Standard Japanese Edition, version 1.1; Scientific Consulting, Apex, NC, USA).

Statistical methods

The ORR, percentage CR, and their 95% confidence intervals (CIs) were calculated with per protocol sets (PPS) of data for all eligible patients and full analysis sets (FAS) of data for all enrolled patients under the F-distribution. The median PFS time, time to CR (TTCR) and time to response (TTR), and their 95% CIs were estimated for all eligible and evaluative patients using the method of Kaplan and Meier, and were compared using the log–rank test. In addition, pretreatment factors affecting the ORR and PFS were analyzed for all eligible and evaluative patients by univariate and multivariate analyses using Fisher's exact test, Wilcoxon's rank sum test, the log–rank test, the logistic regression model or Cox's proportional hazard regression model.

All statistical analyses were performed using SAS software (version 6.12; SAS Institute, Cary, NC, USA). Data used for theses analyses were finally confirmed on March 31, 2004.

Results

Patient characteristics

A total of 69 patients were enrolled from 21 institutions (see Appendix I); 34 patients were allocated to Arm C and 35 patients to Arm S. Patient characteristics at study entry are summarized in Table 1. The median age was 52 years (range, 26–69 years). The major characteristics of the two arms were very similar in both the enrolled and eligible patients. Retrospectively, we analyzed the Follicular Lymphoma International Prognostic Index (FLIPI) in all patients.[29] FLIPI was equally distributed between the two arms. Twenty-eight patients (82%) in Arm C and 30 patients (86%) in Arm S were judged

Table 1. Patient characteristics

Factor	Enrolled (n = 69)			Eligible (n = 66)		
	Arm C	Arm S	Total	Arm C	Arm S	Total
Sex						
Female	18	18	36	17	18	35
Male	16	17	33	15	16	31
Age (years)						
Median	53	50	52	54.5	49.5	52.5
Range	36–65	26–69	26–69	36–65	26–69	26–69
Performance status (ECOG)						
0	29	30	59	28	29	57
1	5	5	10	4	5	9
Histopathology (REAL)†						
Follicular, grade 1	12	11	23	11	11	22
Follicular, grade 2	21	19	40	20	19	39
Follicular, grade 3	0	2	2	0	2	2
Marginal zone B-cell	1	0	1	1	0	1
Low grade B-NHL, NOS‡	0	2	2	0	2	2
No specimen submitted§	0	1	1	0	0	0
Clinical stage (Ann Arbor)						
III	14	15	29	13	14	27
IV	20	20	40	19	20	39
B-symptoms						
Absent	30	33	63	29	32	61
Present	4	2	6	3	2	5
LDH						
Normal	32	31	63	31	30	61
Elevated	2	4	6	1	4	5
No. of extranodal sites						
0–1	25	26	51	24	25	49
≤2	9	9	18	8	9	17
International Prognostic Index						
Low	21	21	42	21	20	41
Low-intermediate	12	12	24	10	12	22
High-intermediate	1	1	2	1	1	2
High	0	1	1	0	1	1
Follicular Lymphoma International Prognostic Index						
Low	16	15	31	16	15	31
Intermediate	12	15	27	10	14	25
High	6	5	11	5	5	10

†According to the diagnosis by the central pathology review. ‡Low-grade B-cell non-Hodgkin lymphoma (NHL) not otherwise specified. §Specimen was not submitted to the central pathology review. LDH, lactic dehydrogenase.

Table 2. Response to therapy

Arm		n	No. of patients achieving response						Response rate (95% CI)	
			CR	CRu	PR	SD	PD	NE	%CR	ORR
Arm C	Eligible	32	19 21 30	2	9	1	0	1	66% (47–81%)	94% (79–99%)
	Enrolled	34	21 23 33	2	10	1	0	0	68% (50–83%)	97% (85–100%)
Arm S	Eligible	34	22 23 33	1	10	0	0	1	68% (50–83%)	97% (85–100%)
	Enrolled	35	21 23 33	1	10	0	0	2	66% (44–81%)	94% (81–99%)

Response to each therapy was evaluated according to the International Workshop Criteria for Non-Hodgkin's Lymphoma. CI, confidence interval; CR, complete response; CRu, complete response/unconfirmed; NE, not evaluative due to insufficient follow-up; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

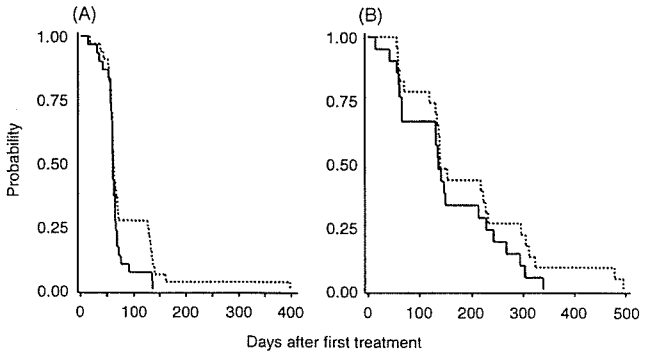

Fig. 1. (A) Time to response (TTR) and (B) time to complete response (TTCR). Medians were estimated by the Kaplan-Meier method. A total of 63 patients (Arm C [-], 30; Arm S [---], 33) were analyzed for TTR, and 44 patients (Arm C, 21; Arm S, 23) for TTCR with per protocol sets of data. Median TTRs in Arm C and Arm S were 61 days (95% confidence interval [CI] 59 to 65 days) and 62 days (95% CI 60–70 days), respectively. The 75th percentile TTRs in Arm C and Arm S were 66 days (95% CI 63 to 76 days) and 140 days (95% CI 66–135 days), respectively ($P = 0.0994$, log–rank test). Median TTCRs in Arm C and Arm S were 136 days (95% CI 65 to 213 days) and 140 days (95% CI 134–227 days), respectively. The 75th percentile TTCRs in Arm C and Arm S were 228 days (95% CI 141 to 293 days) and 295 days (95% CI 153–323 days), respectively ($P = 0.2201$, log–rank test).

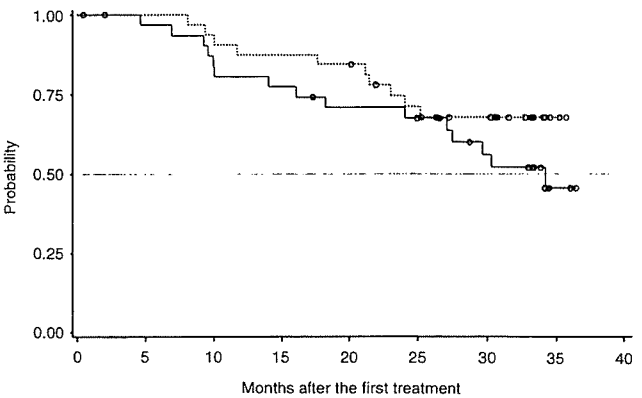

Fig. 2. Progression-free survival (PFS). Medians were estimated by the Kaplan-Meier method. The upper limit of the 95% confidence interval (CI) for Arm C has not yet been determined. A total of 65 patients (Arm C, 32; Arm S, 33) were analyzed with per protocol sets of data. The median PFS time for patients in Arm C (-) was 34.2 months (95%CI, 27.1 months, inestimable), whereas that for patients in Arm S (...) had not yet been reached, with a median follow-up time of 28.2 months. Log–rank test, $P = 0.220$. (o) Censored.

to belong to the low, or low-intermediate risk group categorized by FLIPI. Three patients were judged ineligible by an extramural review committee, because two of them had concomitant active cancer and one had a history of prior chemotherapy, including doxorubicin for the treatment of breast cancer. Sixty-five patients (94%) were confirmed to have FL in the central pathology review.

Response to treatment and survival

Sixty-six eligible patients (Arm C, 32 patients; Arm S, 34 patients) were evaluated with PPSs of data, and 69 patients (Arm C, 34 patients; Arm S, 35 patients) with FASs of data. One patient allocated to Arm C could not be evaluated for response because the first cycle of chemotherapy given was not CHOP (doxorubicin in the CHOP regimen was erroneously replaced with daunorubicin). Two patients (one patient eligible and one ineligible) allocated to Arm S could not be evaluated because they had withdrawn from the study before starting treatment.

