Table 4.
Patient Toxicities: Cladribine plus Mitoxantrone

Toxicity	World Health Organization Grade		
	1/2, n (%)	3, n (%)	4, n (%)
Leukocytopenia	1	9 (15)	50 (81)
Granulocytopenia	1	12 (19)	46 (74)
Thrombocytopenia	15 (24)	8 (13)	8 (13)
Anemia	35 (56)	7 (11)	0
Viral infection	10 (16)	0	0
Bacterial infection	3 (5)	0	0
Nausea/vomiting	15 (24)	2	0
Polyneuropathy	6 (10)	0	0
Lung (pleural fluid, dyspnea)	0	1	0
Hair loss	9 (15)	2	0
Parageusia	9 (15)	0	0
Diarrhea	5 (8)	0	0
Stomatitis	3	0	0

ulocytopenic phase, 8 localized herpes zoster infections, and 1 subcutaneous abscess with micrococcus species (*Gaffkya*). Grade 3 or 4 thrombocytopenia occurred less often and in only 13 (5%) and 11 (4%) of all evaluable cycles, respectively. The median level of $CD4^+$ lymphocytes of only 371/μL before treatment decreased to 142/μL after 2 cycles, 117/μL after 4 cycles, and 93/μL after 6 cycles. Treatment was prematurely discontinued after 2 cycles in 1 case because of persisting leukocytopenia and thrombocytopenia and after 3 cycles in 3 cases and 4 cycles in another 3 cases because of prolonged $CD4^+$ lymphocytopenia at levels below 100/μL (a criterion for discontinuation according to the protocol). Two male patients developed secondary malignancies, 1 case of esophageal cancer and 1 of non-small cell lung cancer in a patient with a history of smoking, at 6 months and 29 months, respectively, after the initiation of mitoxantrone therapy. No other significant infections have been seen since the cessation of therapy after a stable response was reached or during the time interval when a salvage therapy had to be initiated for a recurrence.

6.2.4. Cladribine plus Mitoxantrone: Conclusion

This study demonstrated that the combination of reduced-dose cladribine and mitoxantrone is a highly active regimen in the treatment of indolent NHL. Ninety percent of the 62 patients responded to therapy with a CR rate of 44% and a median remission duration of 25 months. Myelosuppression was the major toxic event of treatment, with granulocytopenias of grade 3 or 4 occurring in 23% and 48% of all cycles, respectively. Because of the addition of mitoxantrone, the hematologic toxicity of mitoxantrone was more distinct from the hematologic toxicity we observed in our previous experience with cladribine monotherapy, in which we observed grade 3 and 4 granulocytopenias in only 14% and 3% of cycles, respectively [62]. Despite the distinct hematologic toxicity, the incidence of infections was rather low, with only 2 patients (3%) experiencing bacterial pneumonias. Otherwise, mitoxantrone was well tolerated by most patients, and there were no acute nonhematologic toxicities and no treatment-related deaths or other severe infectious complications.

Several phase II trials with purine analogue–containing regimens showing promising activity in the treatment of indolent NHL have been reported in the literature. Only a few studies of combination regimens including cladribine are available. Two studies reported results using the standard dose of cladribine combined with mitoxantrone and dexamethasone or prednisone [71,74]. In both studies with heavily pretreated patients, the risk for infectious complications was in a higher range. Saven et al later omitted prednisone from the regimen, because it exacerbated the risk of serious infections without improving the response rates [71].

Therapies with purine analogues are immunosuppressive with a significant drop in and a long-term suppression of $CD4^+$ lymphocyte counts. We attempted to decrease the immunosuppression associated with cladribine therapy by reducing the dosage of 5 mg/m^2 administered per day of cladribine from 5 days to 3 days when we combined it with mitoxantrone. The median count of $CD4^+$ lymphocytes decreased throughout the period of treatment from an already lowered $CD4^+$ cell count of 371/μL before the start of treatment to 142/μL after 2 cycles, 117/μL after 4 cycles, and 93/μL after 6 cycles. Even though these results obtained with reduced-dose cladribine combined with mitoxantrone were not directly comparable with those of our previous study of cladribine monotherapy [62], we found no difference in the decreases in $CD4^+$ cell counts between the 2 studies. This finding is in accordance with the results of recently published studies that also reported no difference in the behavior of $CD4^+$ cell counts when reduced cladribine dosages were used [72]. Conversely, these studies with the lower dosages of cladribine were able to diminish the rate of infections while maintaining similar antitumor activity, compared with the standard dosage regimen of cladribine [72,73].

Forty-eight patients responded to mitoxantrone after the second cycle, 4 patients responded after the third cycle, and 4 patients responded after the fourth cycle. Thus, 2 to 4 courses are sufficient to induce a response. However, the continuation of therapy beyond obtaining remission seems to be necessary because we observed more CRs and a significantly longer overall survival time for patients who had no early discontinuation of therapy and received 5 or 6 cycles of mitoxantrone.

In this trial, the dosage of mitoxantrone was reduced to 12 mg/m^2 on day 1 in previously treated patients to avoid severe hematologic toxicity. Because the response rates of 88% and 95% were similar for previously untreated and pretreated patients in this study and because no difference between the 2 groups was seen in the duration of relapse-free survival and overall survival, we can conclude that the dosage of 12 mg/m^2 mitoxantrone on day 1 only is sufficient when it is combined with cladribine.

