Table 3 Toxicities

Toxicity	Toxicity grade by JCOG toxicity criteria				
	Grade 1	Grade 2	Grade 3	Grade 4	ND#
Hematological					
Leukopenia	11 (8.6%)	49 (38.3%)	60 (46.9%)	7 (5.5%)	
Neutropenia	8 (6.3%)	16 (12.5%)	44 (34.4%)	58 (45.3%)	
Anemia	34 (26.6%)	40 (31.3%)	9 (7.0%)	–	
Thrombocytopenia	11 (8.6%)	3 (2.3%)	1 (0.8%)	1 (0.8%)	
Non-hematological					
Bilirubin elevation	–	16 (12.5%)	4 (3.1%)	1 (0.8%)	
AST elevation	56 (43.8%)	14 (10.9%)	3 (2.3%)	0	
ALT elevation	53 (41.4%)	27 (21.1%)	6 (4.7%)	0	
Cr elevation	6 (4.7%)	2 (1.6%)	0	0	
Hypoxemia	56 (43.8%)	13 (10.2%)	1 (0.8%)	1 (0.8%)	4
Nausea/vomiting	50 (39.1%)	43 (33.6%)	14 (10.9%)	–	
Diarrhea	16 (12.5%)	2 (1.6%)	1 (0.8%)	0	2
Stomatitis	12 (9.4%)	3 (2.3%)	0	0	3
Proteinuria	7 (5.5%)	0	0	0	6
Arrythmia	2 (1.6%)	1 (0.8%)	0	0	4
Phlebitis	–	54 (42.2%)	0	0	2
Dyspnea	5 (3.9%)	5 (3.9%)	1 (0.8%)	0	4
Infection	21 (16.4%)	9 (7.1%)	0	1 (0.8%)	4
Alopecia	63 (49.2%)	40 (31.3%)	–	–	4
Fever (non-infectious)	27 (21.1%)	39 (30.5%)	3 (2.3%)	0	1
Neuropathy	33 (25.8%)	5 (3.9%)	1 (0.8%)	–	3
Constipation	34 (26.6%)	11 (8.6%)	1 (0.8%)	1 (0.8%)	3

Cr creatinine, *ND#* no data collected

hematological toxicity was nausea/vomiting that was observed in 14 patients (10.9%). Other grade 3 non-hematological toxicities were hyperbilirubinemia observed in 4 patients (3.1%), elevation of AST/ALT in 9 patients (7.0%), non-infectious fever in 3 patients (2.3%), and hypoxemia, diarrhea, dyspnea, peripheral neuropathy and constipation in each one patient (0.8% each). The frequent grade 2 non-hematological toxicities (≥30%) were phlebitis, nausea/vomiting, alopecia and non-infectious fever.

Secondary malignancies were observed within 3 years after ABVd therapy in 4 patients (3.1% of 128) throughout the study. Myelodysplastic syndrome (MDS), lung cancer, rectal cancer and gastric cancer were observed in each one patient. Diagnosis of lung cancer and gastric cancer was confirmed at 2.8 and 1.5 years after the treatment, respectively. A patient with MDS had involved-field radiation therapy to left cervical lymphatic area after ABVd therapy. A patient with rectal cancer had mantle-field radiation therapy after ABVd therapy. Each patient with MDS and gastric cancer died from progression of HL, and cerebral infarction followed by pneumonia, respectively. Another two patients died from their secondary malignancies.

Table 4 Responses of eligible patients ($n = 118$)

	Response after Cx		After Cx or Cx and Rx	
	No.	%	No.	%
CR	70	59.3	81	68.6
CRu	5	4.2	15	12.7
PR	32	27.1	10	8.5
NC	5	4.2	5	4.2
PD	5	4.2	6	5.1
NE	1	0.8	1	0.8
CR + CRu (95% CI)	75	63.6 (54.2–72.2%)	96[a]	81.4 (73.1–87.9%)

Cx chemotherapy, *Rx* radiation therapy, *CI* confidential interval

[a] 37 of 96 patients received Rx after the completion of Cx

3.4 Responses

The therapeutic efficacy was evaluated in 118 eligible patients. The ORR after ABVd therapy or ABVd therapy followed by radiation therapy in all 118 eligible patients was 89.8% (95% CI 82.9–94.6%) (Table 4). Seventy-five

Table 5 CR rate (including CRu) and 5-year PFS according to IPS or clinical stage

IPS/stage	CR rate after chemotherapy (%)	CR rate after radiation (%)/5-year PFS (%)
IPS		
0–2	65.4	84.6/83.1
3–7	60.0	75.0/69.0
Clinical stage		
II	67.2	86.9/86.8
III or IV	59.6	75.4/69.2
IIA	67.3	87.8/85.7
IIB, III or IV	60.9	76.8/73.1
Non-bulky IIA	74.4	87.2/87.2
Bulky IIA, IIB, III or IV	58.2	78.5/74.0

IPS International Prognostic Score

patients achieved CR (including CRu) (63.6%; 95% CI 54.2–72.2%) after ABVd therapy and 96 patients achieved CR (including CRu) (81.4%; 95% CI 73.1– 87.9%) after post-ABVd radiation therapy. A total of 39 patients (23 patients in PR and 16 patients in CR or CRu after ABVd) received radiotherapy after the completion of chemotherapy. While a total of 4 patients with initial bulky mass did not receive planned radiation therapy in CR at the end of chemotherapy, 23 patients who achieved PR after 4–8 cycles of ABVd therapy had unplanned radiation therapy. Twenty-one out of the 23 PR patients achieved CR or CRu after unplanned radiation therapy.

The responses according to the IPS category or clinical stage (II vs. III or IV, IIA vs. IIB, III or IV, and non-bulky IIA vs. bulky IIA, IIB, III or IV) are shown in Table 5. After involved-field radiation therapy, CR rate in lower (IPS: 0–2) and higher risk groups (IPS: 3–7) increased from 65.4 to 84.6%, and from 60.0 to 75.0%, respectively. Improved CR rate after radiation therapy was also observed in each clinical stage group. In the group of stage III or IV, the CR rate after ABVd therapy was 58.2%, but after radiation therapy, CR rate was increased to 78.5%.

3.5 Progression-free survival

The PFS curve of all 118 eligible patients is shown in (Fig. 1a), and 5-year PFS was estimated to be 78.4% (95% CI 70.9–85.9%). The PFS was analyzed according to the IPS risk groups and stages. The PFSs at 5 years in lower (IPS of 0–2) and higher risk groups (IPS of 3 or more) were 83.1% (95% CI 74.8–91.5%) and 69.0% (95% CI 54.4–83.7%), respectively (Fig. 1b). The PFSs at 5 years in stage II and III or IV were 86.8% (95% CI 75.4–93.2%) and 69.2% (95% CI 55.1–79.6%), respectively (Fig. 1c). The PFSs at 5 years in stage IIA non-bulky and stage IIA bulky, IIB, III or IV were 87.2 (95% CI 71.9–94.5%) and 74% (95% CI 62.6–82.4%), respectively (Fig. 1d).

3.6 Overall survival (OS)

The OS of all 118 eligible patients is shown in Fig. 2a. There have been 14 deaths among the 118 eligible patients. The OS at 5 years was estimated to be 91.3% (95% CI 86.1–96.5%). Median follow-up time for censored patients was 6.5 years.

The OS was analyzed according to the IPS risk groups and stages. The OS at 5 years in lower (IPS of 0–2) and higher risk groups (IPS of 3 or more) were 93.5% (95% CI 88.0–99.0%) and 87.0% (95% CI; 76.4–97.6%), respectively (Fig. 2b). The OS in stage II and III or IV were 96.7% (95% CI 87.5–99.2%) and 85.3% (95% CI 72.8–92.4%), respectively (Fig. 2c). The OS at 5 years in stage IIA non-bulky and stage IIA bulky, IIB, III or IV were 97.4% (95% CI 83.2–99.6%) and 88.2% (95% CI 78.5–93.7%), respectively (Fig. 2d).

4 Discussion

Our present phase II study confirmed that ABVd with lower dose of dacarbazine is as effective for Japanese patients with newly diagnosed HL with stages II–IV as ABVD with full dose of dacarbazine for the Western patients.

ABVD has been considered the standard of care for advanced-stage HL after the publication of a landmark CALGB study [7] and other large-scaled randomized phase III studies [9, 22–24]. The German Hodgkin Study Group has developed a dose-escalated and accelerated combined-modality program, escalated BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, prednisone, and procarbazine) plus radiation (+RT) for newly diagnosed patients with unfavorable stage IIB, III or IV [20]. The most recent analysis, also with a median follow-up of 9.3 years, included 1,196 evaluable patients and demonstrated superior freedom from treatment failure and overall survivals for the patients treated with escalated BEACOPP + RT [25]. In freedom from treatment failure (88% at 5 years), escalated BEACOPP + RT seems to be superior to failure-free survival (61% at 5 years) in ABVD therapy in CALGB study [8, 25]. Italian group compared ABVD with BEACOPP and CEC in randomized trial for the initial treatment of advanced HL [26]. Although BEACOPP may be associated with an improved PFS, it has greater toxicity; because of the success of salvage regimens, improvements in PFS may not translate to improved OS. Thus, ABVD still represents the standard initial treatment regimen for advanced HL.