As shown in Table 2, similar results of the ORRs and the percentage CRs were obtained in Arm C and Arm S. The ORRs and percentage CRs calculated with PPSs and FASs were similar. Kaplan-Meier curves of TTR and TTCR were plotted for eligible and evaluative patients in each arm, as shown in Fig. 1. Although the median TTRs for patients in Arm C and Arm S were not different (61 days *versus* 62 days, respectively), the 75th percentile TTRs for patients were shorter in Arm C (66 days) than Arm S (127 days), with no statistical difference ($P = 0.0994$, log–rank test). The median TTCRs were similar in Arm C and Arm S (136 days and 140 days, respectively). As shown in Fig. 2, the median PFS time for patients in Arm C ($n = 32$) was 34.2 months

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Table 3. Hematological toxicity

Toxicity	Arm	*n*	Grade 0–2	Grade 3	Grade 4
Any hematological toxicity	Arm C	34	2 (6%)	3 (9%) 32 (94%)	29 (85%)
	Arm S	33	0 (0%)	10 (30%) 33 (100%)	23 (70%)
Leukopenia	Arm C	34	5 (15%)	16 (47%) 29 (85%)	13 (38%)
	Arm S	33	3 (9%)	23 (70%) 30 (91%)	7 (21%)
Neutropenia	Arm C	34	2 (6%)	3 (9%) 32 (94%)	29 (85%)
	Arm S	33	1 (3%)	9 (27%) 32 (97%)	23 (70%)
Thrombocytopenia	Arm C	34	32 (94%)	1 (3%) 2 (6%)	1 (3%)
	Arm S	33	33 (100%)	0 (0%) 0 (0%)	0 (0%)
Anemia	Arm C	34	31 (91%)	3 (9%)	–
	Arm S	33	31 (94%)	2 (6%)	–

Hematological toxicity was evaluated according to the JCOG Toxicity Criteria, an expanded version of the NCI-CTC version 1.0. All hematological toxicities (possibly related to rituximab, or unknown relationship to rituximab) observed during the treatment and follow-up period (for 6 months after the last cycle of CHOP for Arm C, and for 4 months after the last rituximab infusion for Arm S) are listed.

(95%CI, 27.1 months – inestimable), whereas that for patients in Arm S ($n = 33$) had not yet been reached, with a median follow-up time of 28.2 months. One patient (#38) in Arm S died of tumor progression 730 days after the first treatment. No other patients died within approximately 3 years of observation.

Adverse events

Information about AEs was available for 67 patients (Arm C, 34 patients; Arm S, 33 patients) who received protocol treatment. Hematological toxicity was documented at its highest grade throughout the study period. As shown in Table 3, major hematological toxicity was neutropenia; grade 3 or greater neutropenia was observed in 32 patients (94%) in Arm C and in 33 patients (100%) in Arm S; grade 4 neutropenia was seen in 29 patients (85%) in Arm C and in 23 patients (70%) in Arm S. All hematological toxicities were controllable and reversible, although some patients required hematopoietic cytokines.

Grade 3 or greater non-hematological AEs observed during treatment and initial follow-up periods are listed in Table 4. A total of 11 patients (Arm C, seven patients, 21%; Arm S, four patients, 12%) developed 14 events of grade 3 or greater non-hematological adverse events. All non-hematological toxicities were reversible. There was no therapy-related death.

Prognostic factors

Pretreatment factors affecting ORR and PFS were analyzed. Because the sample size of each arm was small, analyses were not performed separately for the two arms, but results were pooled ($n = 64$). There were two factors affecting ORR when analyzed by the Wilcoxon's rank sum test. Patients with PS 0 (41CR, 13PR, 1NC, 0 PD) demonstrated a superior response to those with PS 1 (3CR, 6PR, 0NC, 0PD) ($P = 0.0182$, Wilcoxon's rank-sum). Patients with a tumor size <5 cm (32CR, 6PR, 1NC, 0 PD) had a superior response to those with tumors equal to 5 cm (12CR, 13PR, 0NC, 0 PD) ($P = 0.0066$, Wilcoxon's rank-sum).

However, no factor significantly affected PFS. Multivariate analyses were also performed using the same factors, excluding IPI. There was no factor that independently affected ORR and PFS.

HACA and pharmacokinetics of rituximab

Out of 67 patients who received rituximab, HACA assays were performed for 65 patients (Arm C, 33; Arm S, 32) at 8 months after treatment, and for 64 patients (Arm C, 33; Arm S, 31) at 10 months after treatment. No patient developed HACA. For all 27 patients (Arm C, 14; Arm S 13) who received four rituximab infusions and whose planned monitoring of

Table 4. Grade 3 or greater-non-hematological adverse events

Arm	Patient	Serious adverse event†	Grade‡	Onset timing	Relating drug (causative)
Arm C ($n = 32$)	#04	Hyperglycemia	3	6th cycle (day 4)	CHOP (diabetes)
	#07	Hyperglycemia	3	4th cycle (day 2)	CHOP, rituximab
	#13	Hypertension	3	1st cycle (day 3)	CHOP, rituximab
	#21	Total bilirubin elevation	3	2nd cycle (day 5)	– (constitutional)
	#23	Abdominal pain	3	1st cycle (day 9)	CHOP, rituximab
	#58	Acute cholangitis with elevated AST and ALT	3	3rd cycle (day 10)	CHOP, rituximab
	#59	Hyperglycemia, hypertension	3	5th cycle (day 6)	CHOP, rituximab
Arm S ($n = 33$)	#25	Total bilirubin elevation	3	6th cycle (day 132)	– (constitutional)
	#56	Diarrhea	4	1st cycle (day 13)	– (alimentary)
		Febrile neutropenia	3	3rd cycle (day 12)	CHOP
		Interstitial pneumonia	3	3rd cycle (day 15)	CHOP
	#62	Total bilirubin elevation	3	4th cycle (day 7)	CHOP
	#69	AST and ALT elevation	3	1st cycle (day 10)	CHOP
				2nd cycle (day 8)	CHOP
				6th cycle (day 29)	CHOP

†Grade 3 or greater adverse events other than hematological toxicities that were observed during the treatment and follow-up period (for 6 months after the last cycle of CHOP for Arm C, and for 4 months after the last rituximab infusion for Arm S). ‡JCOG Toxicity Criteria, an expanded version of the NCI-CTC, version 1.0.

Table 5. Pharmacokinetic parameters of rituximab

Arm		Dose (mg/day)	AUC (μg. h/mL)	Cmax† (μg/mL)	$T_{1/2}$ (h)	Clearance‡ (litter/h)	MRT (h)	Vd (litter)
Arm C	Mean	593.9	372 498.9	262.5	232.3	0.0259	335.1	4.49
(n = 14)	SD	51.1	111 660.4	73.2	113.8	0.0301	164.2	0.66
Arm S	Mean	596.4	418 901.3	433.5	356.9	0.0128	514.9	5.57
(n = 13)	SD	82.6	107 002.6	134.9	163.4	0.0077	235.9	1.95

†Actual measured value. ‡Calculated under the one-compartment model. Time points for serum collection were as follows; Arm C: before, and 10 min and 2 days after each rituximab infusion, and 1 week, 1, 4 and 6 months after the sixth rituximab infusion. Arm S: before, 10 min after each rituximab infusion and 2 days, 1 and 2 weeks, and 1 and 4 months after the sixth rituximab infusion. AUC, area under the curve; Cmax, maximum concentration; $T_{1/2}$, elimination half-life; MRT, mean residence time; Vd, volume of distribution.

serum rituximab levels were completed, pharmacokinetic parameters were calculated throughout the four infusions. As shown in Table 5, Arm S showed higher values for the parameters of area under the curve (AUC), maximum concentration (Cmax), elimination half-life (T1/2), mean residence time (MRT), and volume of distribution (Vd).