Encouraged by the favorable response rates achieved with the combination of cladribine and mitoxantrone in the treatment of indolent NHL, we have initiated a phase III study to compare mitoxantrone with a standard regimen consisting of chlorambucil, mitoxantrone, and prednisone. This randomized study is ongoing, and results are expected soon. Our data indicate that the regimen of cladribine plus mitoxantrone broadens the range of therapeutic options available for indolent NHL.

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Japanese multicenter phase II and pharmacokinetic study of rituximab in relapsed or refractory patients with aggressive B-cell lymphoma

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Japanese multicenter phase II and pharmacokinetic study of rituximab in relapsed or refractory patients with aggressive B-cell lymphoma

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Background: To evaluate the efficacy and feasibility of rituximab monotherapy in Japanese patients with relapsed or refractory aggressive B-cell lymphoma.

Patients and methods: Sixty-eight patients were treated with rituximab at 375 mg/m^2 by eight consecutive weekly infusions. Pretreatment variables affecting overall response rate (ORR) and progression-free survival (PFS) and the relationship between pharmacokinetic parameters and efficacy were analyzed.

Results: The ORRs of 68 enrolled patients and 57 eligible patients were 35% [95% confidence interval (CI) 24% to 48%] and 37% (95% CI 25% to 51%), respectively. Median PFS of 53 evaluable patients was 52 days, whereas time to progression of 21 eligible responders was 245 days. Mild to moderate infusion-related toxicities were observed frequently at the first infusion, but all of them were reversible. Elevated lactate dehydrogenase (LDH) and refractoriness to prior chemotherapy were unfavorable factors affecting ORR and PFS (P <0.01). Serum trough levels of rituximab and area under the concentration–time curve for responders were higher than for non-responders (P <0.05).

Conclusions: Eight consecutive weekly infusions of rituximab have significant anti-lymphoma activity for relapsed or refractory aggressive B-cell lymphoma. Several pretreatment variables and serum rituximab levels are useful for predicting its efficacy.

Key words: aggressive B-cell lymphoma, pharmacokinetics, prognostic factor, rituximab

Introduction

In recent years, the incidence of non-Hodgkin's lymphoma (NHL) has been increasing not only in western countries but also in Japan, although the absolute number of patients with NHL is relatively small in Japan compared with that in the USA or Europe [1]. According to a recent clinicopathological investigation of malignant lymphoma in Japan, B-cell NHL accounted for 74% of total NHL cases, and its major subtype was diffuse large B-cell lymphoma (DLBCL) [2]. Another clinicopathological study in Japan revealed that, according to the Revised European and American Lymphoma (REAL) classification [3], 59% of peripheral B-cell neoplasms were DLBCL [4]. Aggressive NHL, represented by DLBCL, is classified as a curable disease. However, the cure rate brought about by standard chemotherapy is as low as 30–40% [5, 6]. Accordingly, a new agent with enhanced therapeutic efficacy is highly desirable.

Rituximab, a mouse-human chimeric anti-CD20 monoclonal antibody, was the first monoclonal antibody approved for the treatment of malignant neoplasms by the Food and Drug Administration in the United States, and its efficacy against indolent B-cell lymphoma has been established [7–9]. Its efficacy against aggressive B-cell lymphoma has also been demonstrated by Coiffier et al. in their monotherapy study and combination study with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) comparing CHOP alone in Europe [10, 11]. In the USA, Vose et al. reported promising results of a phase II study of CHOP combined with rituximab [12]. However, the efficacy of rituximab mono-

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therapy against aggressive B-cell lymphoma, especially for relapsed or chemotherapy-refractory patients, has not been extensively studied.

Previously, we conducted multicenter phase I and II studies of rituximab in Japan [9, 13]. In a pivotal phase II study, by employing a dose of 4 weekly infusions at 375 mg/m^2 in relapsed indolent B-cell lymphoma and mantle cell lymphoma (MCL), we confirmed its remarkable efficacy [9]. Being encouraged by the high efficacy and acceptable toxicity profiles of rituximab in our previous studies, we planned to investigate the potential use of this chimeric antibody for the treatment of Japanese patients with recurrent or chemotherapy-refractory aggressive B-cell lymphoma. In the present multicenter phase II study, we evaluated the efficacy and toxicity of rituximab at the dose of 375 mg/m^2 by eight consecutive weekly infusions. We also analyzed pretreatment variables affecting overall response rate (ORR) and progression-free survival (PFS). In addition, the relationship between pharmacokinetic (PK) parameters and efficacy was analyzed.

Patients and methods

Study design and end points

This study was a single agent, multicenter phase II trial. The primary end point was the ORR in all eligible patients. Secondary end points included time to progression (TTP) in all eligible and evaluable responders. The expected ORR (P_1) was set at 30% based on the results of the preceding phase II studies in aggressive B-cell lymphoma and MCL [8, 10, 14], while the threshold response rate (P_0) was set at 15%. The number of patients required for this study was 53 ($\alpha = 0.05$ and $1 - \beta = 0.8$) when calculated in accordance with Fleming's two-stage testing procedure [15]. However, assuming that up to 20% of patients may be ineligible, mainly due to inaccurate histological diagnoses at participating institutions, we planned to enroll 67 patients. All patients were followed up either until disease progression or for at least 6 months from the first infusion of rituximab. PFS in all eligible patients, including nonresponders, and toxicities in all treated patients were also evaluated.