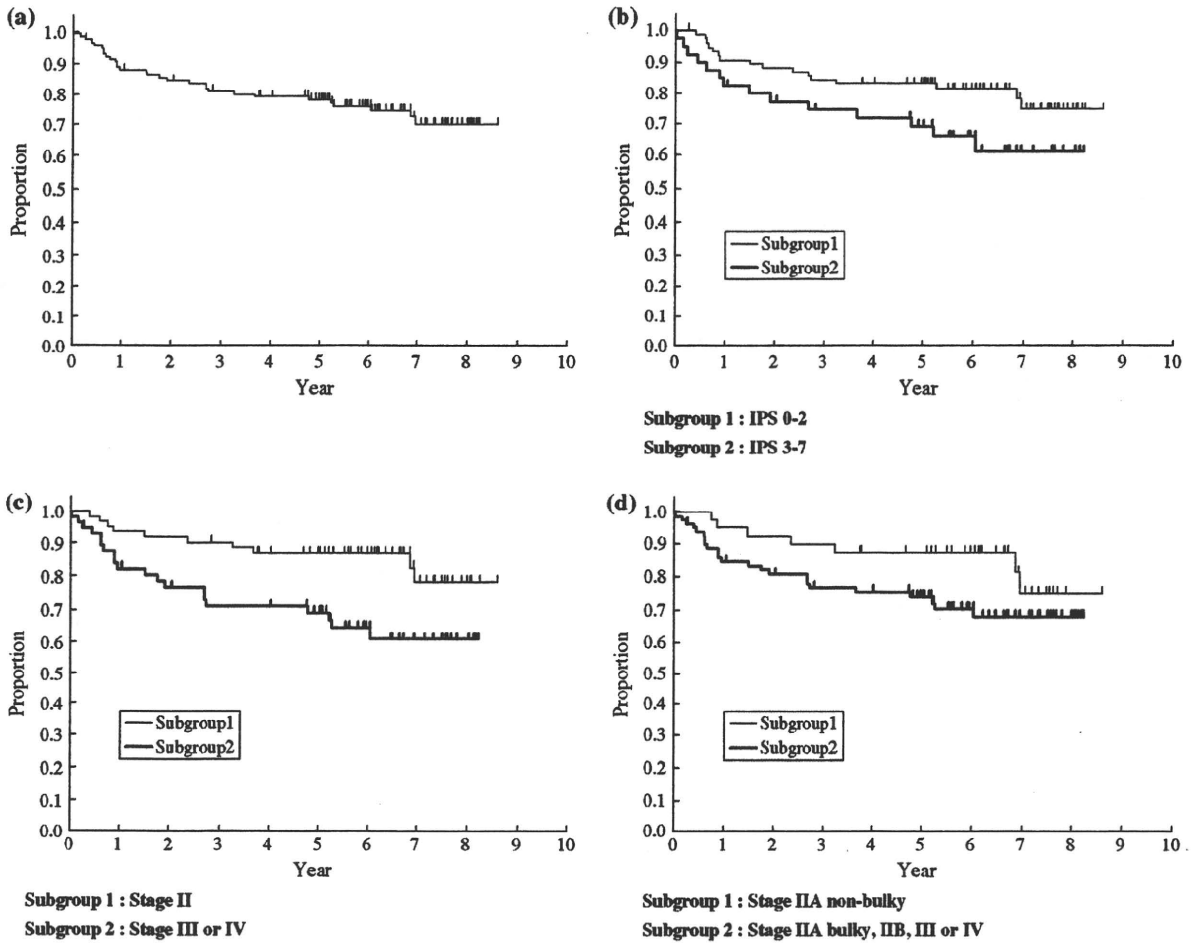

Fig. 1 Progression-free survival. *Tick marks* indicate censored data. **a** All 118 eligible patients. **b** Comparison according to IPS risk groups. *Fine* and *bold lines* represent lower risk group categorized by IPS of 0–2 ($n = 78$) and higher risk group categorized by IPS of 3 or more ($n = 40$), respectively. **c** Comparison according to stages. *Fine* and *bold lines* represent stage II group (subgroup 1; $n = 61$) and stage III or IV group (subgroup 2; $n = 57$), respectively. **d** Comparison according to stages in consideration of bulky disease and B symptom. *Fine* and *bold lines* represent stage IIA non-bulky group (subgroup 1; $n = 39$) and stage IIA bulky, IIB, III or IV group (subgroup 2; $n = 79$), respectively

Hoskin et al. reported the results of the randomized comparison of the Stanford V regimen and ABVD followed by involved-field radiation therapy in both arms to sites of previous bulk (>5 cm) in the treatment of advanced HL conducted by United Kingdom National Cancer Research Institute Lymphoma Group [27]. In that UK study, patients with stage IIB, III, or IV disease or with stages I–IIA disease with bulky disease (>5 cm) or other adverse features were eligible. There was no evidence of a difference in ORR, projected 5-year PFS and OS (92, 76 and 90%, respectively, for ABVD; 91, 74 and 92%, respectively, for Stanford V). They concluded that the efficacies of Stanford V and ABVD were comparable when given in combination with appropriate radiotherapy, and that ABVD is likely to remain standard therapy for initial treatment of HL.

In the present study, stages II–IV were eligible, while in CALGB study and German Hodgkin's Lymphoma Study Group's trial, stages IIIA2, IIIB, IVA or IVB, and unfavorable stages IIB–IV were eligible, respectively. Thus, direct comparison of efficacies of the present study with those in CALGB study or German Hodgkin's Lymphoma Study Group's trial is difficult. However, in UK study, stages I–IIA disease with bulky disease (>5 cm) or other adverse features and stage III or IV were eligible. And, the percentage of patients with stage III or IV in the present study and UK study was 48 and 54%, respectively. In the present study, CR rate and 5-year PFS of the patients with IIA-bulky, IIB, III, or IV (67% of all eligible patients) were 78.5 and 74%, respectively, while CR rate and 5-year PFS of the patients in ABVD arm in UK study were 67 and

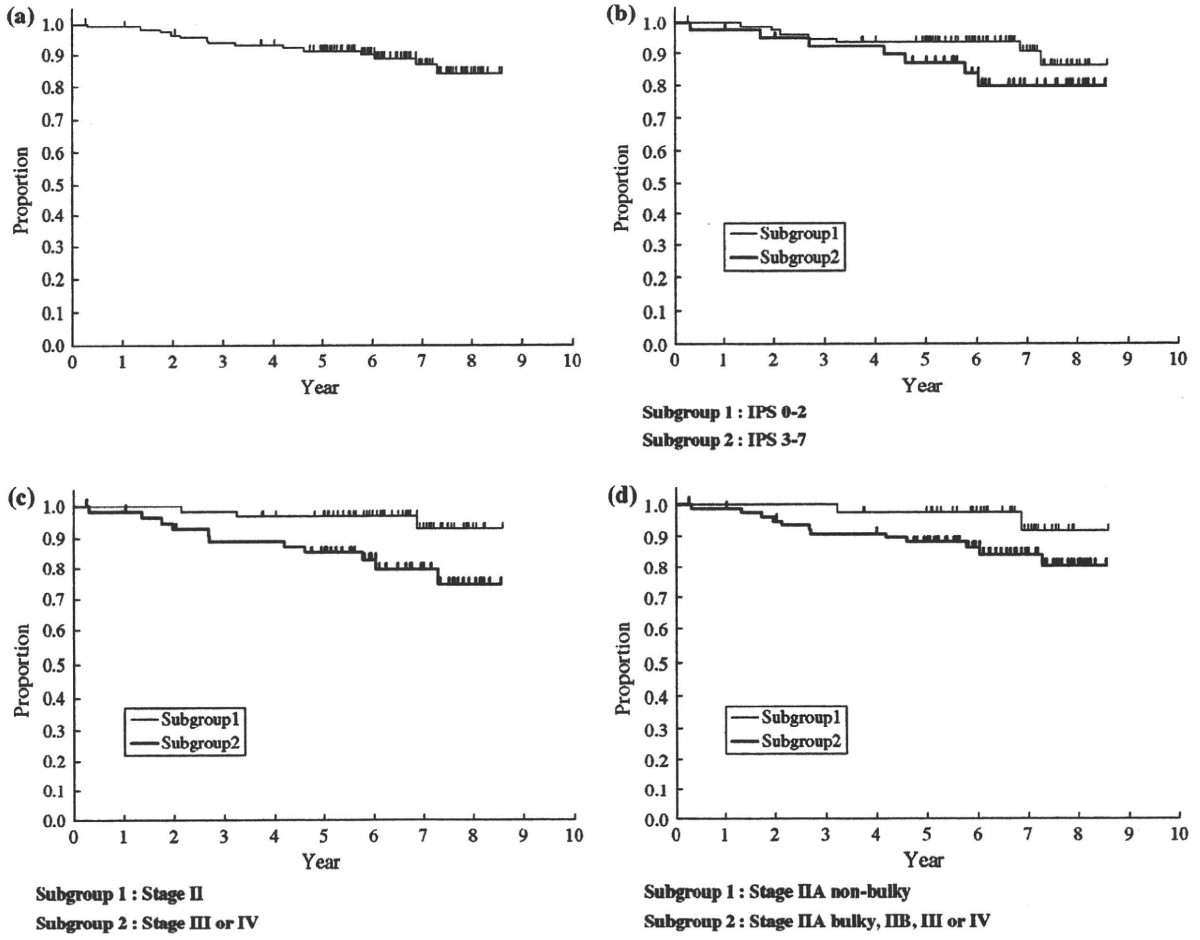

Fig. 2 Overall survival. *Tick marks* indicate censored data. **a** All 118 eligible patients. **b** Comparison according to IPS risk groups. *Fine* and *bold lines* represent lower risk group categorized by IPS of 0–2 ($n = 78$) and higher risk group categorized by IPS of 3 or more ($n = 40$), respectively. **c** Comparison according to stages. *Fine* and *bold lines* represent stage II (subgroup 1; $n = 61$) group and stage III or IV group (subgroup 2; $n = 57$), respectively. **d** Comparison according to stages in consideration of bulky disease and B symptom. *Fine* and *bold lines* represent stage IIA non-bulky (subgroup 1; $n = 39$) and stage IIA bulky, IIB, III or IV group (subgroup 2; $n = 79$), respectively

76%, respectively. Thus, the efficacies of ABVd therapy of advanced-stage patients in the present study seem to be comparable to those of ABVD therapy in UK study, although there is a limitation due to historical comparison.