Discussion

In this randomized phase II trial, we have demonstrated that the combined use of rituximab and CHOP yielded an ORR of 94% and 97%, and a percentage CR of 66% and 68% in the concurrent arm and the sequential arm, respectively. These ORRs and percentage CRs are superior to those reported for combination chemotherapy regimens containing anthracycline without rituximab, which were conducted after stringent clinical staging with CT. The percentage CR obtained by six to eight cycles of CHOP chemotherapy in untreated patients (n = 83) with FL was reported to be 36% (90%CI, 27–46%).[30] The ORR and percentage CR of CHOP chemotherapy obtained by Kimby *et al.* in their randomized study comparing chlorambucil plus prednisone *versus* CHOP in symptomatic low-grade NHL (n = 127), were 60% and 18%, respectively.[31]

Data of the present study was comparable to the preceding study on CHOP combined with rituximab in patients with indolent B-NHL regarding efficacy and tolerability. Although the precise schedule of the administration of rituximab in the first phase II study of R-CHOP reported by Czuczman *et al.* was not the same as that of the present study, the concept of concurrent use is identical between their trial and Arm C in the present study.[14] However, the percentage CR of Arm C is less than that of Czuczman *et al.*'s trial, and the median PFS of Arm C appears to be shorter in the present study, although more than 82% of all enrolled patients in our study were in the low or low-intermediate risk group by FLIPI. In Czuczman *et al.*'s trial, as the last two infusions of rituximab were administered 1 month after the sixth CHOP cycle, like in our sequential arm, the design of Czuczman *et al.*'s trial had characteristics of both the concurrent arm and the sequential arm. So it is possible that the higher percentage CR and longer PFS in Czuczman *et al.*'s trial compared to our concurrent arm were partly due to the mixed design of the administration schedule of rituximab, in addition to the possible selection bias in phase II studies.

The South-west Oncology Group (SWOG) in the USA studied six cycles of CHOP followed by four weekly infusions of rituximab in newly diagnosed patients with FL at advanced stages (31% with bulky disease and 30% with B-symptoms). Sixteen (19%) of the 84 evaluative patients had an improved tumor response after rituximab treatment, with an ORR of 72%, including 54% with a CR or CRu. The PFS was 76% at the median follow-up of 2.7 years.[32] The PFS data of the sequential arm in our trial is similar to that of the SWOG trial.

Cancer and Leukemia Group B (CALGB) conducted a randomized phase II study to explore a more suitable administration schedule of rituximab with fludarabine in previously untreated chronic lymphocytic leukemia (CLL) patients.[33] Patients randomly received either six monthly courses of fludarabine concurrently with rituximab followed 2 months later by four weekly doses of rituximab for consolidation therapy, or fludarabine alone followed 2 months later by rituximab consolidation therapy. The ORR with the concurrent regimen was 90% compared to 77% with the sequential regimen. With a median follow-up time of 23 months, the number of relapsed patients was 18 (35%) in the concurrent regimen and 15 (28%) in the sequential regimen. Although PFS and survival appeared to be somewhat longer with the sequential treatment, CALGB concluded that the concurrent use of rituximab and fludarabine was superior. Our randomized phase II study for indolent B-cell NHLs showed similar percentage ORRs and percentage CRs between the two arms, and a seemingly longer PFS in the sequential arm. Because patients in the concurrent arm in the CALGB study received consolidated administration of rituximab after induction therapy, the concurrent arm in the CALGB study had characteristics of the concurrent arm and sequential arm of our present study.

In a randomized phase III study that compared eight cycles of R-CVP to CVP for previously untreated patients with advanced FL, a significantly prolonged TTP of R-CVP was reported (median 32 months *versus* 15 months for CVP; $P < 0.0001$).[18] The median TTP of R-CVP was similar to the median PFS of Arm C in our study. As the toxicity is stronger in CHOP than CVP, it is worthwhile to conduct a randomized phase III trial to compare R-CHOP to R-CVP.

The maintenance use of rituximab after first-line rituximab therapy was also reported to prolong PFS or event-free survival (EFS).[34,35] Future trials to explore the role of maintenance use of rituximab after first-line rituximab containing chemotherapy like Arm C are warranted.

About 25% of patients in Arm S did not achieve a response (PR or higher) before the initiation of rituximab treatment, despite the completion of six cycles of CHOP. In Arm C, more than 90% of patients showed a response after the six cycles of CHOP plus rituximab. The same tendency was also shown in the TTCR, as shown in Fig. 1B. The TTCR of each patient in Arm C was relatively shorter than that in Arm S.

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While grade 3 or greater non-hematological AEs were observed in 11 patients (Arm C, seven patients, 21%; Arm S, four patients, 13%), both arms were well tolerated. Two patients were withdrawn from the study before completion of the planned treatment by AE. One patient in Arm C developed acute cholangitis after the third cycle of CHOP plus rituximab. The other patient in Arm S developed interstitial pneumonia after the third cycle of CHOP. Both patients fully recovered. Hematological toxicities were observed in all treated patients; grade 4 neutropenia was frequent and was observed in 85% of patients in Arm C and in 70% in Arm S. However, these hematological toxicities were manageable with or without supportive care using hematopoietic growth factor. No patient was withdrawn from the study due to hematological toxicity. Grade 3 or greater thrombocytopenia was rare in Arm C and absent in Arm S. Although hematological and non-hematological toxicities were slightly more frequent in Arm C, toxicities were clinically acceptable in both arms.

In conclusion, CHOP combined with rituximab was highly effective in untreated patients with indolent B-NHL, especially FL, either in a concurrent or sequential combination, with acceptable toxicities. Although the time to achieve a response was more rapid with the concurrent combination than the sequential combination, PFS appeared to be slightly longer with the sequential combination, although the difference was not statistically significant. We conclude that both combination schedules deserve further investigation. Considering the promising results of rituximab maintenance therapy reported by other investigators, it would be worthwhile to conduct future trials to establish the role of rituximab maintenance after concurrent and sequential combinations of rituximab plus CHOP therapy.

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Appendix

Participating institutions and principal investigators of the IDEC-C2B8 Study Group included: Sapporo National Hospital (K. Aikawa, M. Nakata), Sapporo Hokuyu Hospital (M. Kasai, Y. Kiyama), Tochigi Cancer Center (Y. Kano, M. Akutsu), International Medical Center of Japan (A. Miwa, N. Takesako), National Cancer Center Hospital East (K. Itoh, T. Igarashi, K. Ishizawa), National Cancer Center Hospital (K. Tobinai, Y. Kobayashi, T. Watanabe), Tokyo Medical University (K. Ohyashiki, T. Tauchi), Tokai University School of Medicine (T. Hotta, T. Sasao), Hamamatsu University School of Medicine (K. Ohnishi), Aichi Cancer Center Hospital (Y. Morishima, M. Ogura, Y. Kagami), Nagoya University School of Medicine (T. Kinoshita, T. Murate, H. Nagai), Nagoya National Hospital (K. Tsushita, H. Ohashi), Mie University School of Medicine (S. Kageyama, M. Yamaguchi), Kyoto Prefectural University of Medicine (M. Taniwaki), Kyoto University School of Medicine (H. Ohno, T. Ishikawa), Shiga Medical Center for Adults (T. Suzuki), Center for Cardiovascular Diseases and Cancer, Osaka (A. Hiraoka, T. Karasuno), Hyogo Medical Center for Adults (T. Murayama), Hiroshima University School of Medicine (A. Sakai), National Kyushu Cancer Center (N. Uike), Nagasaki University School of Medicine (T. Maeda, K. Tsukasaki).

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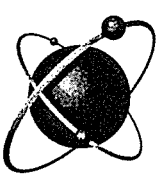
4. Antibody Therapy for Malignant Lymphoma

Kensei Tobinai



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□ SYMPOSIUM II □

4. Antibody Therapy for Malignant Lymphoma

Kensei Tobinai

Abstract

Rituximab, a genetically engineered, chimeric anti-CD20 monoclonal antibody, induces the apoptosis of B-lymphoma cells, in addition to the lyses by complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC), as shown in Fig. 1 (1). A Japanese phase I study of rituximab in relapsed or refractory patients with B-cell non-Hodgkin's lymphoma (B-NHL) showed an overall response rate (ORR) of 64% (7/11) with minimal toxicities. Elimination half-life ($T_{1/2}$) of serum rituximab was 445± 361 hours, and the serum rituximab was detectable at three months. In the subsequent phase II study, 90 relapsed or refractory patients with indolent B-NHL or mantle cell lymphoma (MCL) were treated with rituximab at 375 mg/m^2×4 weekly infusions. ORRs in indolent B-NHL and MCL were 61% (37/61) and 46% (6/13), respectively. In this presentation, the results of clinical trials of antibody therapy of malignant lymphoma are summarized, focusing on the two recent Japanese multicenter trials of rituximab and a Japanese feasibility study of anti-CD20 radioimmunotherapy with yttrium-90-lableled ibritumomab tiuxetan (2).