Eligibility criteria

Patients were enrolled from 22 institutions (see Acknowledgements for a list of participating investigators and institutions) from July 1999 to December 2000. Study subjects consisted of patients with aggressive B-cell lymphoma who had relapsed or were refractory to conventional chemotherapy. The pathology of the lymphoma was to be consistent with MCL, DLBCL, Burkitt's lymphoma or high-grade B-cell lymphoma Burkitt-like according to the REAL classification [3]. Transformed lymphomas from indolent B-cell lymphoma were allowed to be included. The expression of CD20 antigen on the lymphoma cells was confirmed either by immunohistochemical analysis or by flow cytometry using B1 [16] or L26 [17] anti-CD20 antibody. Eligible patients had to have at least one measurable lesion, which had to be ≥2 cm in the greatest diameter if the patient had only one measurable lesion. The last chemotherapy cycle had to have been completed at least 2 weeks prior to study entry and have had no influence on the evaluation of rituximab efficacy and organ function. Patients were between 20 and 74 years of age and with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of ≤2 [18]. All patients were expected to survive for >2 months. Patients had to have no other malignancies, serious illness or infection, and had to have adequate organ functions; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) <4 × upper limit of normal (ULN), total bilirubin <2 × ULN, serum creatinine <1.5 × ULN and PaO2 ≥65 mmHg. Absolute neutrophil count was ≥1200/μl and platelet count ≥75 000/μl.

Patients meeting any one of the following criteria were excluded from the study: a history of treatment with a murine, chimeric or humanized monoclonal antibody; >1000/μl lymphoma cells in peripheral blood (PB); symptomatic central nervous system (CNS) involvement or a history of CNS involvement of lymphoma; seropositive for hepatitis B virus surface antigen, hepatitis C virus antibody or human immunodeficiency virus (HIV) antibody; pregnancy or potential pregnancy; and HIV-related lymphoma. Patients who had received hematopoietic cytokines, such as granulocyte colony-stimulating factor (G-CSF), within 1 week before enrolment were also excluded. All patients were required to stay in hospital for ≥2 days after the first infusion of rituximab.

Each patient signed an informed consent form at the time of study entry. The study was approved by the institutional review board of each institution.

Central review of pathology

Biopsy specimens from all enrolled patients were reclassified by a central pathology review committee according to the REAL classification. Thin-layer preparations on glass slides of lymphoma tissues obtained at the initial diagnosis and/or at relapse were collected following the patient's entry onto the study. These specimens were stained with hematoxylin–eosin. In addition, immunohistochemical staining was also conducted using anti-CD20 (L26), anti-CD3, anti-CD10 and anti-cyclin D1 antibodies [19, 20]. Hematoxylin–eosin and immunohistochemically stained preparations were examined by the central pathology review committee composed of the following three hematopathologists: Y. Matsuno, S. Nakamura and S. Mori. The diagnosis by the central pathology review committee was regarded as the final one in cases where there was a discrepancy between the diagnoses of each institution and the committee.

Rituximab administration and premedication

Rituximab (IDEC-C2B8) manufactured by Genentech, Inc. (San Francisco, CA, USA) was supplied by Zenyaku Kogyo, Co. Ltd (Tokyo, Japan) as a liquid preparation containing 10 mg/ml rituximab in a 10-ml vial, which was stored at 2–8°C until use. The dosage and schedule of rituximab in this study was 375 mg/m^2 and eight consecutive weekly infusions, respectively. Rituximab and pre-medication were given to patients as previously described [9]. Standard supportive care was provided, with the exception of corticosteroids which might affect the evaluation of tumor response. Rituximab infusion was to be discontinued if grade 3 or 4 non-hematological toxicities other than fever occurred during infusion. The use of other anticancer agents and radiotherapy was prohibited during the study period. In most patients, the second and subsequent infusions were conducted in an outpatient setting.

Monitoring of patients

In the 2 weeks prior to enrolment, patients underwent pretreatment tumor assessment at all sites where a tumor could be evaluated or measured using routine computed tomography (CT) scans. Gallium-67 (^{67}Ga) scintigraphy and endoscopic examinations were performed if necessary. In patients with leukemic transformation, tumor cell counts in the PB or bone marrow (BM) were examined by either microscopy or flow cytometry. Clinical observations and routine laboratory examinations were carried out before rituximab administration and 2 days after the first infusion, and were repeated weekly during rituximab administration and approximately every month thereafter. B- (CD19- and CD20-positive cells) and T-lymphocytes (CD3-positive cells) counts in PB and determination of serum immunoglobulins were also performed periodically.

Adverse events (AEs) and adverse drug reactions (ADRs)

Any detrimental change in a patient's condition was considered to be an AE. All AEs associated with rituximab administration or where the relationship to rituximab was unknown were regarded as ADRs. The ADRs were graded according to toxicity criteria of the Japan Clinical Oncology Group (JCOG) [21], an expanded version of the National Cancer Institute–common toxicity criteria (version 1.0).

Human anti-chimeric antibody (HACA) and serum rituximab levels

The presence of HACA in serum was monitored immediately before the first rituximab infusion, and 3 and 6 months thereafter using an enzyme-linked immunosorbent assay (ELISA) as described previously [9, 13, 22, 23]. Serum rituximab levels were assayed in 12 patients who signed another informed consent form for participating in this PK study. During weeks 1 and 8 of treatment, serum was collected immediately before starting the infusion and at 10 min, and 24, 48 and 120 h after completion of the infusion. During weeks 2 and 7, the samples were collected immediately before starting the infusion and at 10 min after the completion of each infusion. Additional samples were taken at 1, 4 and 16 weeks after the final infusion. The PK parameters were calculated using the software WinNonlin PK (WinNonlin Standard Japanese Edition, version 1.1; Scientific Consulting, Apex, NC, USA).

