

Institutional report - Thoracic general

Thymoma needs a new staging system

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Abstract

Despite the wide use of the Masaoka staging system for thymoma, the distribution of survival by stage group is not well balanced. The new staging systems for testing were defined as follows: stage I was created by merging Masaoka's stages I and II, and stage IV remained unchanged. Stages II and III were defined as thymomas with invasive growth and the following combinations of tumor diameter and number of involved structures/organs. Scheme 1: stage II included tumors less than 10 cm in diameter and involving one neighboring structure/organ. Stage III included tumors with all combinations of diameter and number of involved structures/organs other than those in stage II. Scheme 2: stage II included tumors of all combinations other than those in stage III. Stage III included tumors 10 cm or more in diameter and involving two or more structures/organs. The survival curves were assessed for 138 patients treated at the National Cancer Center, Tokyo. The 10-year survival rates for each stage according to the Masaoka, Scheme 1, and Scheme 2 systems were as follows: stage I (100%, 100%, 100%), stage II (100%, 86%, 83%), stage III (70%, 64%, 34%), and stage IV (34%, 34%, 34%), respectively. The survival curves for Scheme 1 gave the most balanced distribution of survival in each staging group. By considering both tumor diameter and number of involved structures/organs, Masaoka's stages I–III could be rearranged with more balanced distribution of survival.

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1. Introduction

Thymoma is a neoplasm that arises from the epithelial cells of the thymus [1]. Due to their low incidence, wide range of histological appearance, and unique biologic behavior, their histological classification and a suitable staging system have been the subject of controversy for many years [2–4]. In 1999, a new histological classification was promulgated by WHO, in which thymic epithelial tumors were defined as types A, AB, B1, B2, B3, and C [5]. Type 'C' is thymic carcinoma with apparent cytological atypia. Our previous study on 130 resected thymomas demonstrated that this WHO histologic classification is an important indicator of the prognosis [6].

The TNM staging system has been applied to most malignant tumors, with an exact definition of the T- (tumor), N- (lymph node), and M- (distant metastasis) denominators. The purpose of this approach is to identify a relatively

homogeneous group of patients with a similar prognosis, a 'stage group', to help determine a suitable treatment strategy [7]. There is currently no authorized staging system available for thymic epithelial tumors. The degree of tumor invasion described by the surgeon has long been respected as the single most important factor in predicting the patient's prognosis [8]. In clinical practice, the Masaoka system [9], which is based on the degree of 'invasiveness' into the capsule and neighboring structures, has been used either tentatively or conventionally. However, several problems have become apparent with this system. Recent advances in treatment strategies for thymic epithelial tumors highlight the need for a TNM-type staging system in this field.

In this retrospective study, we proposed two staging systems based on the tumor diameter and number of structures/organs involved by the tumor. Their suitability for predicting the prognosis was assessed by comparing the survival curves for the Masaoka system and our proposed systems.

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2. Material and methods

2.1. Patients

From 1962 to 2000, 152 patients with thymoma were treated at the National Cancer Center Hospital, Tokyo. Thirteen patients who underwent an initial surgery at another hospital and for whom there were not enough tissue specimens for pathological review were excluded from the present study. One additional patient for whom there were not enough clinical data was also excluded. Therefore, a total of 138 patients with thymoma was considered for the present study. The patients' clinical features were retrospectively studied by an extensive review of their medical records with regard to any allied disease, mode of operation, perioperative therapy, mode of recurrence, and prognosis. The 58 men and 80 women (male to female ratio, 0.72) ranged in age from 15 to 83 years (mean age, 54 ± 14 years). As for allied diseases, myasthenia gravis (MG) and pure red cell aplasia (PRCA) were seen in 12% and 2%, respectively. Among the 138 patients, 131 underwent surgical resection regardless of its completeness, and the remaining seven were treated with non-surgical therapy such as chemotherapy and/or radiation because of the extent of the disease. As for the mode of operation for thymoma, thymomectomy (resection of the tumor only), thymothymomectomy (total thymectomy including the thymoma and neighboring structures if necessary), and exploration were performed in 53, 42, and 5%, respectively. The resection was 'complete (no macroscopic/microscopic residual tumor)' in 95% of 131 resections. Tumors were smaller than 10 cm in diameter in 112 patients (81%), and 10 cm and more in diameter in 26 patients (19%). The histological subtype was determined according to the 1999 WHO classification as type 'A' ($n = 19$), 'AB' ($n = 57$), 'B1' ($n = 18$), 'B2' ($n = 32$), and 'B3' ($n = 12$) using hematoxylin-eosin-stained formalin-fixed paraffin sections of surgically resected or biopsy specimens of the tumor. Patients with thymic carcinoma ('C' by the WHO classification) or thymic neuroendocrine tumor were excluded from this study. Nodal involvement was not seen in any one of the patients.

2.2. Clinical stage

The Masaoka system has most commonly been used to stage thymoma [9]. This system is summarized as follows: stage I, macroscopically completely encapsulated tumors without microscopic capsular invasion; stage II, tumors with macroscopic invasion into surrounding fatty tissue or mediastinal pleura, or tumors with microscopic invasion into capsule; stage III, tumors with macroscopic invasion into neighboring structures/organs; stage IV, tumors with pleural or pericardial dissemination or with lymphogenous/hematogenous metastases. To establish a staging system which better characterizes the extent of the disease in terms

of the prognosis in each stage category, new schemes were introduced based on the results of our previous multivariate analysis [6], where the tumor diameter (10 cm cut-off) had a significant association with the prognosis. Two new staging systems, Scheme 1 and Scheme 2, were established as shown in Table 1. Briefly, in both systems, stage I was defined as tumors without any invasion into other structures/organs regardless of capsular involvement, and stage IV was identical to that in Masaoka's system. Tumors that invaded into neighboring structures/organs were defined as either stage II or III based on a combination of tumor diameter and number of involved structures/organs. In Scheme 1, stage II included tumors that were less than 10 cm in diameter and involved only one neighboring structure/organ. All other combinations of diameter and number of involved structures/organs were included in stage III. In Scheme 2, stage III included tumors that were 10 cm or more in diameter and involved two or more structures/organs. All other combinations of diameter and number of involved structures/organs were included in stage II.