Key words: antibody therapy, malignant lymphoma, CD20, rituximab, radioimmunotherapy

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1. Chimeric Anti-CD20 Antibody, Rituximab

1) Phase II study of rituximab in relapsed or refractory aggressive B-NHL

To evaluate the efficacy and feasibility of rituximab monotherapy in Japanese patients with relapsed or refractory aggressive B-NHL, a multicenter phase II study was conducted (3). Sixty-eight patients were enrolled and treated with rituximab at 375 mg/m^2 by eight consecutive weekly infusions. The ORRs of the 68 enrolled patients and of the 57 eligible patients were 35% and 37%, respectively. The median progression-free survival (PFS) of the 53 evaluable patients was 52 days, whereas the time to progression of the 21 eligible responders was 245 days. Elevated serum lactate dehydrogenase (LDH) and refractoriness to prior chemotherapy unfavorably affected ORR and PFS ($P<0.01$). Serum trough levels of rituximab and the area-under-the-curve (AUC) for responders were higher than for non-responders ($P<0.05$). In conclusion, treatment with eight weekly infusions of rituximab has significant anti-lymphoma activity for relapsed or refractory aggressive B-NHL (3).

2) Randomized phase II study of rituximab plus CHOP (R-CHOP) in untreated indolent B-NHL

To explore the more promising administration schedule of R-CHOP for indolent B-NHL for further investigations, this randomized phase II study was conducted (4). Untreated patients with advanced, indolent B-NHL were randomized to receive either six courses of CHOP concurrently with rituximab (Arm C) or sequential six courses of CHOP followed by six courses of weekly rituximab (Arm S). The primary endpoint was ORR. Sixty-nine patients were randomized to Arm C (n=34) and Arm S (n=35). ORR with Arm C was 94% (95% confidence interval [CI], 79-99%) including 66% of complete response (CR) compared with 97% (95% CI, 85-100%) including 68% of CR with Arm S. Patients with Arm C experienced more grade 4 hematologic toxicities (85% vs. 70%) and grade 3 or 4 non-hematologic toxicities (15% vs. 9%) as compared with Arm S. Both arms were well tolerated. With a median follow-up time of 28.2 months, the median PFS time was 1,026 days in Arm C, and has not been reached in Arm S ($P=0.227$). IgH/Bcl-2 copy numbers, especially in peripheral blood, decreased more rapidly in Arm C than in Arm S. In conclusion, R-CHOP is highly effective for untreated indolent B-NHL either by concurrent or sequential combination. The time to

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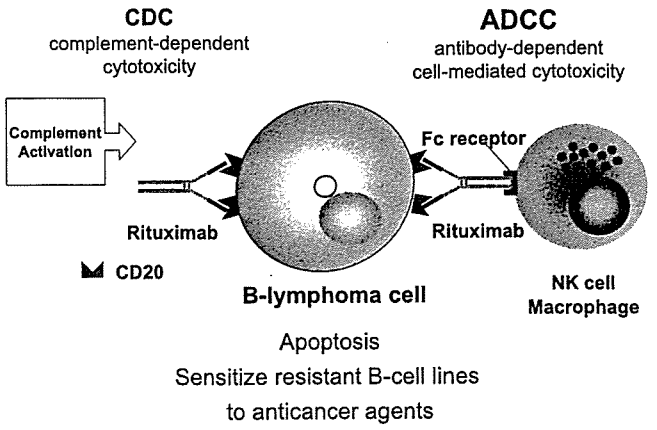

Figure 1. Putative Mechanism of Action of Rituximab.

response was more prompt with the concurrent combination, whereas PFS appears to be longer with the sequential combination. Minimal residual disease (MRD) can be effectively eradicated either by the concurrent or sequential combination;however, rapid clearance of MRD by the concurrent combination may not lead to the prolongation of PFS.

2. Radioimmunotherapy of B-cell NHL with Yttrium-90-labeled, Murine Anti-CD20 Antibody, Ibritumomab Tiuxetan

Ibritumomab is a murine anti-CD20 monoclonal antibody that was engineered to form rituximab. Tiuxetan is a MX-DTPA linker chelator that is attached to ibritumomab to form ibritumomab tiuxetan (Zevalin™). The ibritumomab tiuxetan is radiolabeled with either ^{111}In (^{111}In-Zevalin™) for imaging or dosimetry studies or with ^{90}Y (^{90}Y-Zevalin™) for therapy of B-NHL (5). Between 2002 and 2003, a phase I and feasibility study of ibritumomab tiuxetan was conducted for Japanese patients with B-NHL at the National Cancer Center. In this study, ten patients had relapsed or refractory B-NHL, including nine with follicular lymphoma and one with mantle cell lymphoma, and eight of them had been treated with rituximab. The encountered toxicities were primarily hematologic. None of the three patients in the 0.3 mCi/kg cohort developed critical toxicities, whereas two of the six patients in the 0.4 mCi/kg cohort developed critical toxicities, including one patient who developed long-lasting neutropenia and thrombocytopenia. Among the ten enrolled patients, seven showed objective responses, including five patients achieving CR and two patients achieving partial response (PR). Based on these results, we concluded that yttrium-90-labeled, murine anti-CD20 antibody, ibritumomab tiuxetan is highly effective for relapsed or refractory patients with indolent B-NHL with acceptable toxicities, and that the recommended phase II dose was 0.4 mCi/kg. Subsequently, we conducted a pivotal multicenter phase II study of ibritumomab tiuxetan for relapsed or refractory indolent B-NHL.

Summary

Rituximab, a chimeric anti-CD20 antibody, was approved for indolent B-NHL in Japan in 2001, and was approved for aggressive B-NHL in 2003. French, US and German phase III studies indicated that rituximab plus CHOP is a new standard therapy for aggressive B-NHL. A Japanese phase I study of an anti-CD20 radioimmunoconjugate, ibritumomab tiuxetan, showed high efficacy with acceptable toxicities, and a pivotal phase II study was completed. Monoclonal antibody therapies will have significant roles in the treatment of malignant lymphoma.

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Phase I study of radioimmunotherapy with an anti-CD20 murine radioimmunoconjugate (^{90}Y-ibritumomab tiuxetan) in relapsed or refractory indolent B-cell lymphoma

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We conducted a phase I study to evaluate the safety and efficacy of radioimmunotherapy with yttrium-90-ibritumomab tiuxetan (Y2B8) in Japanese patients with relapsed or refractory indolent B-cell lymphoma. Indium-111-labeled ibritumomab tiuxetan (In2B8; 3.5 or 5 mCi [129.5 or 185 MBq]) was administered on day 1, followed by serial gamma-camera imaging to investigate the distribution of In2B8 in the whole body of patients and to judge the feasibility of Y2B8 administration. Y2B8 with a dose of 0.3 mCi/kg (11.1 MBq/kg) or 0.4 mCi/kg (14.8 MBq/kg) was administered on day 8. Grade 4 neutropenia and grade 3 thrombocytopenia were observed in three of nine of the patients evaluated for safety. Critical toxicities (prolonged thrombocytopenia or severe non-hematological toxicities) were observed in two of six patients in the 0.4 mCi/kg (14.8 MBq/kg) dose group but were not seen in any of the three patients in the 0.3 mCi/kg (11.1 MBq/kg) dose group. The non-hematological toxicities of the nine patients were of grade 2 or less, except in two patients who had been heavily treated previously. They experienced critical toxicities such as infection, diarrhea, hyponatremia and prolonged thrombocytopenia, as well as other frequent grade 2 non-hematological toxicities. Although the pharmacokinetic profiles were similar to those in the US study, one of the two patients was clarified retrospectively as showing abnormal biodistribution of In2B8 in the bone marrow, as judged by an independent third party panel of radiologists. Five of the 10 participants achieved complete responses or unconfirmed complete responses and two partial responses. In conclusion, the recommended dose of Y2B8 for the subsequent phase II study for Japanese patients is 0.4 mCi/kg (14.8 MBq/kg). This dose of radioimmunotherapy was feasible when patients with altered biodistribution of In2B8 were excluded, and it was highly effective. (*Cancer Sci* 2005; 96: 903–910)