Response, progression-free survival (PFS) and time to progression (TTP)

Tumor lesions were observed by physical examination weekly during rituximab administration and by CT scans and physical examination approximately every 4 weeks thereafter. Response was assessed according to protocol-defined World Health Organization (WHO) criteria and the International Workshop NHL response criteria (IWRC) described by Cheson et al. [24], but is reported here as IWRC because those are the current standards. PFS was defined for all patients, including the non-responders, as the interval from the day of the first rituximab infusion to the day on which progression or death due to any cause was observed, while the TTP was defined for all responders as the interval from the day of the first infusion to the day on which progression was observed.

Central review of CT films

CT films of all responders were centrally reviewed by an independent CT review committee consisting of the following three radiologists: T. Terauchi (National Cancer Center Hospital, Tokyo), S. Nawano (National Cancer Center Hospital East, Kashiwa) and M. Matsusako (St Luke's International Hospital, Tokyo). When there was a discrepancy between the tumor-size evaluations by each institution and by the committee, the evaluation by the central review committee was regarded as the final evaluation.

Statistical methods

ORR and its 95% confidence interval (CI) were calculated for all eligible patients under F-distribution. Median TTP and PFS as well as the 95% CIs were estimated for all eligible and evaluable patients using the method of Kaplan and Meier [25]. In addition, pretreatment factors affecting the ORR and PFS were analyzed for all eligible and evaluable patients. Factors selected for multivariate analyses were as follows: gender; age (<60 versus ≥60 years); ECOG PS (0 versus 1–2); Ann Arbor clinical stage (I–II versus III–IV); B-symptom (presence versus absence); pathology (MCL versus all other aggressive B-cell NHL); LDH (normal versus elevated); number of extranodal lesions (0–1 versus ≥2); BM involvement; the largest tumor size (<5 cm versus ≥5 cm); number of relapses (0 versus 1–2); number of prior chemotherapy treatments (one regimen versus two or three regimens); and response to the last chemotherapy treatment (responder versus non-responder). In univariate analyses, Fisher's exact probability test was used for factors affecting ORR, and the log-rank test for those affecting PFS. In the multivariate analyses, a logistic regression model [stepwise procedure with entry and stay probability (P) levels ≤0.25 and ≤0.15, respectively] was used for factors affecting ORR, and Cox's proportional hazard regression model (stepwise procedure with entry and stay P levels ≤0.25 and ≤0.15, respectively) for those affecting PFS [26]. The relationship between PK parameters and response was analyzed by Student's t-test. All statistical analyses were performed using SAS software (version 6.12; SAS Institute, Cary, NC, USA).

Results

Patients' characteristics

A total of 68 patients were enrolled in the study. The characteristics of the patients at entry are summarized in Table 1. There were 47 males and 21 females; median age was 63 years (range 20–74). One patient was withdrawn from the study before the initiation of rituximab treatment since she was found to have received four regimens of prior chemotherapy. Six patients were judged ineligible due to inappropriate pathology in the central pathology review: five follicular center lymphomas and one low-grade B-cell lymphoma (not otherwise specified). In addition, four patients were judged ineligible by the extramural review committee; two of them had received corticosteroid until the initiation of rituximab treatment, one was positive for hepatitis C virus antibody and the remaining one had concomitant gastric cancer. However, the characteristics were similar between the 68 enrolled patients and the 57 eligible patients. There were 10 patients (15%) with clinical stage I or II disease at the time of enrolment, but the remaining 58 patients (85%) had either stage III or IV disease. Thirty-seven (54%) of 68 enrolled patients had extranodal diseases. BM involvement was found in 15 patients (22%). Thirty-one patients (46%) belonged to high or high-intermediate risk groups according to the international prognostic index (IPI) [27]. All patients had received at least one chemotherapy regimen. The most commonly used chemotherapy regimens prior to study entry were CHOP or CHOP-like regimens; 87% of enrolled patients had received them. Of 68 enrolled patients, 10 patients had a history of autologous hematopoietic stem cell transplantation (AHSCT), as shown in Table 1. No patients had received monoclonal antibody therapy.

Central pathology review

A central pathology review was performed on all tissue specimens, except for one patient who was withdrawn from study before initiating rituximab treatment. Patients were re-categorized according to the REAL classification, as shown in Table 1. The agreement between the diagnosis by each institution and that by the central pathology review committee was 91% (61/67 patients). Among the 57 eligible cases, DLBCL accounted for 50 cases (88%) and MCL for five cases (9%).

