G. Treatment and survival of 38 female breast lymphomas: a population-based study with clinical and pathological reviews. *Ann Hematol* 2003;**82**:397–404.
15. Liu MT, Hsieh CY, Wang AY, Pi CP, Chang TH, Huang CC, Huang CY. Primary breast lymphoma: a pooled analysis of prognostic factors and survival in 93 cases. *Ann Saudi Med* 2005;**25**:288–93.
16. Choo SP, Lim ST, Wong EH, Tao M. Breast lymphoma: favorable prognosis after treatment with standard combination chemotherapy. *Onkologie* 2006;**29**:14–18.
17. Ryan GF, Roos DR, Seymour JF. Primary non-Hodgkin's lymphoma of the breast: retrospective analysis of prognosis and patterns of failure in two Australian centers. *Clin Lymphoma Myeloma* 2006;**6**:337–41.
18. Niitsu N, Okamoto M, Nakamine H, Hirano M. Clinicopathologic features and treatment outcome of primary breast diffuse large B-cell lymphoma. *Leuk Res* 2008;**32**:1837–41.
19. Avilés A, Delgado S, Nambo MJ, Neri N, Murillo E, Cleto S. Primary breast lymphoma: results of a controlled clinical trial. *Oncology* 2005;**69**:256–60.
20. Avilés A, Castañeda C, Neri N, Cleto S, Nambo MJ. Rituximab and dose dense chemotherapy in primary breast lymphoma. *Haematologica* 2007;**92**:1147–8.
21. Lin Y, Guo XM, Shen KW, Wang JL, Jiang GL. Primary breast lymphoma: long-term treatment outcome and prognosis. *Leuk Lymphoma* 2006;**47**:2102–9.
22. Ribrag V, Bibeau F, El Weshi A, Frayfer J, Fadel C, Cebotaru C, Laribi K, Fenaux P. Primary breast lymphoma: a report of 20 cases. *Br J Haematol* 2001;**115**:253–6.
23. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the Committee on Hodgkin's disease staging classification. *Cancer Res* 1971;**31**: 1860–1.
24. The international non-Hodgkin's lymphoma prognostic factors project: a predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med* 1993;**329**:987–94.
25. Cheson BD, Horning SJ, Coiffier B, *et al.* Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. *J Clin Oncol* 1999;**17**:1244–53.
26. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958;**53**:457–81.
27. Swerdlow SH, Campo E, Harris NL, *et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th edn. Lyon, France: IARC Press, 2008.
28. Hugh JC, Jackson FI, Hanson J, Poppema S. Primary breast lymphoma. An immunohistologic study of 20 new cases. *Cancer* 1990;**66**:2602–11.
29. Ganjoo K, Advani R, Mariappan MR, McMillan A, Horning S. Non-Hodgkin lymphoma of the breast. *Cancer* 2007;**110**:25–30.
30. Yoshida S, Nakamura N, Sasaki Y, Yoshida S, Yasuda M, Sagara H, Ohtake T, Takenoshita S, Abe M. Primary breast diffuse large B-cell lymphoma shows a non-germinal center B-cell phenotype. *Mod Pathol* 2005;**18**:398–405.
31. Yamamoto W, Tomita N, Watanabe R, Hattori Y, Nakajima Y, Hyo R, Hashimoto C, Motomura S, Ishigatsubo Y. Central nerve system involvement in diffuse large B-cell lymphoma. *Eur J Haematol* 2010;**85**:6–10.
32. Lin YC, Tsai CH, Wu JS, Huang CS, Kuo SH, Lin CW, Cheng AL. Clinicopathologic features and treatment outcome of non-Hodgkin lymphoma of the breast–a review of 42 primary and secondary cases in Taiwanese patients. *Leuk Lymphoma* 2009;**50**:918–24.

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Central nervous system event in patients with diffuse large B-cell lymphoma in the rituximab era

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Central nervous system (CNS) events, including CNS relapse and progression to CNS, are known to be serious complications in the clinical course of patients with lymphoma. This study aimed to evaluate the risk of CNS events in patients with diffuse large B-cell lymphoma in the rituximab era. We performed a retrospective survey of Japanese patients diagnosed with diffuse large B-cell lymphoma who underwent primary therapy with R-CHOP chemoimmunotherapy between September 2003 and December 2006. Patients who had received any prophylactic CNS treatment were excluded. Clinical data from 1221 patients were collected from 47 institutions. The median age of patients was 64 years (range, 15–91 years). We noted 82 CNS events (6.7%) and the cumulative 5-year probability of CNS events was 8.4%. Patients with a CNS event demonstrated significantly worse overall survival ($P < 0.001$). The 2-year overall survival rate after a CNS event was 27.1%. In a multivariate analysis, involvement of breast (relative risk [RR] 10.5), adrenal gland (RR 4.6) and bone (RR 2.0) were identified as independent risk factors for CNS events. We conclude that patients with these risk factors, in addition to patients with testicular involvement in whom CNS prophylaxis has been already justified, are at high risk for CNS events in the rituximab era. The efficacy and manner of CNS prophylaxis in patients for each involvement site should be evaluated further. (*Cancer Sci* 2012; 103: 245–251)

The central nervous system (CNS) is thought to be a sanctuary for lymphoma cells from systemic chemoimmunotherapy, such as rituximab (R) plus CHOP (cyclophosphamide [CPA], doxorubicin [adriamycin, ADR], vincristine [VCR] and prednisolone [PSL]), because standard doses of these drugs do not adequately penetrate the CNS. Occurrence of a CNS event, defined as CNS relapse during systemic complete remission or CNS progression during concurrent systemic active lymphoma, is associated with extremely poor prognosis, with median survival of <6 months.[1–6] Many studies concerning CNS prophylaxis have been conducted; however, the efficacy of such prophylaxis in preventing CNS events is controversial.[5,7–12] The discrepancies between reports might be due to the differences in the various subtypes of lymphoma histology and the variability of treatment of CNS prophylaxis.[13–16] In addition, R has had a substantial impact on outcomes in patients with diffuse large B-cell lymphoma (DLBCL).[17] It is thus necessary to re-evaluate the risk of CNS events in the R era.

The present study comprises a multicenter retrospective analysis of patients with uniform DLBCL histology who have undergone uniform treatment with R-CHOP, widely accepted as the standard therapy in the R era. Patients who received any CNS prophylactic treatment, such as intrathecal chemotherapy, intraveneous high-dose methotrexate or whole brain irradiation, were excluded to evaluate the natural risk of CNS events in R-CHOP therapy. This study also took particular note of the evaluation of various extranodal involvement sites at presentation.

Materials and Methods

Patients, diagnosis, treatment and inclusion/exclusion criteria. In October 2009, the Bay-area Lymphoma Information Network (Bay-LINK) in Japan, a cooperative study group consisting of the Cancer Institute of the Japanese Foundation for Cancer Research and the Yokohama City University Hematology Group, performed a mail and e-mail survey about CNS involvement in patients with DLBCL. By June 2010, all clinical data had been collected by Bay-LINK.

All patients had been diagnosed with *de novo* DLBCL and had undergone primary therapy between September 2003 and December 2006. R was approved for the treatment of CD20-positive aggressive B-cell lymphoma in Japan in September 2003 by the Ministry of Health, Labour and Welfare. Patients with distinct forms of DLBCL, such as intravascular lymphoma, primary effusion lymphoma and primary mediastinal large B-cell lymphoma, were excluded from the study. Primary CNS lymphoma and intraocular lymphoma were also excluded in this study. Pathological diagnosis was made by the pathologists in

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