Combined-modality treatment (CMT) using multidrug chemotherapy and radiotherapy is currently considered the standard of care in early stage HL. Its role in advanced stages, however, continues to be debated [28, 29]. In the present study, radiotherapy was planned to deliver to the initial sites of bulky mass for the patients in CR after ABVd therapy. As a result, it is possible that radiation to patients in PR might contribute to good PFS in the present study. Those tendencies were reported in UK study [27].

In the present study, 39 patients (33% of all eligible patients) were stage IIA without bulk disease (non-bulky) defined as early stage. Although CMT of short courses of ABVD followed by involved-field radiation therapy has been established as a standard of care in early stage HL since 2004 [30], extended-field radiation therapy was utilized as a standard of care of early stage HL, and full courses of chemotherapy was considered as an optional therapy in late 1980s to early 1990s when the present study was planned. Meyer et al. [31] reported the results of a randomized trial conducted by National Cancer Institute of Canada Clinical Trials Group (NCIC) and the ECOG comparing ABVD chemotherapy alone with CMT in patients with early stage HL. The 5-year PFS of patients with non-bulky stage IIA in the present study, and 5-year freedom from disease progression of patients with favorable early stage in NCIC and ECOG study was 87.2 and 87%,

respectively. Although a direct comparison is difficult, the outcome of early stage HL in the present study seems to be comparable with that in the NCIC and ECOG study.

According to the international comparison of survival data by Cancer Registries [32], OS at 5 years of the patients with HL treated in 1997–1999 was 68.3% in Japan, while it was 84.9% in the USA and 83.0% in the Europe in the same period. One of the reasons why the 5-year survival rate in Japan was about 15% lower than those in the USA and Europe is that dacarbazine was not commercially available in 1990s, then ABVD therapy could not be delivered to Japanese patients with HL. In this context, the present study contributed to get a governmental approval of dacarbazine for HL in 2000s and may contribute to improve greatly the treatment outcome for patients with HL in Japan.

As reported previously [7], major toxicity was grade 4 neutropenia (45.3%) in ABVd therapy in the present study. However, no severe (grade 3 or 4) infection was observed in the present study. As Boeti et al. reported, ABVD administration irrespective of granulocyte counts may allow the treatment to be given at full dose without delays or significant number of infective episodes [33]. Although four kinds of grade 4 non-hematological toxicity were observed in 4 patients as described above, all toxicities were transient, and all patients fully recovered. Although ABVd regimen contains doxorubicin and bleomycin, no severe cardiac toxicity such as congestive heart failure or ischemic heart disease, and no severe pulmonary toxicities were observed in the present study. Although the dose of dacarbazine in the present study was as same as that in JCOG8905, the incidence of grade 3/4 nausea/vomiting was decreased from 25%/12% in JCOG8905 to 11%/−% in the present study [12]. This improvement was mainly due to the development of 5HT3 antagonist such as granisetron that was launched in May 1992 in Japan. Since acute emesis due to dacarbazine is now well known to be controlled by these 5HT3 antagonists and new anti-emetic drug called substance P antagonist, dose reduction of dacarbazine in ABVD therapy may not be quite necessary.

In this study, four secondary malignancies including one MDS and three solid tumors developed after the completion of protocol treatment. Although ABVD therapy was reported to be less leukemogenic or carcinogenic [7, 34], development of MDS in one patient in the present study might be related to ABVd regimen. Although radiation is known to be carcinogenic, a patient with rectal cancer received mantle-field radiation without any radiation to abdomen. Thus, this case with rectal cancer also seems to be incidental development. Other two patients with lung cancer and gastric cancer, respectively, received only ABVd therapy. It might be less possible that ABVd therapy caused these solid cancers.

In summary, the present phase II study, JCOG 9305, showed the expecting efficacy and acceptable toxicity of ABVd therapy including post-chemotherapeutic involved-field radiation therapy for previously untreated patients with stage II–IV HL, despite the dose reduction of dacarbazine to two-thirds of that in the original ABVD therapy. These data should be useful for future clinical trials.

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Appendix

Participating institutions and principal investigators of the JCOG9305 study included: Sapporo National Hospital (C. Mikuni), Sapporo Hokuyu Hospital (M. Kasai), Akita University School of Medicine (A. Miura), Iwaki Kyoritsu General Hospital(T. Sai), Ota Nishinouchi Hospital (S. Ohta), National Cancer Center Hospital East (T. Ohtsu), National Cancer Center Hospital (K. Tobinai), Kyorin Medical University (K. Kawano), Tokyo Metropolitan Komagome Hospital (T. Sasaki), National Medical Center (A. Togawa), The 3rd Hospital of Tokyo Jikei Medical School (F. Mizoroki),Tokai University School of Medicine (T. Hotta), Niigata Cancer Center (T. Chou), Saku General Hospital (S. Seki), Hamamatsu University School of Medicine (K. Ohnishi), Aichi Cancer Center Hospital (Y. Morishima), Nagoya University School of Medicine (T. Kinoshita), Nagoya National Hospital (M. Tanaka), Fujita Health University (M. Hirano), Mie University School of Medicine (H. Shiku), Kyoto Prefectural University of Medicine (M. Taniwaki), Kyoto University School of Medicine (H. Ohno), Shiga Medical Center for Adults (T. Suzuki), Ohtsu Red Cross Hospital (T. Ohno), Osaka Red Cross Hospital (K. Nasu), Kansai Medical School (S. Fukuhara), Tenri Yorozu Hospital (Y. Ohno), Hiroshima Red Cross Atomic Bomb Hospital (H. Asaoku), Kagawa Medical School (M. Nagai), Shikoku National Hospital (K. Okabe), Sasebo Municipal General Hospital (S. Ikeda), National Kyushu Cancer Center (N. Uike), Nagasaki University School of Medicine (M. Tomonaga), Kagoshima University (S. Hanada), Kagoshima Municipal Hosipital (M. Tara).

Central review of pathological diagnosis

Reviewers included Drs. Yoshihiro Matsuno (National Cancer Center Hospital, Tokyo), Shigeo Nakamura (Aichi Cancer Center Hospital, Nagoya), Tadashi Yoshino (Okayama University, Okayama), Koichi Oshima and Masahiro Kikuchi (Fukuoka University, Fukuoka), and Kiyoshi Mukai (Tokyo Medical University) as pathologists for the Pathology Panel, and Masanori Shimoyama (National Cancer Center Hospital) as a hematologist for the Panel.

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Randomized phase II study of concurrent and sequential combinations of rituximab plus CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone) chemotherapy in untreated indolent B-cell non-Hodgkin lymphoma: 7-year follow-up results

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Rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP) is one of the most frequently applied initial treatments for indolent B-cell non-Hodgkin lymphoma (B-NHL); however, information on its long-term outcome is limited. Untreated patients in the concurrent arm (Arm C) received six R (375 mg/m^2) treatments, 2 days prior to each cycle of CHOP, and patients in the sequential arm (Arm S) received 6 weekly R (375 mg/m^2) treatments following six cycles of CHOP. Sixty-nine patients were randomized but two patients were withdrawn before receiving the protocol treatment. Sixty-five patients (94%) had follicular lymphoma, and 37 (55%) were at low risk, 23 (34%) at intermediate risk and seven (10%) at high risk according to the Follicular Lymphoma International Prognostic Index. We previously reported that the overall response rate (ORR) in Arm C and in Arm S was 94% and 97%, respectively. The median progression-free survival (PFS)/7-year PFS rate in Arm C, Arm S and all 67 assessable patients was 2.4 years/23% (95% confidence interval [CI], 9–40%), 3.8 years/41% (95% CI, 23–57%) and 2.8 years/32% (95% CI, 20–45%), respectively. There was no significant difference between the two arms ($P = 0.107$). The overall survival (OS) of the 67 patients was 95% at 7 years. In conclusion, R-CHOP is a highly effective initial treatment for untreated indolent B-NHL in terms of ORR and OS; however, its long-term PFS is not good enough either in concurrent or sequential combination, warranting further investigations on post-remission therapy. (*Cancer Sci* 2010; 101: 2579–2585)

Since the introduction of a chimeric anti-CD20 monoclonal antibody, rituximab, into the treatment of B-cell non-Hodgkin lymphoma (B-NHL),[1–5] various clinical trials have been carried out to investigate the efficacy of its combination with conventional chemotherapies, and currently, the combination of rituximab with chemotherapy is regarded as a routine modality for the treatment of B-NHL.[6–12] Rituximab plus cyclophosphamide, doxorubicin, vincristine and prednisolone (R-CHOP) has been regarded as the gold standard for the treatment of diffuse large B-cell lymphoma.[9–12]