Early termination of rituximab treatment

Rituximab treatment was discontinued early in the course of the treatment period because of disease progression in 22 patients (33%). One patient who turned out not to meet the eligibility

Table 1. Patient characteristics

Characteristics	No. of cases		Characteristics	No. of cases	
	Enrolled	Eligible		Enrolled	Eligible
No. of patients	68	57	No. of extranodal diseases		
Median years of age (range)	63 (20–74)	63 (34–74)	0	31	24
Gender, male/female	47/21	40/17	1	21	18
Performance status (ECOG)			≥2	16	15
0	38	30	Bone marrow involvement		
1	23	21	Positive	15	14
2	7	6	Negative	53	43
Histology (REAL)			Tumor size, cm		
Diffuse large B-cell lymphoma	52	50	≥5	30	25
Mantle cell lymphoma	7	5	<5	38	32
Other aggressive B-cell lymphoma	2	2	LDH		
Follicular center lymphoma	5	0	Normal	26	22
Low-grade B-NHL not specified	1	0	Elevated	42	35
Specimen not available	1	0	No. of prior chemo-Tx		
Clinical stage at entry (Ann Arbor)			1	22	18
I	2	2	2	26	22
II	8	7	3	19	17
III	14	11	4	1	0
IV	44	37	Prior AHSCT		
B-symptoms			No	58	48
Present	16	15	Yes	10	9
Absent	52	42	International prognostic index		
No. of relapses			Low	15	12
0 (primary refractory)	26	19	Low-intermediate	22	18
1	33	30	High-intermediate	21	18
2	9	8	High	10	9
Response to prior chemo-Tx					
Responder	41	36			
Non-responder	27	21			

AHSCT, autologous hematopoietic stem cell transplantation; Chemo-Tx, chemotherapy; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; NHL, non-Hodgkin's lymphoma.

criteria during rituximab treatment was withdrawn early from the study. No patient developed grade ≥3 non-hematological toxicity requiring the discontinuation of rituximab treatment. Thus 44 of 67 patients (66%) completed the planned rituximab treatment.

ORR, PFS and TTP

Fifty-seven eligible patients were evaluated for response to rituximab on a protocol-compatible (PC) basis, whereas 68 patients were evaluated on an intention-to-treat (ITT) basis. As shown in Table 2, the ORRs on the basis of PC and ITT were 37% (21/57; 95% CI 25–51%) and 35% (24/68; 95% CI 24–48%), respectively.

Among 57 eligible patients, 11 patients had a washout period <4 weeks (21–26 days, eight cases; 18 days, one case; 17 days, one case; 15 days, one case). None of the 11 patients responded to the last prior salvage chemotherapy (three SD and eight PD), and they all had massive tumor lesions immediately before rituximab treatment. Only one patient responded to rituximab (one CR, one SD, eight PD, and one not evaluable).

Median PFS and the 95% CI were estimated by the Kaplan–Meier method for all eligible patients on the basis of PC and for all enrolled patients on the basis of ITT. However, unevaluable patients (use of steroid or anti-cancer agents, four patients; early withdrawal from the study, two patients; and inadequate measurement of tumor lesion, two patients) were excluded from the estimation of PFS. Median PFSs for all eligible and evaluable patients ($n = 53$) and for all enrolled and evaluable patients ($n = 60$) were 52 days (95% CI 33–111 days) and 61 days (95% CI 41–156 days), respectively, as shown in Figure 1. The median TTP of 21 eligible responders was 245 days (95% CI 176–435 days; Figure 1).

Table 2. Responses

	n	No. of patients						ORR, % (95% CI)
		CR	PR	CR+PR	SD	PD	NE	
Intention to treat	68	15	9	24	9	27	8	35 (24–48)
Protocol compatible	57	15	6	21	5	27	4	37 (25–51)

Responses to rituximab were evaluated according to the International Workshop NHL response criteria. No patient showed CR/unconfirmed.
CI, confidence interval; CR, complete response; NE, not evaluable due to insufficient follow-up; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