Indolent B-cell non-Hodgkin lymphoma (NHL) is known to be radiosensitive, as 50% of the patients with localized disease are curable by external-beam radiotherapy. However, 70–80% of patients with indolent B-cell NHL have widespread disease at initial presentation, such that conventional external-beam radiotherapy is not applicable to most patients. To deliver radiation directly to the tumor cells and to minimize the exposure of normal tissue to radiation, radioimmunotherapy (RIT) has been developed. The most common radionuclides used for RIT are iodine-131 (^{131}I) and yttrium-90 (^{90}Y). ^{131}I is available commercially and is familiar to most nuclear medicine departments. Despite these advantages, ^{131}I has several limitations including the long 8-day half-life of the radioisotope, dehalogenation of iodinated antibody in various tissues, hypothyroidism and the gamma emission of ^{131}I, which requires the isolation of patients to prevent exposure of family members and the public to radiation.[1,2]

The radiopharmaceutical ^{90}Y-labeled ibritumomab tiuxetan (Y2B8) is an anti-CD20 murine monoclonal antibody (immunoglobulin G1 kappa) radiolabeled with the isotope ^{90}Y. This agent binds to the surface of B-cell NHL cells that express CD20 antigen, where the decay of accumulated ^{90}Y produces a lethal dose of the beta particle component, thereby causing tumor necrosis. Its mean path length is approximately 5 mm, and therefore irradiation of adjacent normal organs is kept to a minimum. Because it has a different mechanism of action to chemotherapy, Y2B8 is expected to be more suitable for the treatment of bulky or poorly vascularized tumors. It is also expected to be more effective than unconjugated antibody therapy.[3,4]

Previous clinical studies have shown that Y2B8 has a high overall response rate (ORR), exceeding that of the chimeric anti-CD20 monoclonal antibody rituximab alone in patients with chemotherapy-resistant or relapsed follicular or low-grade B-cell NHL.[5] A high ORR has been achieved especially in rituximab-refractory cases.[6]

There have been no previous reports on clinical studies of RIT in Japan. This treatment option is awaited by patients suffering from indolent B-NHL. The feasibility and pharmacokinetics

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of this treatment in Japanese patients are in need of assessment. We therefore conducted a phase I and feasibility study of Y2B8 for relapsed or refractory indolent B-cell NHL to evaluate the safety and efficacy of this treatment, and to estimate the recommended dosage for a subsequent phase II study in Japanese patients.

Patients and Methods

Patient population

The protocol required the relapsed or refractory patients to have histologically confirmed indolent B-cell NHL according to the International Working Formulation[7] (A–E) and the Revised European–American Lymphoma classification[8] (small lymphocytic, lymphoplasmacytoid, mantle cell, follicular center [grades I, II, and III], marginal zone B-cell and splenic marginal zone lymphoma). Those patients who met the following criteria were enrolled in the study: bidimensionally measurable disease with the longest diameter equal to 2.0 cm by computed tomography scan or magnetic resonance imaging; a demonstrable monoclonal CD20-positive B-cell population in lymph nodes by flow cytometry or immunohistochemical examination; neither prior therapy with rituximab for 6 months nor antineoplastic therapy 4 weeks prior to enrollment; and a performance status of 0 or 1 according to the Eastern Cooperative Oncology Group scale.[9] In addition, patients had to be 20–74 years old, not pregnant or lactating, using accepted birth control methods, and had to have a life expectancy of ≥ 3 months. Within 1 week of enrollment patients were required to have acceptable hematological status (neutrophil counts $\geq 1200/mm^3$ and platelet counts $\geq 150\,000/mm^3$), hepatic function (aspartate aminotransferase [AST], alanine aminotransferase [ALT] and alkaline phosphatase levels ≤ 2.5 times normal, total bilirubin level ≤ 2.0 mg/dL) and renal function (serum creatinine concentration ≤ 2.0 mg/dL). Patients were excluded from the study if they had the following conditions: prior stem cell transplantation; external beam radiation therapy to bilateral ilium or whole abdomen; central nervous system lymphoma; chronic lymphocytic leukemia; human immunodeficiency virus-related lymphoma, lymphoma cell counts $> 5000/mm^3$ in peripheral blood; and $\geq 25\%$ bone marrow involvement with lymphoma cells. The study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan, and written informed consent was obtained from all patients.

Study design

The study was an open-label, dose-escalation trial in which imaging with indium-111 (^{111}In)-labeled ibritumomab tiuxetan (In2B8) and administration of 0.3 or 0.4 mCi/kg (11.1 or 14.8 MBq/kg) of Y2B8 were carried out. At first, three patients were enrolled in the 0.3 mCi/kg (11.1 MBq/kg) dose group. When no critical toxicities occurred in these three patients, six patients were enrolled in the 0.4 mCi/kg (14.8 MBq/kg) dose group. When critical toxicities occurred in two or fewer of the six patients, 0.4 mCi/kg (14.8 MBq/kg) was considered to be the recommended dosage of Y2B8 for the subsequent phase II study. The toxicity was evaluated according to the National Cancer Institute's Common Toxicity Criteria, version 2.0 (http://ctep.cancer.gov/reporting/CTC-3test.html). Critical toxicities were defined as: (1) grade 3 or 4 non-hematological toxicities; (2) thrombocytopenia ($< 25\,000$ cells/mm^3) lasting for 3 weeks or more; and (3) grade 4 neutropenia (absolute neutrophil counts < 500 cells/mm^3) lasting for 3 weeks or more.

Y2B8 was to be injected when evaluation of the data obtained by imaging with In2B8 did not reveal an altered biodistribution. Gamma camera imaging was carried out at 2–6 h, 24–48 h, 48–72 h and 90–120 h after injection of In2B8. Altered biodistribution was defined as follows: (1) the blood pool is not visualized on the first image indicating the rapid clearance of In2B8 by the reticuloendothelial system such as the liver, the spleen and the bone marrow; and (2) diffuse uptake of In2B8 in the normal lungs or kidneys is more intense than that in the liver on the image at 48–72 or 90–120 h.

The treatment regimen consisted of the following components: rituximab, In2B8 and Y2B8. Rituximab was provided by Zenyaku Kogyo (Tokyo, Japan). In2B8 and Y2B8 were provided by Nihon Schering KK (Osaka, Japan). The administration of 250 mg/m^2 of rituximab on days 1 and 8 was carried out as described previously.[10,11] Clinical sites were provided with the unlabeled antibody–chelate conjugate, ibritumomab tiuxetan, and radiolabeling was carried out on site with either ^{111}In for gamma camera imaging or ^{90}Y for therapy. Radiometal incorporation of the radiolabeled antibody was determined by instant thin-layer chromatography (TLC) and was required to be 95% or more prior to injection. Radioactivity of ^{90}Y on the TLC was measured by counting bremsstrahlung using a gamma counting system calibrated for ^{90}Y.[12] On day 1, a lead-shielded syringe was filled with a total of 129.5 MBq (only for patient 3) or 185 MBq of the radiolabeled In2B8 solution. ^{111}In-radioactivity in the filled syringe was measured with a dose calibrator and the radioactivity and time of measurement were recorded. Immediately after the rituximab infusion, In2B8 was injected into a line placed in the patient's forearm via a 0.22-μm filter through the infusion port over exactly 10 min. The line was followed by flushing with at least 10 mL of normal saline after the In2B8 injection. The injected ^{111}In-radioactivities were determined by subtracting the radioactivities of the empty syringe and the line for injection from the recorded radioactivities of the syringe filled with In2B8.