Indolent B-NHL, of which the representative histopathological subtype is follicular lymphoma, is characterized by an advanced stage at presentation, lack of symptoms, indolent behavior and a long natural history in most patients. Various treatment strategies, including a watch and wait policy until progression,[13–15] cytotoxic chemotherapy with a single agent or in combination,[6,14–16] antibody therapy[17–20] and stem cell transplantation[21] have been tested; however, the majority of patients remain incurable, and no standard therapy has been established.[7,22] In 1999, Czuczman *et al.* reported the initial results of the first phase II study of R-CHOP in mostly untreated patients with indolent B-NHL, showing a high overall response rate (ORR) of 95% (38/40).[23] Currently, R-CHOP is one of the most frequently applied chemotherapeutic regimens for the treatment of untreated indolent B-NHL.[24]

In 1999, we initiated a multicenter, randomized phase II study to compare CHOP combined with rituximab concurrently or sequentially in previously untreated indolent B-NHL of advanced stage. The primary objective of the study was to select a promising combination regimen for further investigation in view of ORR as the primary end-point. Our initial analysis at a median follow-up time of 28.2 months demonstrated that both arms were equally highly effective, with 94% or higher ORR, including almost 70% complete response (CR) with acceptable toxicities. Thus, we concluded that R-CHOP is highly effective for untreated indolent B-NHL, either concurrently or in sequential combination, and that both combination schedules deserve further investigation.[25]

Several randomized studies have suggested the advantage of R-CHOP over CHOP alone in the treatment of follicular or indolent B-NHL.[26,27] However, the majority of studies investigating R-CHOP were carried out by combining rituximab concurrently with CHOP, and studies on CHOP followed by rituximab or other combination schedules are limited.[6,28] More importantly, long-term follow-up results after rituximab-containing chemotherapy

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for indolent B-NHL have not been sufficiently elucidated, except for the US phase II study of R-CHOP[23,29] and the European phase III study of rituximab plus cyclophosphamide, vincristine and prednisone (R-CVP) *versus* CVP alone.[30] In the former US phase II study reporting 9-year follow-up results, the authors claimed prolonged clinical and molecular remission, with time to progression (TTP) of 82.3 months; however, this single-arm phase II study has several limitations, including a small sample number, unusual combination schedule and heterogeneous populations.[29] In the latter European phase III study of a fairly large number of patients (n = 321), the chemotherapeutic regimen is not CHOP but CVP.[30] Thus, information regarding the long-term therapeutic outcome after R-CHOP for indolent B-NHL is still quite limited. From the viewpoints described above, this 7-year follow-up study provides additional information regarding the response durability of R-CHOP for untreated indolent B-NHL.

Patients and methods

This long-term follow-up study with a median follow-up time of 7.1 years regarding progression-free survival (PFS) and overall survival (OS) is an update of a Japanese phase II, open-label, multicenter, randomized study. Detailed descriptions of the study design and the initial results after the induction phase have been reported previously.[25] Briefly, untreated patients diagnosed as having indolent B-NHL of advanced stage, exclusive of mantle cell lymphoma, were randomized to either the concurrent arm (Arm C), where rituximab 375 mg/m^2 was given 2 days prior to each cycle of CHOP in six cycles, or the sequential arm (Arm S), where rituximab 375 mg/m^2 was given six times weekly following six cycles of CHOP. Sixty-nine patients were enrolled, and the primary end-point of ORR was 94% (30/32; 95% confidence interval [CI], 79–99%) for Arm C and 97% (33/34; 95%CI, 85–100%) for Arm S.[25]

As described in our previous manuscript, approval was obtained from the institutional review boards of all participating institutions. Written informed consent was obtained from all patients before enrollment in accordance with the Declaration of Helsinki.[25] Participating institutions and principal investigators of the IDEC-C2B8 Study Group are shown in the Appendix.

In this 7-year follow-up study, of the 69 patients enrolled, two patients who had never received the protocol treatment were removed from analyses. The remaining 67 patients who had received the allocated arm treatment were included in the analytical data set. The International Workshop Response Criteria for NHL published in 1999 was applied to evaluate the tumor response.[31] Tumor progression was monitored at approximately 3-month intervals for the first 12 months, and every 4–6 months thereafter until lymphoma progression or for 7 years.

Adverse events (AE) of the delayed type were also monitored for 7 years or at least until lymphoma progression, and were graded according to the toxicity criteria of the Japan Clinical Oncology Group,[32] an expanded version of the Common Toxicity Criteria version 1.0 by the National Cancer Institute (Bethesda, MD, USA).

The PFS data for patients who were transferred to other institutions were censored on the last date of assessment. Survival information for patients who were transferred to other institutions was obtained by questionnaire addressed to the hospital doctor in charge. The PFS and OS were analyzed using the Kaplan–Meier method, and the log-rank test was carried out to examine differences between cohorts. Statistical Analysis System (SAS, Cary, NC, USA), version 9.1, was used for analyses.

Results

Patient characteristics. Sixty-seven patients who received either Arm C (n = 34) or Arm S (n = 33) were evaluated. The majority of patients had follicular lymphoma (FL) accounting for 96% (64 patients) of the 67 assessable patients revealed by the central pathology review.[25] The allocated protocol treatment was not completed in two of the 67 patients due to grade 3 AE. One had cholangitis after the third cycle of R-CHOP in Arm C, and the other had interstitial pneumonia after the third cycle of CHOP in Arm S. The protocol treatment was completed in the remaining 65 patients. Patient characteristics at entry are shown in Table 1.

Of the 67 patients evaluated, two patients were judged "ineligible" for ORR analysis at the induction phase: one patient was ineligible due to concomitant kidney cancer, and his follow up was censored at his last observation; and the other patient was ineligible due to a prior history of anthracycline use for breast cancer, which was recognized after completion of the protocol treatment, and her lymphoma progression had been confirmed before judging her ineligibility. In addition, one patient who received erroneous protocol treatment was included in this long-term follow-up analysis, that is, a patient who erroneously received daunorubicin (50 mg/m^2) instead of doxorubicin in the first cycle of CHOP.

Progression-free survival. All 67 patients who received the protocol treatment were followed up for 7 years or until lymphoma progression, and their PFS and OS were updated. Fourteen patients (Arm C, five patients; Arm S, nine patients) were followed up until 7 years without progression. Kaplan–Meier plots of the PFS for all 67 patients (Arm C + Arm S) and for each treatment arm are shown in Figure 1.

As shown in Table 2, the median PFS of Arm C, Arm S and all 67 patients was 2.4, 3.8 and 2.8 years, respectively; the 7-year PFS rate of Arm C, Arm S and all 67 patients was 23%, 41% and 32%, respectively. There was no significant difference between the two arms (P = 0.107, log-rank test).

Overall survival. Kaplan–Meier plots of OS for the 67 patients and for each arm are shown in Figure 2. Three patients died from lymphoma progression during the 7-year follow up. Fifty-eight patients were confirmed to have survived for 7 years. However, for the remaining six patients, complete survival information could not be obtained because of a change of abode as well as institution. The estimated 7-year OS rate for Arm C, Arm S and all 67 patients was 97%, 94% and 95%, respectively, as shown in Table 2.

PFS and OS by the Follicular Lymphoma International Prognostic Index (FLIPI). As 64 (96%) of the 67 patients had follicular lymphoma, PFS curves by FLIPI[33] are shown in Figure 3. The FLIPI scoring was also applied to the remaining three patients diagnosed as having marginal zone B-cell lymphoma or low-grade B-NHL, not otherwise specified. The median PFS for the low-, intermediate- and high-risk groups was 4.0, 2.5 and 1.5 years, respectively, and the 7-year PFS for the low-, intermediate- and high-risk groups was 39%, 24% and 21%, respectively, as shown in Table 3. There were no statistical differences among the low-, intermediate- and high-risk groups.

Late-onset adverse events. Clinically significant AE that occurred during the induction phase, that is, from the start of protocol treatment to 6 months after the last cycle of CHOP, were reported in our previous paper.[25] In the present report, we focused on the late onset AE observed during the 7-year follow up.

All three deaths were associated with lymphoma progression, and no patients died due to AE associated with the protocol treatment. Serious AE were documented in four patients during the follow-up period: prosthetic aortic valve replacement, cerebral hemorrhage, bodyweight gain and stomach cancer. It is unlikely that these four AE were directly associated with the protocol treatment. Besides, no late-onset cardiac dysfunctions associated with the R-CHOP treatment were documented during the follow-up period.