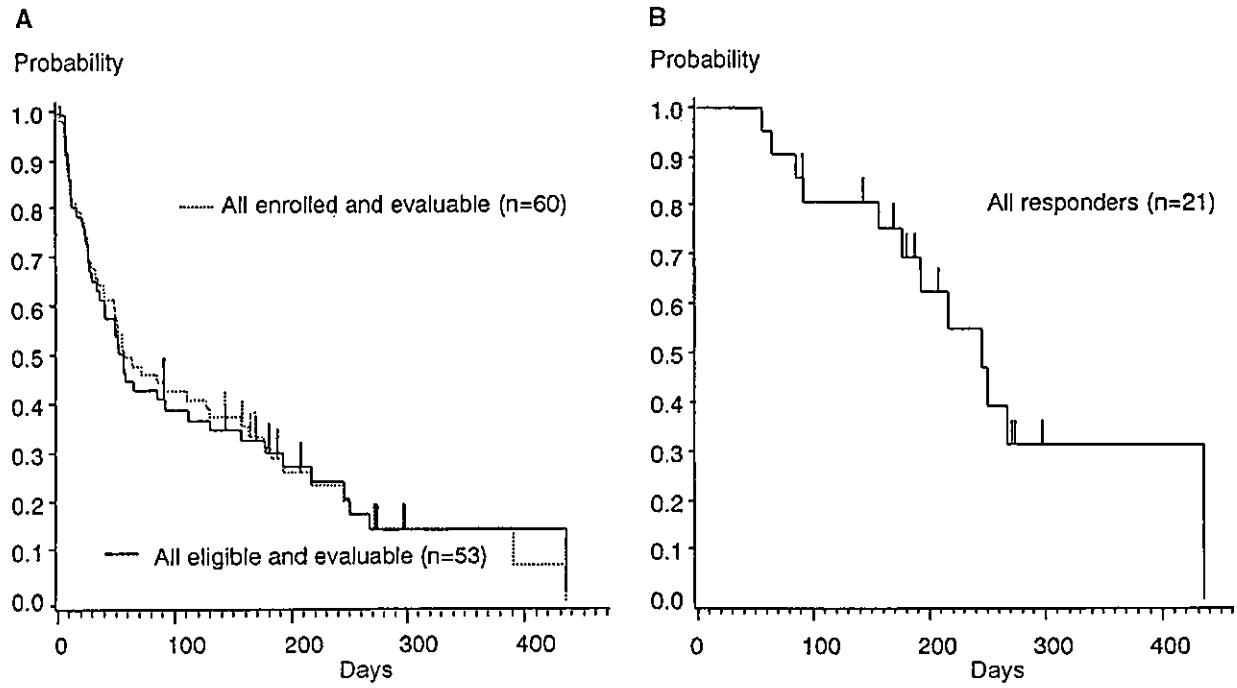

Figure 1. (A) Progression-free survival (PFS) and (B) time to progression (TTP). The median PFS values for all eligible and evaluable patients (n = 53) and for all enrolled and evaluable patients (n = 60) were 52 days [95% confidence interval (CI) 33–111] and 61 days (95% CI 41–156), respectively. The median TTP for all 21 eligible responders was 245 days (95% CI 176–435).

Non-hematological toxicities

Non-hematological toxicities were evaluated for all 67 patients who received at least one infusion of rituximab. Fifty-nine patients (88%) developed non-hematological toxicities. Commonly observed toxicities were infusion-related symptoms including fever, chills, burning sensation, headache, asthenia, pain, throat discomfort, perspiration and pruritus, most of which did not exceed grade 2, as shown in Table 3. These symptoms generally occurred during the first infusion. They were effectively managed with prophylactic or supportive antihistamines and antipyretics, and generally resolved within 24 h. Infusion-related ADRs decreased at subsequent infusions.

One patient developed a grade 3 upper-respiratory infection 3 months after completion of the planned rituximab treatment. Hematological testing indicated that the patient had also developed grade 4 neutropenia. Supportive care with antibiotics, G-CSF and immunoglobulin preparations was performed under hospitalization, and he recovered 9 days after the onset of infection.

Hematological toxicities

Twenty-nine patients (43%) developed hematological toxicities, as shown in Table 3. Grade 4 toxicities were observed in four patients (6%), including one case of leukopenia (2%) and four of neutropenia (6%). Out of the four patients, three had a history of receiving autologous peripheral blood stem cell transplantation. While one patient required G-CSF, the remaining three recovered without any medical intervention.

Abnormal laboratory findings

As also shown in Table 3, 20 patients (30%) had abnormal laboratory values for which a relationship to rituximab was not clearly ruled out. Elevation of hepatic enzymes (AST, ALT or ALP) and/

Table 3. Adverse drug reactions (n = 67)

JCOG toxicity grading	No. of patients				Total, n (%)
	Grade 1	Grade 2	Grade 3	Grade 4	
Non-hematological toxicity	31	27	1	0	59 (88)
General					
Fever	18	23	0	0	41 (61)
Chills	17	3	0	0	20 (30)
Burning sensation	16	0	0	0	16 (24)
Headache	14	2	0	0	16 (24)
Asthenia	13	1	0	0	14 (21)
Pain	9	2	0	0	11 (16)
Thirst	5	0	0	0	5 (8)
Numbness	2	1	0	0	3 (5)
Flu-like reaction	3	0	0	0	3 (5)
Facial flushing	3	0	0	0	3 (5)
Back pain	3	0	0	0	3 (5)
Infection	0	0	1	0	1 (2)
Cardiovascular					
Hypotension	7	0	0	0	7 (10)
Tachycardia	4	0	0	0	4 (6)
Respiratory					
Throat discomfort	10	0	0	0	10 (15)
Cough	4	0	0	0	4 (6)
Rhinorrhea	3	0	0	0	3 (5)
Digestive					
Nausea	8	0	0	0	8 (12)
Vomiting	0	3	0	0	3 (5)
Nervous system					
Dizziness	3	0	0	0	3 (5)
Skin/appendages					
Sweating	8	2	0	0	10 (15)
Pruritus	9	0	0	0	9 (13)
Rash	5	1	0	0	6 (9)
Hematological toxicity	9	9	7	4	29 (43)
Leukopenia	11	9	4	1	25 (37)
Neutropenia	6	5	7	4	22 (33)
Anemia	0	0	0	0	0 (0)
Thrombocytopenia	2	0	0	0	2 (3)
Abnormal laboratory findings	12	7	0	1	20 (30)
ALT (s-GPT)	5	0	0	1	6 (9)
AST (s-GOT)	6	0	0	1	7 (10)
Total bilirubin	–	1	1	0	2 (3)
ALP	4	0	0	0	4 (6)
Hyponatremia	2	1	0	0	3 (4)
Hyperglycemia (n = 61)	0	2	0	0	2 (3)
Proteinuria (n = 58)	2	2	0	0	4 (7)
Hematuria (n = 58)	1	2	0	0	3 (5)

An adverse drug reaction was defined as any adverse event which was related to rituximab or whose relationship to rituximab was unknown. Grading was made according to the Japan Clinical Oncology Group Toxicity Criteria, an expanded version of the National Cancer Institute–common toxicity criteria, version 1.0. Frequent (>3%) or grade 3 non-hematological toxicities and abnormal laboratory findings, and all hematological toxicities observed during treatment and during the follow-up period (for 6 months after the first rituximab infusion) are listed.