On day 8, the patients received a second dose of rituximab followed by Y2B8. The individual volume for injection of 0.3 mCi/kg (11.1 MBq/kg) and 0.4 mCi/kg (14.8 MBq/kg) was determined by the actual measured radioactivity of ^{90}Y and the bodyweight of each patient. Radioactivity of ^{90}Y was measured by counting bremsstrahlung using a dose calibrator.[12] The calibration factor for ^{90}Y measurement was determined in advance. The calculated dose or up to a maximum of 1184 MBq was injected in a way similar to that described for In2B8.

Determination of the blood-to-urine ratios of radioactivities after In2B8 injection was also carried out. For the pharmacokinetic analyses, the area under the concentration–time curve (AUC) and the half-life ($t_{1/2}$) of ^{90}Y in blood were estimated from the data of ^{111}In radioactivities. For efficacy analyses, computed tomography scans of measurable lesions were repeated on all patients 4 and 8 weeks after the infusion of Y2B8. Their responses were assessed according to the International Workshop NHL Response Criteria.[13]

Laboratory testing to monitor the toxicities included hematology and serum chemistry assays, immunoglobulin

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concentrations, and human antichimeric antibody (HACA)/human antimurine antibody (HAMA) assays.

Cases were handled according to the following criteria. For efficacy analysis, all patients who received the investigational products were included, unless they had critical protocol violation. The patients were excluded from the analysis of critical toxicities if they withdrew or discontinued, or if they deviated seriously from the protocol. For the purpose of collecting safety data, a treatment period was defined as the time from the first rituximab infusion to 12 weeks after Y2B8 injection.

The primary variable of the present study is the number of patients who developed critical toxicities. The secondary, safety variables include the incidence of adverse events, the incidence of adverse drug reactions and changes in the laboratory values. The secondary, efficacy variables are the best ORR (the incidence of responders showing complete response (CR), unconfirmed complete response (CRu) and partial response (PR). In addition to the efficacy evaluation carried out at each participating institute, an independent, third-party panel of two radiologists (TT and SN) carried out a central evaluation.

Results

Patients

Eleven patients were enrolled in this study. One patient did not receive the study treatment because of herpes simplex infection. Table 1 lists the demographic and clinical characteristics of 10 patients who received Y2B8. Nine patients with follicular lymphoma and one with mantle cell lymphoma completed one course of ibritumomab tiuxetan treatment defined by the study. Eight of 10 patients were treated previously with rituximab. Patients 8 and 10 had been heavily pretreated with more than seven antineoplastic regimens. Nine patients completed the study, including the observation period. However, one patient (patient 4 in the 0.3 mCi/kg [11.1 MBq/kg] dose group) discontinued the study prematurely due to early death from disease progression during the observation period, and was therefore withdrawn from the critical toxicity analysis (Table 2). The actual mean radiochemical purities of ^{111}In and ^{90}Y were 99.2% (range: 98.6–99.6) and 98.2% (range: 96.7–98.9), respectively.

Safety evaluation

The safety and efficacy results of the radioimmunotherapy are summarized in Table 2. All of the observed hematological and non-hematological toxicities that were considered to be definitely, probably or possibly related to either In2B8 or Y2B8 are listed in Table 3. Hematological toxicities were frequently encountered as reported previously.[3,5,6] The neutrophil and the platelet counts at baseline and nadir are shown in Tables 4 and 5, respectively. The median days from baseline to nadir of the neutrophil and platelet counts were 49.5 and 43 days, respectively, after In2B8. Grade 4 neutropenia was observed in three patients and all but patient 6 had bone marrow involvement with follicular lymphoma cells at baseline (Table 4). Grade 3 thrombocytopenia was found in three patients (Table 5). One patient who experienced both neutropenia and thrombocytopenia required treatment with granulocyte colony-stimulating factor (G-CSF) for more than 2 weeks (Table 4) and platelet transfusions for more than 5 weeks (Table 5) (patient 10, described in detail later). All patients recovered their levels of hematological parameters to grade 1 or less during the study period, except for one patient (patient 10) who received alternative further treatment for progressive disease.

Sera of 10 patients were assayed for HAMA and HACA at baseline and at 4, 8 and 12 weeks after Y2B8 injection. Neither HAMA nor HACA was detected in any measurements.

The relatively frequent events among the non-hematological adverse events that were definitely, probably or possibly related to ibritumomab tiuxetan were nausea and vomiting, and dermatitis (Table 3). The non-hematological toxicities observed were generally mild, but those of grade 3 or greater, which were considered to be critical toxicities, occurred in two of nine patients (patients 8 and 10) (Table 2). These two patients had been heavily pretreated with more than seven regimens.

Because none of the three patients in the 0.3 mCi/kg (11.1 MBq/kg) dose group and two of the six patients in the 0.4 mCi/kg (14.8 MBq/kg) dose group developed critical toxicities, the recommended dose for the subsequent phase II study of ibritumomab tiuxetan in Japan was judged as 0.4 mCi/kg (14.8 MBq/kg), the same dose as that approved in the USA and the Europe Union.

Patient 8 was a 71-year-old woman with a concomitant illness of chronic cystitis. She had been treated with eight prior

Table 1. Patient demographic and clinical characteristics

Dose (mCi/kg)	Patient	Age (years)	Sex	Clinical stage†	Histology	BM lymphoma cells (%)‡	No. prior Cx regimens	
							Total no.	Including rituximab
0.3	1	64	M	IV	FL	0	1	1
(11.1 MBq/kg)	2	44	M	III	FL	0	3	1
	3	48	F	IV	FL	0 to < 5	2	
	4	60	F	III	FL	0	4	3
0.4	5	69	M	IV	MCL	0 to < 5	3	1
(14.8 MBq/kg)	6	53	F	I	FL	0	3	
	7	65	F	III	FL	0	4	1
	8	71	F	II	FL	0	8	3
	9	53	M	III	FL	0 to < 5	3	1
	10	53	M	IV	FL	5 to < 20	9	2

†Ann Arbor classification at the time of enrollment in this study. ‡Proportion of bone marrow involvement with lymphoma cells at baseline. BM, bone marrow; No., number; Cx, chemotherapy; F, female; M, male; FL, follicular lymphoma; MCL, mantle cell lymphoma.

Table 2. Adverse drug reaction and clinical response

Dose (mCi/kg)	Patient	G4 hematological ADR	≥ G2 non-hematological ADR†	Critical toxicity	No. prior Cx regimens		Response to Y2B8§	BM lymphoma cells (%)¶
					Total no.	Including rituximab		
0.3	1	0	0	–	1	1	CRu	0
(11.1 MBq/kg)	2	0	3	–	3	1	CR	0
	3	1	2	–	2		PR	0 to < 5
	4	NE	NE	NE	4	3	PD	0
0.4	5	0	1	–	3	1	SD	0 to < 5
(14.8 MBq/kg)	6	1	3	–	3		CR	0
	7	0	0	–	4	1	CRu	0
	8	0	7(2)	Non-hematol: 2	8	3	CR	0
	9	0	0	–	3	1	PR	0 to < 5
	10	1	4(2)	Hematol: 1‡	9	2		5 to < 20
				Non-hematol: 2			PD	

†The number reflects the number of ADR that were considered to be definitely, probably or possibly related to either In2B8 or Y2B8. The number in the parentheses indicates that of ≥ grade 3 non-hematological ADR. ‡Prolonged thrombocytopenia (< 25 000 cells/mm³). §Judged by the independent third party panel of two radiologists as central assessment. ¶Proportion of bone marrow involvement with lymphoma cells at baseline. ADR, adverse drug reaction; BM, bone marrow; CR, complete response; CRu, unconfirmed complete response; G, grade; hematol, hematological toxicity; NE, not evaluable; PR, partial response; PD, progressive disease; SD, stable disease.