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Table 1. Patient characteristics

Factors	Enrolled			Evaluated		
	Arm C (n = 34)	Arm S (n = 35)	Total (n = 69)	Arm C (n = 34)	Arm S (n = 33)	Total (n = 67)†
Sex						
Female	18	18	36	18	17	35
Male	16	17	33	16	16	32
Age (years)						
Median	53	50	52	53	49	52
Range	36–65	26–69	26–69	36–65	26–69	26–69
Performance status (ECOG)						
0	29	30	59	29	28	57
1	5	5	10	5	5	10
Histopathology (REAL)‡						
Follicular, grade 1	12	11	23	12	11	23
Follicular, grade 2	21	19	40	21	18	39
Follicular, grade 3	0	2	2	0	2	2
Marginal zone	1	0	1	1	0	1
Low-grade B-NHL, NOS§	0	2	2	0	2	2
No specimen¶	0	1	1	0	0	0
Clinical stage (Ann Arbor)						
III	14	15	29	14	13	27
IV	20	20	40	20	20	40
B symptoms						
Absent	30	33	63	30	31	61
Present	4	2	6	4	2	6
LDH						
Normal	32	31	63	32	29	61
Elevated	2	4	6	2	4	6
Number of extranodal sites						
0 or 1	25	26	51	25	24	49
2 or more	9	9	18	9	9	18
International prognostic index						
Low	21	21	42	21	19	40
Low-intermediate	12	12	24	12	12	24
High-intermediate	1	1	2	1	1	2
High	0	1	1	0	1	1
Follicular Lymphoma International Prognostic Index						
Low	18	20	38	18	19	37
Intermediate	13	11	24	13	10	23
High	3	4	7	3	4	7

Arm C, concurrent arm; Arm S, sequential arm; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; REAL, revised European–American classification of lymphoid neoplasms. †Two patients who withdrew before the protocol treatment initiation with the allocated arm were removed from evaluation. ‡According to the diagnosis by the Central Pathology Review Committee. §Low-grade B-cell non-Hodgkin lymphoma (B-NHL), not otherwise specified (NOS). ¶Specimen for central pathology review was not submitted.

Discussion

The 7-year OS of patients with indolent B-NHL treated with R-CHOP in the present study was excellent, with 97% for Arm C and 94% for Arm S, which appeared to be closely associated with the excellent ORR by R-CHOP, with 94% for Arm C and 97% for Arm S;[25] however, the 7-year PFS is not satisfactory, with 23% for Arm C and 41% for Arm S. More than 50% of patients relapsed within 4 years, and no clear plateau of the PFS curve was observed in either arm. Czuczman *et al.* reported a somewhat longer TTP of 82.3 months by R-CHOP alone.[29] The reason for the difference in PFS and TTP between the two studies is unclear, but might be partly explained by the small sample numbers and different background factors, including histopathological subtypes, different administration schedules of rituximab, etc. Although they claimed a long response durability in their 9-year follow-up study, more than half of the patients relapsed.[29] Taken together, it appears reasonable to consider that most patients with indolent B-NHL are still incurable by R-CHOP alone.

One of the large-scale clinical trials investigating the efficacy of R-CHOP for indolent B-NHL is a German study for untreated follicular lymphoma.[26] According to the updated results at a median 58-month follow up, the improvement of time to treatment failure (TTF), response duration (RD) and OS remained consistently superior in the R-CHOP group compared with the CHOP alone group;[34] however, the number of patients free from treatment failure decreased in a linear manner over time, even in the R-CHOP group. The patients enrolled in the German study were secondarily randomized after remission induced by R-CHOP, and received post-remission therapy with interferon alpha maintenance or myeloablative chemoradiotherapy with autologous hematopoietic stem cell transplantation. The estimated 5-year TTF of 65% appears to be longer than the 7-year PFS of 32% in the present study, possibly suggesting the prolonging remission effect of the post-remission therapy.

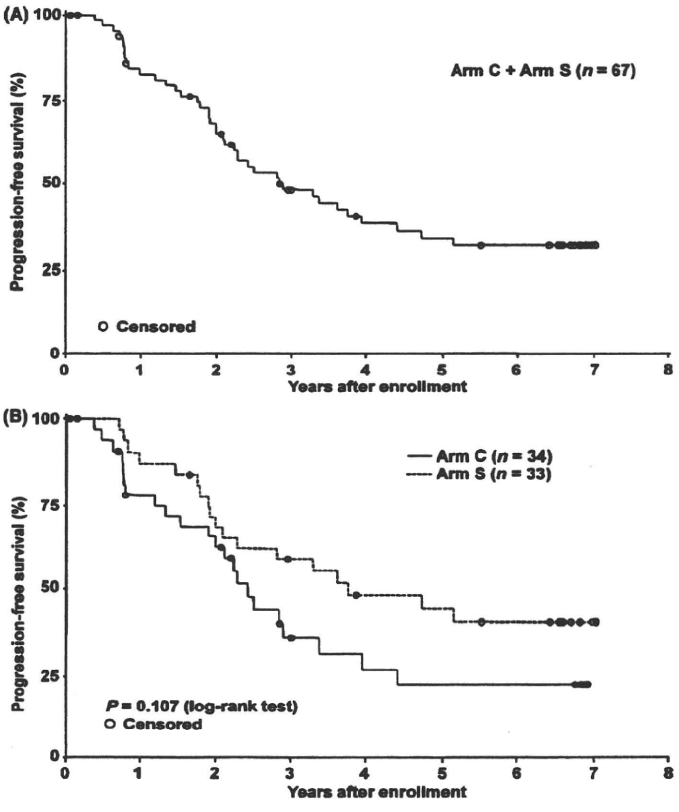

Fig. 1. Progression-free survival (PFS). (A) The PFS of 67 assessable patients. Median PFS with 95% confidence interval (95% CI) was estimated to be 2.8 years with a 95% CI of 2.1–4.4 years. (B) The PFS by the treatment arm. Median PFS with the 95% CI of the concurrent arm (Arm C) (n = 34) and the sequential arm (Arm S) (n = 33) were estimated to be 2.4 years with a 95% CI of 1.9–3.4 years, and 3.8 years with a 95% CI of 2.1– years, respectively. There was no statistical difference between the two arms using log-rank test (P = 0.107).

Table 2. Progression-free survival (PFS) and overall survival (OS) by treatment arm

Cohort	n	Median PFS (years)		7-year PFS (%)		7-year OS (%)	
		95% CI		95% CI		95% CI	
Arm C†	34	2.4	1.9–3.4	23	9–40	97	79–100
Arm S‡	33	3.8	2.1–	41	23–57	94	78–98
ALL (Arm C + S) §	67	2.8	2.1–4.4	32	20–45	95	86–98

There was no statistical difference between Arm C and Arm S in the progression-free survival (P = 0.1071 by log-rank test). CI, confidence interval. Tumor progression was evaluated according to the International Workshop Response Criteria for Non-Hodgkin Lymphoma, at approximately 3-month intervals for the first 12 months and every 4–6 months thereafter. †Patient group treated with rituximab plus CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) concurrently 2 days prior to each CHOP cycle, repeating six cycles every 3 weeks. ‡Patient group treated with six cycles of CHOP repeated every 3 weeks, followed by rituximab given six times weekly. §All 67 patients were treated with either the concurrent arm (Arm C) or the sequential arm (Arm S) in this study.

The maintenance use of rituximab is a promising option for prolonging PFS in the treatment of indolent B-NHL. The results of the Primary Rituximab Maintenance (PRIMA) study, where rituximab was combined concurrently with a CHOP-like or fludarabine-containing regimen for induction in untreated follicular lymphoma patients with high tumor burden, and responders were randomized to either maintenance with rituximab given bimonthly for 2 years or observation, are expected to show a prolonging PFS effect in the maintenance rituximab arm, considering several preceding studies suggesting its usefulness in the treatment of indolent B-NHL, including a German study,[35] European study,[27] US studies[36,37] and Swiss study.[38]

Another potentially effective strategy for prolonging PFS after R-CHOP might be radioimmunotherapy. The researchers of the Southwest Oncology Group (SWOG) in the United States reported a favorable 5-year PFS rate of 67% in a phase II study of CHOP followed by idodine-131(^{131}I)-labeled anti-CD20 monoclonal antibody, tositumomab, for untreated follicular lymphoma patients of advanced stages.[39] The authors claimed that the 5-year estimate of PFS was 23% better than the corresponding figures for patients treated with previous SWOG protocols with CHOP alone.[6,39] Based on the encouraging results of the phase II study, they completed patient enrolment into the subsequent phase III trial comparing R-CHOP. Another anti-CD20 radioimmunoconjugate, yttrium-90 (^{90}Y)-labeled ibritumomab tiuxetan, was also evaluated for advanced-stage follicular lymphoma in first remission in an international phase III study.[40] ^{90}Y-ibritumomab tiuxetan consolidation significantly prolonged median PFS compared with the no consolidation arm (36.5 *vs* 13.3 months; hazard ratio, 0.465; $P < 0.0001$). Although this phase III study clearly demonstrated the efficacy of ^{90}Y-ibritumomab tiuxetan as post-remission therapy in follicular lymphoma, most enrolled patients (86%, 350/409) had not received rituximab-containing chemotherapy as the initial treatment.