or total bilirubin was observed in 10 patients (15%). Out of the 10, one patient, who had developed a flu-like syndrome, asthenia and jaundice 5 days after the final rituximab infusion, demonstrated grade 4 AST and ALT elevations along with grade 3 total bilirubin elevation. He was diagnosed as having developed acute hepatitis and was hospitalized. The patient had a history of TT virus infection and the TT virus-DNA [28, 29] was detected at the time of the event, while hepatitis B virus surface, core and envelope antigens were all negative; antibodies to hepatitis A and C virus were also negative. The patient recovered 32 days after the onset of the syndrome with conservative management. In addition to routine laboratory testing, examination of serum C-reactive protein (CRP) was performed for all 67 patients. Elevation (≥1.0 mg/dl) of CRP values was observed in 14 patients (21%). All non-hematological toxicities, including abnormal laboratory findings, were reversible.

Infection

Within 6 months after the initiation of rituximab administration, 37 episodes of infection or suspected infection (events for which antibiotic, anti-fungal and/or anti-viral agents were prescribed) were reported in 28 patients, including one patient who developed a grade 3 upper-respiratory tract infection and the patient described above who developed acute TT virus-positive hepatitis.

Early death

Two patients died within 30 days following the last rituximab infusion. They showed rapid lymphoma progression during rituximab treatment and were withdrawn early from the study. They both received salvage chemotherapy 5 or 7 days after withdrawal and developed grade 4 neutropenia and septic shock leading to death 14 days and 15 days after the initiation of the chemotherapy, respectively.

PB T- and B-cell counts, and serum immunoglobulins

All 67 patients receiving rituximab exhibited a marked decrease in CD19- and CD20-positive cells after the first rituximab infusion (data not shown). On the other hand, no change was observed in CD3-positive cells. Changes in the mean percentage ± standard deviation (SD) of CD19- and CD20-positive cells in the PB from immediately before the first rituximab infusion until 2 days thereafter were 8.5 ± 9.4% to 0.5 ± 0.3% and 9.4 ± 10% to 0.4 ± 0.7%, respectively. There was little change in serum immunoglobulin levels (IgG, IgA and IgM) for 12 months (data not shown).

HACA development

The number of patients whose sera were tested for HACA at 3 and 6 months or thereafter were 40 and 25, respectively. HACA was not detected in these patients.

Factors affecting ORR and PFS

Univariate and multivariate analyses of pretreatment factors affecting ORR and PFS were performed in 53 patients who were eligible and evaluable. As shown in Table 4, elevated LDH and primary chemorefractoriness were found to be unfavorable factors significantly affecting ORR and PFS in the univariate and multivariate analyses. In the univariate analysis, PFS in patients in the low/low-intermediate risk group according to IPI was longer than that in patients in the high-intermediate/high risk group ($P = 0.034$). PFS in patients with a history of AHSCT was also longer than that in patients without it ($P = 0.045$).

Pharmacokinetic parameters and correlation with responses

Serum rituximab levels were determined in seven responders and five non-responders whose planned rituximab treatments were completed. As shown in Table 5, the mean ± SD values of trough levels and AUCs of the responders and the non-responders were 59.7 ± 11.4 and 43.0 ± 6.4 μg/ml and 608 585 ± 147 373 and 383 053 ± 176 903 μg.h/ml, respectively, and there were significant differences between the two groups ($P = 0.021$; $P = 0.037$). In addition, pre-treatment tumor size measured as the sum of the products of the perpendicular diameters (SPD) was inversely correlated with AUC by Spearman's rank order correlation analysis (coefficient: $r = -0.566$, $P < 0.05$) (data not shown). There were no significant differences between the two groups regarding maximum concentration (C_{max}) or serum half-life of rituximab.

Discussion

We report here the findings of a multicenter phase II study in Japan to evaluate the efficacy and feasibility of eight consecutive weekly administrations of rituximab for relapsed or refractory patients with aggressive B-cell lymphoma. The first clinical study of rituximab for aggressive B-cell lymphoma was conducted in Europe by Coiffier et al. [10]. The study evaluated rituximab monotherapy in 54 relapsed or elderly untreated patients with aggressive B-cell lymphoma that mainly consisted of DLBCL. Rituximab was given as two dosing schedules: eight consecutive weekly infusions at 375 mg/m^2 (arm A; $n = 28$), or one infusion at 375 mg/m^2 followed by seven consecutive weekly infusions at 500 mg/m^2 (arm B; $n = 26$). The ORR over the two arms was 31% (17/57) including 9% CR (5/54) on the basis of ITT, and there was little difference between the two arms. The most commonly observed AEs were mild to moderate infusion-related reactions such as fever, rigors, hypotension and dyspnea. Slightly more patients experienced serious AEs related to rituximab at 500 mg/m^2 than at 375 mg/m^2 (three versus six cases).