Table 3. Adverse drug reactions during the treatment period†

Adverse drug reaction‡	0.3 mCi/kg (11.1 MBq/kg) (*n* = 3)			0.4 mCi/kg (14.8 MBq/kg) (*n* = 6)		
	Grade 2	Grade 3	Grade 4	Grade 2	Grade 3	Grade 4
Hematological						
Anemia	1			1	1	
Thrombocytopenia		1		1	2§ (#10)	
Lymphocytopenia		3		1	4	
Neutropenia		2	1		2	2
Non-hematological						
General malaise				1 (#8)¶		
Generalized pain	1					
Dehydration				1 (#8)		
Nausea/vomiting				2 (#8) (#10)		
Diarrhea					1 (#10)	
Constipation				1 (#8)		
Fever				1		
Infection with grade 3 or 4 neutropenia					1 (#8)	1 (#10)
Stomatitis	1					
Allergic conjunctivitis				1		
Episcleritis	1					
Dermatitis				2		
Folliculitis	1					
Tinea pedis	1					
Elevated bilirubin				1 (#8)		
Low albumin				1 (#10)		
Hyponatremia					1 (#8)	

†The treatment period includes the 1-week imaging period and 12 weeks after Y2B8 injection. ‡Includes all the grade ≥ 2 events that occurred during the treatment period and were considered to be definitely, probably or possibly related to either In2B8 or Y2B8 in nine patients (excluding patient 4). §One patient (patient 10) developed thrombocytopenia (< 25 000 cells/mm³) lasting for more than 3 weeks, and this was considered to be one of dose-limiting toxicities. ¶The number in parentheses (#) is the patient number who developed dose-limiting toxicities.

regimens for follicular lymphoma. She developed grade 2 nausea on day 10, vomiting on day 12, and dehydration on day 13. She received hydration and, consequently, her serum sodium concentration declined to 128 mEq/L on day 14. She developed the first symptom of cystitis on day 3 and then the cystitis deteriorated on day 49. Symptoms and signs disappeared on day 57 through the administration of ciprofloxacin hydrochloride. Although the hematuria was mild, the latter event was considered to be grade 3 non-hematological toxicity (infection with grade 3 or 4 neutropenia; Table 3) due to coexisting grade 3 neutropenia.

Patient 10 was a 53-year-old man with a history of chronic bronchitis and he had been suffering from stage IV follicular lymphoma for four years. He had received nine prior

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Table 4. Neutrophil counts at baseline and nadir

Dose (mCi/kg)	Patient	Baseline value (cells/mm³)	Nadir			Duration with grade 4 (days)§	BM lymphoma cells (%)¶
			Value (cells/mm³)	Grade†	Days from baseline to nadir‡		
0.3	1	2257	850	3	56	–	0
(11.1 MBq/kg)	2	2438	936	3	49	–	0
	3	2168	262	4	46	1	0 to < 5
0.4	5	4293	1521	1	60	–	0 to < 5
(14.8 MBq/kg)	6	1482	460	4	58	3	0
	7	3036	882	3	58	–	0
	8	3783	921	3	44	–	0
	9	3477	1725	1	50	–	0 to < 5
	10	4359	184	4	38	15	5 to < 20

†Toxicity grade of NCI-CTC version 2.0. Grade 4 was defined as absolute neutrophil count < 500 cells/mm³. ‡Days after In2B8 injection. §Days from the first date in grade 4 before nadir to the last date in grade 4 after nadir. ¶Proportion of bone marrow involvement with lymphoma cells at baseline. BM, bone marrow.

Table 5. Platelet counts at baseline and nadir

Dose (mCi/kg)	Patient	Baseline value (× 10⁴ cells/mm³)	Nadir			Platelet transfusion period
			Value (× 10⁴ cells/mm³)	Grade†	Days from baseline to nadir‡	
0.3	1	17.3	8.0	1	43	–
(11.1 MBq/kg)	2	17.7	8.0	1	43	–
	3	17.4	2.6	3	51	–
0.4	5	25.9	7.3	2	39	–
(14.8 MBq/kg)	6	20.5	8.9	1	37	–
	7	23.4	4.0	3	44	–
	8	18.3	7.9	1	35	–
	9	29.1	12.3	0	35	–
	10	18.9	1.2	3	53	37

†Toxicity grade of NCI-CTC version 2.0. Grade 3 was defined as platelet count ≥ 10 000 cells/mm³ and < 50 000 cells/mm³. ‡Days after In2B8 injection.

antineoplastic regimens, including rituximab and fludarabine phosphate. The patient was judged cytologically to have 12.3% bone marrow involvement with lymphoma cells according to the baseline evaluation, but the flow cytometric analysis by CD45-gating showed that CD20-positive cells occupied 53.5% of the nucleated cells in the bone marrow. His gamma camera imaging 2.7 h after In2B8 injection showed blood-pool radioactivity and therefore the institutional radiologists did not consider him to have altered biodistribution of the radioisotope according to the protocol definition. He received the 0.4 mCi/kg (14.8 MBq/kg) dose of Y2B8 on day 8. On day 30 his platelet count declined to 19 000/mm³ and he required the first platelet transfusion. The bone marrow examination on day 35 showed hypoplasia mainly infiltrated with small lymphocytes accounting for 92% of all the nucleated cells in the bone marrow. On day 37 he developed pharyngeal pain, productive cough and a fever of 38.6°C. Although ceftazidime and lenograstim (granulocyte colony-stimulating factor [G-CSF]) were promptly administered to him, his blood pressure dropped to 83/55 mmHg. Because he was diagnosed as being in suspected septic shock (infection with grade 3 or 4 neutropenia; Table 3), dopamine hydrochloride was administered for 1 day. The chest X-ray showed pleural effusion in the left thorax, and cytological examination of the cells aspirated from the left pleural effusion revealed infiltration of lymphoma cells. Blood cultures were negative for bacteria. He became afebrile with stable vital signs by the next day. He was kept on lenograstim until day 56 and required platelet transfusions for more than 5 weeks until day 67. On day 65, the bone marrow aspiration showed 40% involvement with lymphoma cells. He was diagnosed as having progressive disease and the next treatment was started on day 88.

Efficacy evaluation

Efficacy was evaluated in the 10 patients who received the study treatment (Table 2). In total, the best ORR was 70% (seven of 10 intent-to-treat patients), with the combined CR/CRu rate of 50%. Objective responses were observed in three of four (75%) patients in the 0.3 mCi/kg (11.1 MBq/kg) dose group, including one CR, one CRu and one PR. Among the six patients in the 0.4 mCi/kg (14.8 MBq/kg) dose group, two patients achieved CR, one CRu and one PR. Only patients who had no bone marrow involvement at baseline were able to achieve a CR or a CRu (Table 2). Of nine patients with follicular lymphoma, seven responded to ibritumomab tiuxetan. One patient with mantle cell lymphoma had a stable disease (SD) as the reduction in the sum of the products of the greatest diameters (SPD) of his diseases was 27.7%. Of

Table 6. Pharmacokinetics for ^{90}Y in blood

Dose (mCi/kg)	Parameters	*n*	Mean (h)	SD (h)	Median (h)	Range (h)	Patient 10† (h)
0.3	Effective AUC∞	4	38.0	4.2	39.4	31.9–41.1	–
(11.1 MBq/kg)	Biological AUC∞	4	80.9	16.0	85.9	58.6–93.2	–
	Effective $t_{1/2}$	4	34.7	2.1	34.8	32.3–37.1	–
	Biological $t_{1/2}$	4	76.4	10.1	76.2	65.1–88.4	–
0.4	Effective AUC∞	5	38.2	6.0	36.3	30.3–44.9	8.9
(14.8 MBq/kg)	Biological AUC∞	5	95.0	26.8	88.3	67.2–136.7	11.1
	Effective $t_{1/2}$	5	39.3	4.3	37.9	35.5–46.7	27.1
	Biological $t_{1/2}$	5	106.8	37.5	92.9	79.7–172.9	47.0
All	Effective AUC∞	9	*38.1*	5.0	38.8	30.3–44.9	–
	Biological AUC∞	9	88.7	22.6	88.3	58.6–136.7	–
	Effective $t_{1/2}$	9	*37.2*	4.1	37.1	32.3–46.7	–
	Biological $t_{1/2}$	9	93.3	31.6	88.4	65.1–172.9	–

AUC, area under the concentration *vs* time curve from dosing time extrapolated to infinity; biological, decay corrected; effective, non-decay corrected; SD, standard deviation; $t_{1/2}$, terminal half-life. †Patient 10, who showed a different biodistribution of In2B8 from the others, was excluded from summary statistics and his data are described separately.

the eight patients who were treated previously with rituximab, two achieved CR, two CRu and one PR (Table 2).