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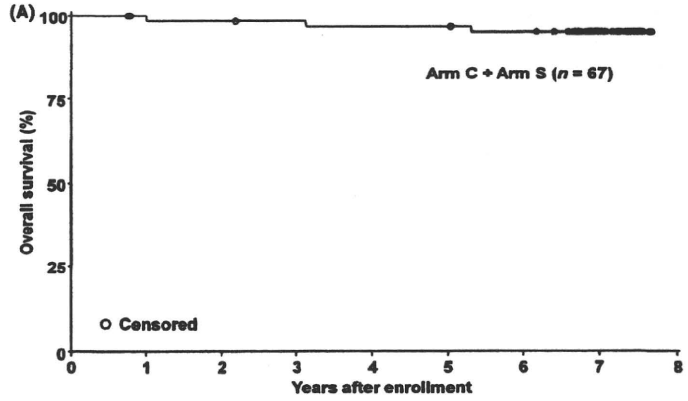


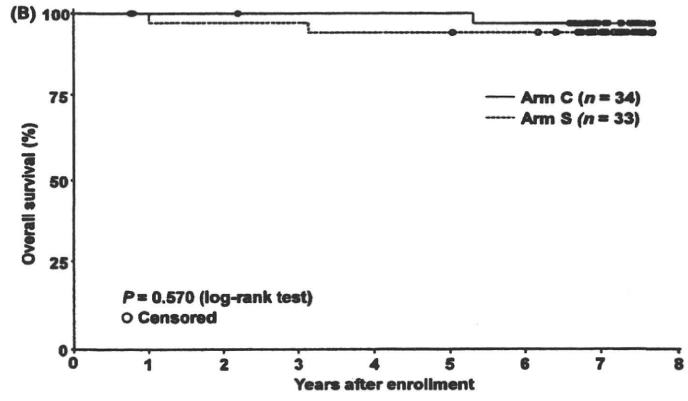
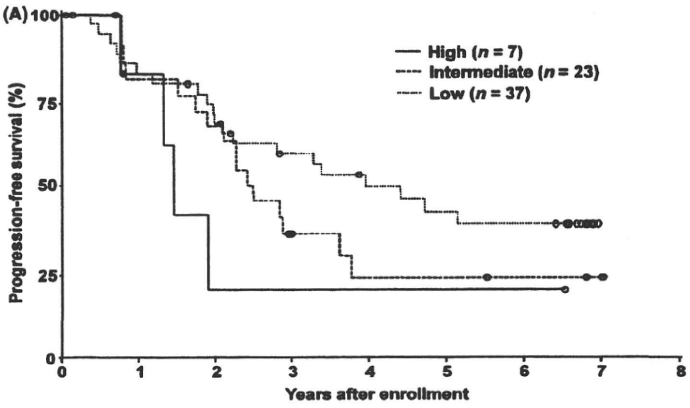


Fig. 2. Overall survival (OS). (A) The OS of the 67 assessable patients. The 7-year OS rate with 95% confidence interval (CI) was estimated to be 95% with a 95% CI of 86–98%. (B) The OS by the treatment arm. The 7-year OS rate with a 95% CI of the concurrent arm (Arm C) ($n = 34$) and the sequential arm (Arm S) ($n = 33$) was estimated to be 97% with a 95% CI of 79–100%, and 94% with a 95% CI of 78–98%, respectively. There was no statistical difference between the two arms using log-rank test ($P = 0.570$).



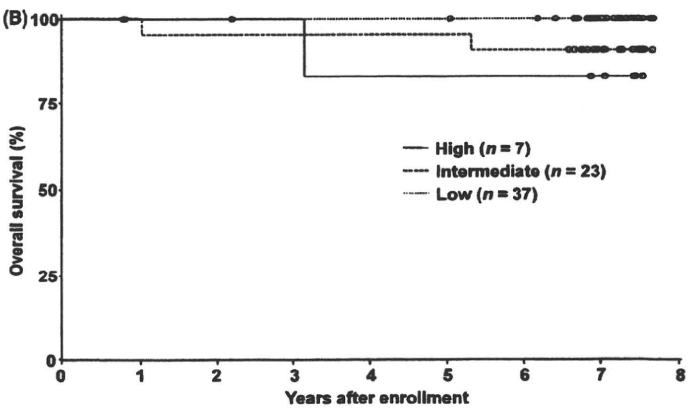


Fig. 3. The progression-free survival (PFS) and overall survival (OS) according to the Follicular Lymphoma International Prognostic Index (FLIPI). (A) The PFS according to the FLIPI risk group. The median PFS with 95% confidence intervals (CI) of low-risk ($n = 37$), intermediate-risk ($n = 23$) and high-risk ($n = 7$) groups was 4.0 years with a 95% CI of 2.2– years, 2.5 years with a 95% CI of 1.9–3.8 years, and 1.5 years with 95% CI of 1.3– years, respectively. (B) The OS according to the FLIPI risk group. The 7-year OS rate with 95% CI of low-risk ($n = 37$), intermediate-risk ($n = 23$) and high-risk ($n = 7$) groups was 100% with a 95% CI of 100–100%, 91% with a 95% CI of 69–98%, and 83% with a 95% CI of 27–97%, respectively. (O), censored.

Table 3. Progression-free survival (PFS) and overall survival (OS) by the Follicular Lymphoma International Prognostic Index

FLIPI	*n*	Median PFS (years)		7-year PFS (%)		7-year OS	
			95% CI		95% CI		95% CI
Low	37	4.0	2.2–	39	22–55	100	100–100
Intermediate	23	2.5	1.9–3.8	24	8–44	91	69–98
High	7	1.5	1.3–	21	1–60	83	27–97

Tumor progression was evaluated according to the International Workshop Response Criteria for Non-Hodgkin Lymphoma at approximately 3-month intervals for the first 12 months and every 4–6 months thereafter. There were no statistical differences among the low-, intermediate- and high-risk groups. CI, confidence interval; FLIPI, Follicular Lymphoma International Prognostic Index.

Therefore, to elucidate the exact role of anti-CD20 radioimmunotherapy as post-remission therapy after rituximab-containing chemotherapy like R-CHOP, prospective comparative studies directly targeting this cohort are needed.

Although the primary end-point of this randomized phase II study is ORR of R-CHOP chemotherapy, and survival parameters such as PFS and OS are among the secondary end-points associated with insufficient statistical power due to small samples, it is noteworthy that the favorable tendency of the sequential combination in the R-CHOP arm was recognized not only in the initial analysis[25] but also in the 7-year follow-up study. The exact reason for this finding is unclear; however, the use of rituximab after reduction of the tumor burden by chemotherapy might be a more promising treatment strategy for prolonging PFS in the treatment of indolent B-NHL, or antibody-dependent cellular cytotoxicity might be more active with intact effector cells recovered after the completion of chemotherapy.

As shown in Table 1, according to the International Prognostic Index (IPI), 96% of assessable patients (64/67) were categorized as a low- or low-intermediate-risk group, and only 4% (3/67) were categorized as a high-intermediate- or high-risk group. As compared with IPI, FLIPI discriminated the three risk groups (55%, 34% and 10%) in a better-balanced manner. As shown in Table 3 and Figure 3, a tendency towards the relationship of PFS and OS with the FLIPI risk groups was recognized, although there were no statistical differences, presumably due to the small samples. FLIPI was developed from the data of follicular lymphoma patients who had been treated in the pre-rituximab era.[33] Thus, the Follicular Lymphoma International Prognostic Index 2,[41] which was recently proposed based on the PFS and OS data of follicular lymphoma patients who were treated in the rituximab era, deserves future investigation.

In conclusion, the combination of rituximab with CHOP either concurrently or sequentially is highly effective for remission induction in previously untreated patients with indolent B-NHL. While the OS was excellent and significant late-onset AE were not observed, the estimated PFS after 7-year follow up without post-remission therapy is not satisfactory either in the concurrent or sequential combination of R-CHOP. Further investigations on post-remission therapy after R-CHOP are warranted.

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Disclosure Statement

The authors have no conflict of interest.

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Appendix

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Phase I study of inotuzumab ozogamicin (CMC-544) in Japanese patients with follicular lymphoma pretreated with rituximab-based therapy

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Inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapeutic agent composed of an anti-CD22 antibody conjugated to calicheamicin, a potent cytotoxic antibiotic, specifically targets the CD22 antigen present in >90% of B-lymphoid malignancies, rendering it useful for treating patients with B-cell non-Hodgkin lymphoma (B-NHL). This phase I study evaluated the safety, tolerability, efficacy, and pharmacokinetics of inotuzumab ozogamicin in Japanese patients. Eligible patients had relapsed or refractory CD22-positive B-NHL without major organ dysfunction. Inotuzumab ozogamicin was administered intravenously once every 28 days (dose escalation: 1.3 and 1.8 mg/m^2). All 13 patients had follicular lymphoma, were previously treated with ≥1 rituximab-alone or rituximab-containing chemotherapy, and were enrolled into two dose cohorts (1.3 mg/m^2, three patients; 1.8 mg/m^2, 10 patients). No patient had dose-limiting toxicities, and the maximum tolerated dose, previously determined in non-Japanese patients (1.8 mg/m^2), was confirmed. Drug-related adverse events (AEs) included thrombocytopenia (100%), leukopenia (92%), lymphopenia (85%), neutropenia (85%), elevated AST (85%), anorexia (85%), and nausea (77%). Grade 3/4 drug-related AEs in ≥15% patients were thrombocytopenia (54%), lymphopenia (31%), neutropenia (31%), and leukopenia (15%). The AUC and C_{max} of inotuzumab ozogamicin increased dose-dependently with pharmacokinetic profiles similar to non-Japanese. Seven patients had complete response (CR, 54%) including unconfirmed CR, four patients had partial response (31%), and two patients had stable disease (15%). The overall response rate was 85% (11/13). Inotuzumab ozogamicin was well tolerated at doses up to 1.8 mg/m^2 and showed preliminary evidence of activity in relapsed or refractory follicular lymphoma pretreated with rituximab-containing therapy, warranting further investigations. This trial was registered in ClinicalTrials.gov (NCT00717925). (*Cancer Sci* 2010; 101: 1840–1845)