The schedule of administration of rituximab in our study was similar to that of arm A of the European study. The ORR obtained in the present study was 35% on the basis of ITT. The seemingly higher ORR in the present study may be ascribed to the difference in the patient pathological demography. The ORR in DLBCL in the present study was 34% (17/50), which was similar to that of the European study (37%, 11/30). The median TTP of responders in the present study was 245 days, which was also comparable with that observed in the European study (246 days+; $n = 17$). There was little difference in the toxicity profiles between the two studies, while the incidence of non-hematological toxicity was higher in the present study. The high incidence of toxicities in the

Table 4. Pretreatment factors affecting response and progression-free survival (PFS) by univariate and multivariate analyses

Factors affecting overall response rate (ORR)				
	ORR, % (95% CI)	Univariate P^a	Multivariate P^b	Odds ratio (95% CI)
LDH				
Normal	65 (41–85)	0.004**	0.003**	0.12 (0.03–0.49)
Elevated	24 (11–42)			
No. of relapses				
0 (Primary refractory)	22 (6–48)	0.081	0.030*	5.81 (1.18–28.5)
Relapsed one or two times	49 (31–66)			
Factors affecting progression-free survival (PFS)				
	Median PFS, days (95% CI)	Univariate P^c	Multivariate P^d	Risk ratio (95% CI)
LDH				
Normal	156 (85–267)	0.002**	0.0002**	4.47 (2.04–9.81)
Elevated	27 (21–48)			
No. of relapses				
0 (primary refractory)	27 (10–52)	0.005**	0.0004**	0.25 (0.12–0.54)
Relapsed one or two times	85 (40–216)			

[a] P value by Fisher's exact test.
[b] P value by logistic regression model (stepwise procedure).
[c] P value by log-rank test.
[d] P value by Cox's proportional hazard model (stepwise procedure).
Statistically significant difference at *P <0.05 and **P <0.01.
CI, confidence interval; LDH, lactate dehydrogenase; ORR, overall response rate.

Table 5. Pharmacokinetic parameters of responders and non-responders

	Responders, mean ± SD (n = 7)	Non-responders, mean ± SD (n = 5)	P^a
Trough (μg/ml)	59.7 ± 11.4	43.0 ± 6.4	0.015[b]
C_{max} (μg/ml)	502.9 ± 123.4	398.8 ± 52.2	0.109
$t_{1/2}$ (h)	517.1 ± 165.9	314.5 ± 153.8	0.057
AUC (μg·h/ml)	608 585 ± 147 373	383 053 ± 176 903	0.037[b]

[a] Student's t-test.
[b] Significant difference at P <0.05.
AUC, area under the concentration–time curve; C_{max}, maximum concentration; SD, standard deviation; $t_{1/2}$, serum half-life.

present study may partially have resulted from the relatively frequent performance of examinations.

One patient developed grade 4 elevations of AST (2564 IU/l) and ALT (3176 IU/l) concomitantly with grade 3 elevation of total bilirubin after completion of the planned infusion, and was diagnosed with acute hepatitis in the present study. Virus testing revealed that hepatitis viruses A, B and C were negative, but TT virus-DNA was present in his serum. TT virus has been reported to be a novel virus associated with elevation of hepatic transaminase in patients with post-transfusion as well as acute and chronic non-A to G hepatitis [28, 29]. Neither hepatomegaly nor space-occupying lesion was observed on CT films in this patient. Pretreatment transaminase levels were all within normal ranges. Moreover, the acute hepatitis resolved without particular treatment, suggesting that TT virus might have been causative for the hepatitis.

The incidence of grade 4 hematological toxicity was 6%, which was very similar to that in the European study (arm A, 6%; arm B, 8%) [10]. Out of four patients who developed grade 4 neutropenia, three had a history of receiving AHSCT. The remaining patient had a history of three regimens of prior chemotherapy. One of the four patients developed a grade 3 respiratory infection 12 weeks after completion of the final rituximab infusion. The neutrophil count at that time was 10/μl. He was effectively treated with G-CSF and antibiotics. This patient also developed grade 2 herpes zoster concomitantly with grade 4 neutropenia 20 weeks after completion of the final rituximab infusion.

According to the International Non-Hodgkin's Lymphoma Prognostic Factors Project, age >60 years, ECOG PS of 2–4, clinical stage III–IV, elevated LDH and extranodal involvement of two or more organs were significant factors unfavorably affecting OS [27]. In the present study, elevated LDH and primary refractoriness to prior chemotherapy were unfavorable factors

affecting both ORR and PFS, while other factors as listed in IPI were not unfavorable. However, when we compared the median PFS of the low/low-intermediate subgroup with that of the high-intermediate/high subgroup, there was a significant difference, suggesting that IPI is an important predictor of efficacy of rituximab monotherapy. Tsai et al. reported that rituximab has significant activity in intermediate-grade B-cell lymphoma that has relapsed after AHSCT [30]. Similar results were obtained in the present study.

The trough levels and AUCs of rituximab were significantly higher in the responders than in the non-responders. Berinstein et al. reported, based on their analyses of the pivotal study in the USA, that there was a correlation between response and serum rituximab level [31]. In our previous study of indolent B-cell lymphoma, patients with higher serum rituximab levels had longer PFS [9]. These results suggest that PK-guided treatment may be worthy of future investigations to further improve the efficacy of rituximab.

In conclusion, rituximab monotherapy is effective in relapsed or refractory patients with aggressive B-cell lymphoma with acceptable toxicity. Several pretreatment variables, including refractoriness to prior chemotherapy, elevated LDH and higher IPI score, and serum rituximab level are useful for predicting the efficacy of rituximab. Further investigations on rituximab-incorporating combination chemotherapy are warranted for improving the outcome in untreated and relapsed or refractory patients with B-cell lymphoma.

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