Pharmacokinetic evaluation

Table 6 shows the descriptive statistics of effective and biological AUC and $t_{1/2}$ for ^{90}Y radioactivity estimated from ^{111}In radioactivity in blood. With regard to the parameters, 'effective' means non-decay corrected data whereas 'biological' means decay corrected data, as described previously.[14] ^{90}Y radioactivity estimated from ^{111}In radioactivity in all of the study patients (except patient 10) showed that the mean effective $t_{1/2}$ for Y2B8 in blood was 37.2 h, which was similar to that of the results reported previously.[14] Although the mean effective $t_{1/2}$ was slightly longer, the data were within the range of the results reported previously.[14] The mean effective AUC value of all of the study patients (except patient 10) was 38.1 h. Patient 10 was found to have a markedly lower AUC and a shorter $t_{1/2}$ of radioactivity in the blood than the other study patients, and therefore his data was described separately.

Imaging

All of the images acquired after In2B8 administration were evaluated by the institutional radiologists and were judged to show a normal biological distribution before Y2B8 administration was allowed. Lymphoma lesions were visualized in eight cases (except for patients 5 and 10). Figure 1 shows a representative gamma scan image obtained from patient 3, which shows the left preauricular lymphoma lesion on the image 24 h after In2B8 injection. In contrast, the gamma imaging of patient 10 showed minimal blood-pool radioactivity with striking contrast images of the vertebral column, whole ribs, pelvis and bilateral femora to other body parts on the scans 24 h after In2B8 injection (Fig. 1). This indicates the accumulation of radioactivity in the bone marrow. He was not considered to show an altered biodistribution of the radioisotope according to the protocol definition because the first image taken 2.7 h after In2B8 injection showed radioactivity in the blood pool. However, the image 24 h after In2B8 injection was judged to be an abnormal biodistribution of ^{111}In2B8 by the independent third-party panel of radiologists.

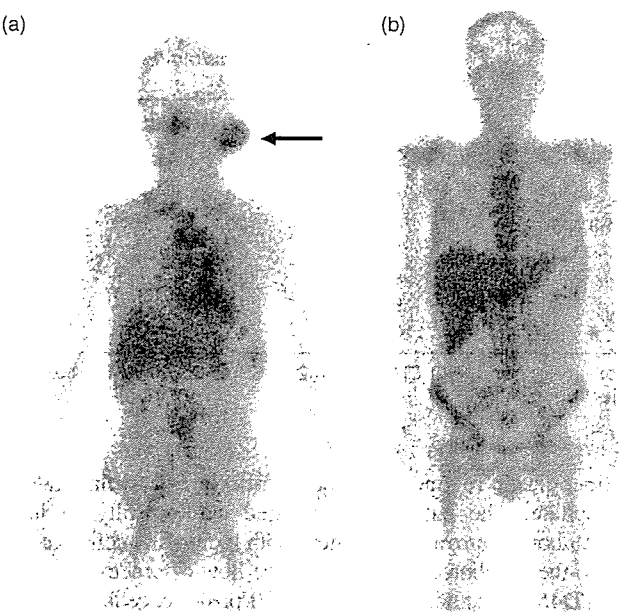

Fig. 1. Representative gamma camera images of patients with (a) expected and (b) altered biodistribution at 24 h after injection of In2B8. (a) The preauricular tumor of patient 3 is visualized clearly (arrow). The blood pool is still visualized, indicating that there is no rapid clearance of ^{111}In. (b) Rapid clearance of ^{111}In from the blood pool of patient 10 is observed. There is intense bone marrow visualization of the pelvis, bilateral femora, the spine and the whole ribs. Tumors are not visualized anywhere in the image.

Discussion

The adverse events observed most frequently in the present study were hematological toxicities, as expected from the previous studies in the USA,[3,5,6] but they were mostly reversible and manageable. Only two patients (patients 6 and 10) needed G-CSF and one patient (patient 10) required transfusions of red blood cells and platelets during the study period. However, all the hematological parameters were recovered to grade 1 or lower during the study period except

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for the platelet count of patient 10. He finally showed progressive disease and this might explain the minimal recovery of the platelet count. Baseline evaluation of his bone marrow involvement with lymphoma cells by cytological examination of bone marrow aspiration turned out to have been underestimated. Except for patient 6, all of the patients who developed grade 4 neutropenia had had bone marrow involvement at the baseline evaluation as previously reported.[6] Patients who had bone marrow involvement should be monitored carefully after treatment with ^{90}Y ibritumomab tiuxetan.

Most of the non-hematological adverse events were mild to moderate and were therefore easily manageable. Grade 3 or 4 non-hematological adverse events were seen in only two (patients 8 and 10) of the nine patients evaluated for critical toxicities. Patients 8 and 10 in the 0.4 mCi/kg (14.8 MBq/kg) dose group had been heavily pretreated with more than seven regimens (Table 1). Other grade 2 non-hematological toxicities were also frequently found in the same two patients (Table 2). Although one of these two patients (patient 8) achieved a CR, those patients who had received a number of prior regimens should be carefully monitored when they receive ^{90}Y ibritumomab tiuxetan. Patient 8 had concomitant cystitis at the time of enrollment. Ibritumomab tiuxetan was reported to infrequently cause mild non-hematological adverse effects but frequently caused hematological toxicities. Therefore, those patients who have chronic infection should be observed cautiously because the illness has the possibility to be deteriorated by transient neutropenia.

In contrast, patient 10 experienced prolonged bone marrow suppression and he was diagnosed as being in suspected septic shock. From the results of the flow cytometric analysis of his bone marrow cells at baseline, there was some possibility that the patient might have more than 25% bone marrow involvement with B-cell lymphoma cells. The gamma camera imaging proved that there was more involvement with B-NHL cells in the bone marrow than had been assessed by cytological examination of the bone marrow aspiration at baseline. The criteria used for evaluation of the gamma camera imaging were thought to be insufficient for detecting abnormalities of excessive bone marrow accumulation of the radiolabeled antibody. For the subsequent phase II study, the criteria used for evaluation of gamma camera imaging was revised so that patients with extensive bone marrow accumulation of ^{111}In could be excluded for the administration of Y2B8. The image from 2 to 24 h after In2B8 injection was to be included in the first image to judge the rapid clearance of In2B8 from the blood pool. Although hematological toxicities were encountered frequently, as reported previously, all patients recovered both their neutrophil and platelet counts, except for patient 10.[3,5,6] We expect that ^{111}In-imaging helps to exclude this kind of patient who cannot recover the hematological parameters or may result in progressive disease.

Considering that five of eight patients who were pretreated with regimens including rituximab achieved a CR, a CRu or a PR (the ORR was 63%), this ORR is higher than that of the rituximab retreatment (38%).[15,16] Most lymphoma lesions were visualized with gamma camera images, except for patient 5 with stable disease of mantle cell lymphoma and patient 10 with progressive disease of follicular lymphoma. To conclude whether the visualization of lymphoma lesions can predict the response to ^{90}Y ibritumomab tiuxetan, further studies are needed.

The pharmacokinetic data of ^{90}Y in the present study were within the range previously reported from the USA.[14] The mean value of effective $t_{1/2}$ showed slight prolongation. The study population was so small and the variability of its value so large that no definite conclusion can be made for Japanese patients. The fact that the mean value of effective AUC was similar to that of previous reports also supported the recommended dose of 0.4 mCi/kg (14.8 MBq/kg) for Japanese patients. In conclusion, the treatment of ^{90}Y-ibritumomab tiuxetan for Japanese patients with relapsed or refractory indolent B-NHL was highly effective and well tolerated, with similar pharmacokinetic profiles to those of patients in the USA. The recommended dose for the subsequent phase II study in Japan was determined as 0.4 mCi/kg (14.8 MBq/kg) for the patients with platelet counts ≥150 000 cells/mm^3 at baseline. The subsequent multicenter phase II study is presently under way to further evaluate the feasibility of a 0.3 mCi/kg (11.1 MBq/kg) dose for patients with platelet counts of 100 000–149 000 cells/mm^3 at baseline, as well as the efficacy and safety of the 0.4 mCi/kg (14.8 MBq/kg) dose.

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Original article

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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 non-responders, 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