The successful use of monoclonal antibodies (mAbs) in the treatment of human diseases has been growing steadily in the past decade. Rituximab, a human-mouse chimeric anti-CD20, unconjugated antibody, was approved in 1997 in the USA as the first mAb for antilymphoma therapy. It is now most commonly used in combination with chemotherapy for first and subsequent lines of therapy in B-cell non-Hodgkin lymphoma (B-NHL), such as diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL).[1–6] However, a subgroup of patients does not respond, and early relapses occur in patients with initial response, thus indicating rituximab resistance. This indicates a clear unmet need to explore alternative antibodies non-cross resistant to rituximab as a therapy for B-NHL. One alternative is inotuzumab ozogamicin (CMC-544), an antibody-targeted chemotherapy agent that specifically targets CD22. Inotuzumab ozogamicin is composed of a recombinant engineered humanized IgG4 anti-CD22 antibody G544 conjugated to calicheamicin, a potent cytotoxic antibiotic derivative.[7]

CD22 is a potential therapeutic target for B-NHL because it is expressed in >90% of B-NHL cells.[8] In addition, CD22 is expressed in mature B cells, but not in their precursor or memory B cells, which may potentially minimize the adverse effect of CD22-targeted treatment on long-term immune function. Moreover, when antibodies bind to the CD22 antigen, the antigen is internalized, that is it is not shed into the extracellular environment.[9]

Both inotuzumab ozogamicin and unconjugated calicheamicin showed potent cytotoxic activity *in vitro* against CD22-positive B cells in preclinical studies.[7] In addition, the unconjugated form of inotuzumab ozogamicin, G544, did not demonstrate any antitumor activity in preclinical studies.[7] Inotuzumab ozogamicin inhibited the growth and the establishment of B-cell lymphomas and induced the regression of large B-cell lymphomas in mouse xenograft models.[7] Furthermore, in preclinical models of disseminated B-NHL in which rituximab was ineffective, treatment with inotuzumab ozogamicin lead to a significant tumor regression and an improvement in survival.[10] This potent cytotoxic activity in preclinical murine models of B-cell lymphomas in which rituximab had failed as a therapeutic agent[11] establishes support for the clinical investigation of inotuzumab ozogamicin for the treatment of CD22-positive B-NHL.

A phase I dose escalation study was previously conducted in the USA and the European Union in patients with relapsed or refractory B-NHL (both FL and DLBCL).[12] In this study, intravenous administration of the drug demonstrated clinical activity in patients with relapsed or refractory B-NHL with clinically manageable thrombocytopenia as the main toxicity. The maximum tolerated dose (MTD) in this non-Japanese patient population was determined to be 1.8 mg/m^2 once every 4 weeks.

The objectives of the present study were to assess the safety, toleralility, efficacy, and pharmacokinetics of inotuzumab ozogamicin in Japanese patients with relapsed or refractory B-NHL who had received prior treatment with rituximab.

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Materials and Methods

Study design. The present trial was an open-label multicenter phase I study in which inotuzumab ozogamicin was administered intravenously (IV) as a single agent to patients with CD22-positive B-NHL once every 28 days (±2 days, 1 cycle) for at least four doses provided that the drug was well tolerated with no evidence of progressive disease (PD). The protocol was approved by the Institutional Review Board of each participating institution, and it conformed to the provisions of the Declaration of Helsinki in 1995 (as revised in Tokyo, 2004). All the patients gave written informed consent.

Patients. Patients were eligible for enrollment if they had a diagnosis of CD22-positive B-NHL, according to the World Health Organization (WHO) classification, version 3.[13] Patients were included if they had progressed after at least one prior chemotherapy regimen for indolent B-NHL, or after one or two chemotherapy regiments, which included anthracyline or anthraquinone for aggressive B-NHL. Other inclusion criteria were age ≥20 and <75 years, a performance status of one or better on the Eastern Cooperative Oncology Group Scale, life expectancy ≥12 weeks, an absolute neutrophil count (ANC) $\geq 1.5 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L, serum creatinine ≤1.5 × upper limit of normal (ULN), urine protein-to-creatinine ratio of ≤0.2, total bilirubin ≤1.5 × ULN, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 × ULN, and at least one measurable lesion ≥1.5 cm in at least one dimension by computer tomography (CT) at inclusion, in an area of no prior radiation therapy, or clear progression in an area that had been previously irradiated.

Dose escalation and toxicity criteria. Dose escalation decisions were based on the toxicities observed in the first 28 days after the administration of the first dose. Patients (three and 10 patients per cohort) could receive more than the four planned doses of inotuzumab ozogamicin if they experienced at least stable disease and tolerated treatment. The starting dose was 1.3 mg/m^2 administered IV once every 28 days, and dose escalation was performed up to the MTD of 1.8 mg/m^2 administered IV once every 28 days. Both the starting dose and the MTD were based on information from a previous clinical trial.[12] The dose escalation in subsequent cohorts was based on the toxicity assessed in the first 28 days after the first dose. Dose escalation continued until three or more patients in a cohort experienced a dose-limiting toxicity (DLT).

A DLT was defined as any of the following that were at least possibly related to inotuzumab ozogamicin during the first 28 days after the first dose: any grade 3 or 4 (National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTC], version 3.0) nonhematologic toxicity (except grade 3 alopecia, nausea, or vomiting unless the patient was receiving optimal medical therapy); febrile neutropenia (grade 4 ANC ≥3-day duration and temperature ≥38.0 C); grade 4 ANC ≥7-day duration; grade 4 thrombocytopenia ≥3-day duration, or any bleeding episode requiring platelet transfusion; or delayed recovery (to grade 1 or baseline, except alopecia or grade 2 nausea or vomiting unless the patient was receiving optimal medical therapy) from a toxicity related to inotuzumab ozogamicin that delayed the initiation of the next dose by more than 3 weeks. Patients who experienced a DLT had the subsequent doses of inotuzumab ozogamicin reduced by one dose level, the maximum allowed dose reduction per patient. Patients who experienced toxicities other than DLTs could receive additional doses of inotuzumab ozogamicin at the same dose if they met the following criteria: recoveries to ≤grade 1 (nonhematologic), or baseline toxicity except alopecia; ANC $\geq 1.5 \times 10^9$/L; platelet count $\geq 75 \times 10^9$/L; serum creatinine ≤1.5 × ULN, and urine protein-to-creatinine ratio of ≤0.2. The maximum number of doses of inotuzumab ozogamicin was 8 for 1.3 mg/m^2 and 7 for 1.8 mg/m^2.

Pharmacokinetics. Timed blood samples for pharmacokinetic analysis were collected for cycles 1–3 at 0 (pre-dose), 1, 4 (cycles 1 and 3 only), 24, 48, 120, 168, 216, 336, and 504 h relative to the start of infusion for each dosing period and at predose only for cycle 4. If the patient received four doses, then the sample had to be drawn before cycle 5. The serum concentrations of inotuzumab ozogamicin and total calicheamicin were determined using a validated enzyme-linked immunosorbent assay.

The noncompartmental pharmacokinetic parameters of inotuzumab ozogamicin and total calicheamicin were estimated using the WinNonlin (version 4.1) program. The parameters which were determined included the following: end-of-infusion peak concentration (C_{max}), area under the concentration-time curve (AUC), clearance (CL), apparent steady-state volume of distribution (V_{ss}), and the terminal-phase elimination half-life ($t_{1/2}$).

Safety. An AE was considered to be treatment emergent if its onset occurred between the first and the last dose, plus a lag of 28 days provided the following criteria were met: (i) the AE was not present before the start of the first dose and did not occur in the patient as a chronic condition; (ii) the AE was present before the start of the first dose or was part of the patient's medical history, but the severity or frequency increased after the start of the first dose.

Efficacy. Patients were evaluable for efficacy if they received ≥2 doses of inotuzumab ozogamicin, had a baseline tumor CT scan and had undergone at least one tumor assessment for response after baseline assessment. In addition, patients with documented PD prior to receiving two doses of inotuzumab ozogamicin were considered evaluable for efficacy. Tumor response was assessed according to the International Workshop Response Criteria for Non-Hodgkin Lymphoma.[14] The overall response rate (ORR) was defined as the percentage of patients meeting the criteria for complete response (CR), unconfirmed complete response (CRu), or partial response (PR). Stable disease (SD) was measured from the start of the treatment until the criteria for PD were met, taking as the reference the smallest measurements recorded since the initiation of treatment.

Statistical analysis. The sample size for this study was determined by clinical rather than statistical considerations. The probabilities of detecting at least one AE of grade ≥3 with six patients receiving inotuzumab ozogamicin were 0.469, 0.822, and 0.984 when the true rates were 0.10, 0.25, and 0.50, respectively. The probabilities of detecting at least one such event in 10 patients receiving treatment were 0.651, 0.944, and 0.999, respectively.

With cohort sizes of three to six patients, if the true underlying rates of DLT were 0.1, 0.2, 0.3, 0.4, and 0.5, there would be a 0.985, 0.905, 0.754, 0.558, and 0.359 chance, respectively, of escalating to the next full dose. The ORR was estimated using an exact confidence interval (CI) approach.

Results

Patients. From March 2007 to July 2008, a total of 13 patients were enrolled in the study; three patients enrolled in the 1.3 mg/m^2 dose cohort and 10 patients in the 1.8 mg/m^2 dose cohort. The summary of demographic and other baseline characteristics for all patients is presented in Table 1. There were seven males and six females, all with a median age of 49 years (range, 43–72 years). All 13 patients had FL. The median number of prior treatment regimens was 1 (range, 1–13). All 13 patients had previous rituximab treatment (monotherapy or in combination with chemotherapy). Patients were categorized in low (38.5%), intermediate (42%), and high (15%) risk groups according to Follicular Lymphoma International Prognostic Index (FLIPI).[15]

Table 1. Demographic and baseline characteristics, safety population

Characteristics	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
Median age, years (range)	57 (51–66)	48 (43–72)	49 (43–72)
Sex, n (%)			
Female	2 (67)	4 (40)	6 (46)
Male	1 (33)	6 (60)	7 (54)
ECOG performance status, n (%)			
0	3 (100)	10 (100)	13 (100)
Primary diagnosis, n (%)			
Follicular lymphoma	3 (100)	10 (100)	13 (100)
FLIPI risk groups, n (%)			
Low	2 (67)	3 (30)	5 (39)
Intermediate	1 (33)	5 (50)	6 (46)
High	0	2 (20)	2 (15)
Number of prior chemo-/immunotherapy regimens, n (%)			
1	2 (67)	6 (60)	8 (62)
2	0	0	0
3	0	1 (10)	1 (8)
≥4	1 (33)	3 (30)	4 (31)

ECOG, Eastern Cooperative Oncology Group; FLIPI, Follicular Lymphoma International Prognostic Index.

Safety. In dose escalation, no patients had DLTs, and the MTD previously determined in non-Japanese patients (1.8 mg/m^2) was confirmed for Japanese patients in this study. The most common drug-related AEs were thrombocytopenia (100% patients); leukopenia (92%); neutropenia, elevated AST, anorexia, and lymphopenia (85%, each); elevated blood fibrinogen (69%); nausea (77%); elevated ALT, elevated alkaline phosphatase, and decreased hemoglobin (54%, each); malaise, elevated blood bilirubin, and headache (46%, each; Table 2(a)).

A summary of drug-related grade 3 or higher AEs is shown in Table 2(b). At least one drug-related grade ≥3 AEs was reported in nine of the 13 (69%) patients. Drug-related grade ≥3 AEs were thrombocytopenia (7 patients, 54%), lymphopenia and neutropenia (4, 31% each), leukopenia (2, 15%), and elevated blood bilirubin and hypokalemia (1, 8% each). Although neither lymphopenia nor leukopenia was reported for the 1.3 mg/m^2 cohort, the overall incidence of drug-related grade ≥3 AEs was comparable between the two cohorts. There were no patients who died during the study.

A total of four patients experienced dose delays, one (33%) patient in the 1.3 mg/m^2 cohort and three (30%) patients in the 1.8 mg/m^2 cohort (Table 3). Each had one delay. The AEs leading to dose delays were neutropenia (3 patients, 23%) and thrombocytopenia (2, 15%). Two (20%) patients in the 1.8 mg/m^2 cohort had one dose reduction (Table 4). Adverse events (AEs) leading to the dose reduction were thrombocytopenia and pleural effusion (1 patient, 8% each). There were no dose reductions in the 1.3 mg/m^2 cohort.

Seven patients discontinued treatment due to AEs: one patient because of grade 2 rash, one patient because of grade 2 urticaria, and five patients because of AEs that required treatment delays of >3 weeks (two patients with prolonged thrombocytopenia, one patient with prolonged thrombocytopenia and neutropenia, one patient with neutropenia and elevated alkaline phosphatase, and one patient with prolonged neutropenia and elevated total bilirubin).

Pharmacokinetics. Pharmacokinetic data after the first dosing were obtained for all 13 patients. The two patients who received 1.8 mg/m^2 inotuzumab ozogamicin and had a dose reduction after cycle 1 were excluded from pharmacokinetic assessments for cycle 2 and thereafter. The mean ± SD serum concentrations of inotuzumab ozogamicin and total calicheamicin *versus* time

Table 2. Inotuzumab ozogamicin-related adverse events, (a) all grades in ≥4 patients (b) grades ≥3

Adverse event, n (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
(a) all grades in ≥4 patients			
Thrombocytopenia	3 (100)	10 (100)	13 (100)
Leukopenia	3 (100)	9 (90)	12 (92)
Lymphopenia	3 (100)	8 (80)	11 (85)
Neutropenia	3 (100)	8 (80)	11 (85)
Aspartate aminotransferase increased	3 (100)	8 (80)	11 (85)
Anorexia	3 (100)	8 (80)	11 (85)
Nausea	3 (100)	7 (70)	10 (77)
Blood fibrinogen increased	2 (67)	7 (70)	9 (69)
Alanine aminotransferase increased	1 (33)	6 (60)	7 (54)
Blood alkaline phosphatase increased	1 (33)	6 (60)	7 (54)
Hemoglobin decreased	1 (33)	6 (60)	7 (54)
Malaise	3 (100)	3 (30)	6 (46)
Blood bilirubin increased	2 (67)	4 (40)	6 (46)
Headache	2 (67)	4 (40)	6 (46)
Constipation	1 (33)	4 (40)	5 (39)
Influenza	1 (33)	4 (40)	5 (39)
Blood lactate dehydrogenase increased	2 (67)	3 (30)	5 (39)
Fibrin D dimer increased	0	5 (50)	5 (39)
Hyperglycemia	1 (33)	4 (40)	5 (39)
Stomach discomfort	1 (33)	3 (30)	4 (31)
Fatigue	0	4 (40)	4 (31)
Hypercholesterolemia	1 (33)	3 (30)	4 (31)
Hypokalemia	2 (67)	2 (20)	4 (31)
Somnolence	2 (67)	2 (20)	4 (31)
Epistaxis	0	4 (40)	4 (31)
Rash	1 (33)	3 (30)	4 (31)
(b) grades ≥3			
Thrombocytopenia	2 (67)	5 (50)	7 (54)
Lymphopenia	0	4 (40)	4 (31)
Neutropenia	1 (33)	3 (30)	4 (31)
Leukopenia	0	2 (20)	2 (15)
Blood bilirubin increased	1 (33)	0	1 (8)
Hypokalemia	1 (33)	0	1 (8)

Table 3. Number (%) of patients reporting adverse events leading to dose delays, safety population

Parameter, n (%)	Inotuzumab ozogamicin treatment		
	1.3 mg/m^2 (n = 3)	1.8 mg/m^2 (n = 10)	Total (n = 13)
No. of patients with dose delays			
No dose delays	2 (67)	7 (70)	9 (69)
One or more dose delays	1 (33)	3 (30)	4 (31)
No. of dose delays per patient*			
One	1 (100)	3 (100)	4 (31)
Any adverse event leading to dose delay†	1 (33)	3 (30)	4 (31)
Neutropenia	1 (33)	2 (20)	3 (23)
Thrombocytopenia	1 (33)	1 (10)	2 (15)

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

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

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

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

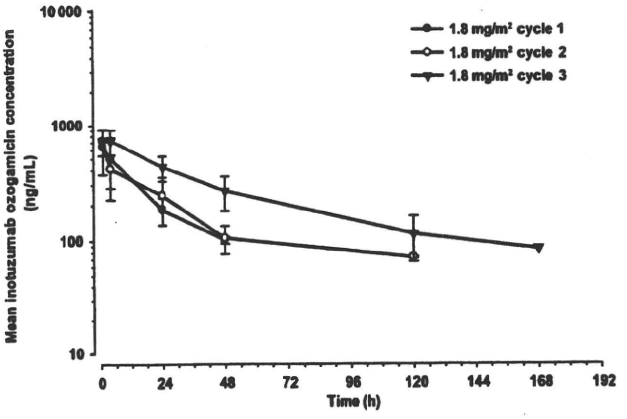

Fig. 1. Mean (SD) serum concentrations of inotuzumab ozogamicin after 1.8 mg/m^2 infusion of inotuzumab ozogamicin once every 4 weeks. Closed circle, cycle 1 (day 1, *n* = 10); open circle, cycle 2 (day 29, *n* = 8); closed triangle, cycle 3 (day 57, *n* = 5).

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

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

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

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

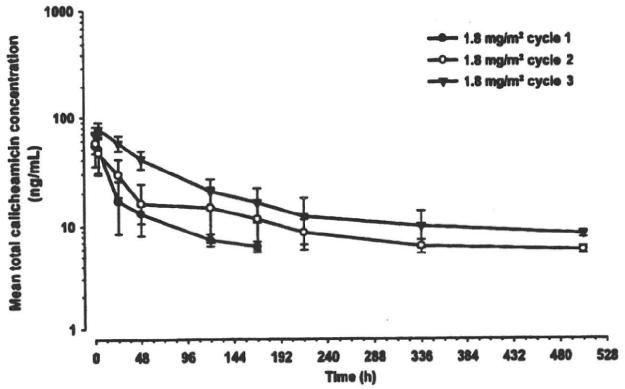

Fig. 2. Mean (SD) serum concentrations of total calicheamicin after 1.8 mg/m^2 infusion of inotuzumab ozogamicin once every 4 weeks. Closed circle, cycle 1 (day 1, *n* = 10); open circle, cycle 2 (day 29, *n* = 8); closed triangle, cycle 3 (day 57, *n* = 5).

Discussion

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

Table 5. Serum pharmacokinetic parameters of inotuzumab ozogamicin

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

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

Table 6. Serum pharmacokinetic parameters of total calicheamicin

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

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

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

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

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

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

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

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

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

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

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

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

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

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Disclosure Statement

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

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