2.3. Statistical analyses

Survival was measured from the day of the operation until death or the last follow-up visit. For patients without surgical treatment, the initial date of any treatment was defined as the first day of treatment. The Kaplan–Meier method was used to estimate the time to death from thymoma-related causes and its 95% confidence interval. Death due to the worsening of MG was included as a thymoma-related death. Differences in survival were evaluated by the log-rank test. Significance was defined as a *P*-value less than 0.05.

3. Results

3.1. Distribution of stage

The distributions of stages of 138 patients according to the Masaoka, Scheme 1, and Scheme 2 systems are as follows: stage I (40, 94, 94), stage II (54, 10, 22), stage III (28, 18, 6), stage IV (16, 16, 16), respectively. Since all tumors limited to within the mediastinal compartment were newly defined as stage I regardless of capsular involvement, the percentage of stage I was more than that in the Masaoka system.

3.2. Prognosis

There were 19 recurrences after the treatment out of 131 patients who underwent surgery. The most common mode of recurrence was pleural dissemination in 11, followed by local regrowth of the tumor in four, pulmonary metastasis in three, and unknown site in one. The number of patients with recurrence by stage (Masaoka, Scheme 1, Scheme 2) was as

Table I
New staging systems for testing (Scheme 1 and Scheme 2)

Stage	Description	
I	Tumors without any invasion into other structures/structures/structures/organs regardless of capsular involvement	
II	Scheme 1: tumors smaller than 10 cm in diameter and involving only one neighboring structure/organ Scheme 2: tumors of all combinations of diameter and number of involved structures/organs other than those in stage III	
III	Scheme 1: tumors of all combinations of diameter and number of involved structures/organs other than those in stage II Scheme 2: tumors 10 cm or more in diameter and involving two or more neighboring structures/organs	
IV	Tumors with pleural or pericardial dissemination (IVa) or lymphatic/vascular metastasis (IVb)	
Invasion	Tumor diameter	
	Less than 10 cm	10 cm or more
Scheme 1		
Single-structure/organ invasion	Stage II	Stage III
Multiple-structure/organ invasion	Stage III	Stage III
Scheme 2		
Single-structure/organ invasion	Stage II	Stage II
Multiple-structure/organ invasion	Stage II	Stage III

follows: stage I (2, 4, 4), stage II (2, 2, 8), stage III (10, 8, 2), and stage IV (5, 5, 5), respectively. There was no special trend in the mode of recurrence according to the stage of any staging system. For all 138 patients, the 5- and 10-year survival rates were 89 and 87%, respectively. The survival curves according to the Masaoka, Scheme 1, and Scheme 2 systems are shown in Figs. 1–3, respectively. In the Masaoka system (Fig. 1), the survival curves for stages I and II were completely superimposed throughout the entire course of observation, which indicated that capsular invasion had no impact on survival. Since there were no events in stages I and II, we could only evaluate the difference in survival between stages III and IV: this difference was significant ($P = 0.027$). In Scheme 1, the survival curves for stages I–IV varied, with the prognosis worsening from stage I to IV (Fig. 2). The differences in survival according to the stage were as follows: between

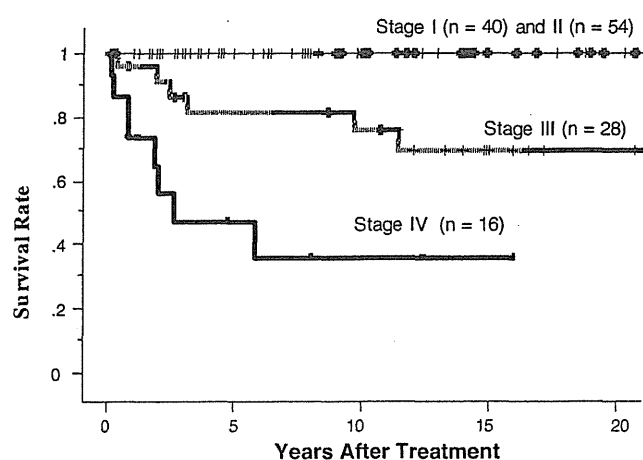


Fig. 1. Survival curves according to stage defined by the Masaoka system. The 5- and 10-year survival rates were 100%, 100% (stage I), 100%, 100% (stage II), 75%, 70% (stage III), and 44%, 34% (stage IV). The difference in survival: between stages III and IV ($P = 0.027$).

stages II and III ($P = 0.13$), stages II and IV ($P = 0.012$), and stages III and IV ($P = 0.18$). In Scheme 2, the survival curves of stages III and IV were almost superimposed (Fig. 3). The differences in survival according to the stage were as follows: between stages II and III ($P = 0.003$) and stages II and IV ($P = 0.003$).

4. Discussion

Thymoma is a tumor that arises from thymic epithelial cells and has unique clinicopathological properties [1,2]. In the earlier phase of the disease, tumors are well encapsulated with dense fibrous tissue, and behave like benign tumors. In the later phase, however, they break the capsule and invade neighboring structures. Nevertheless,

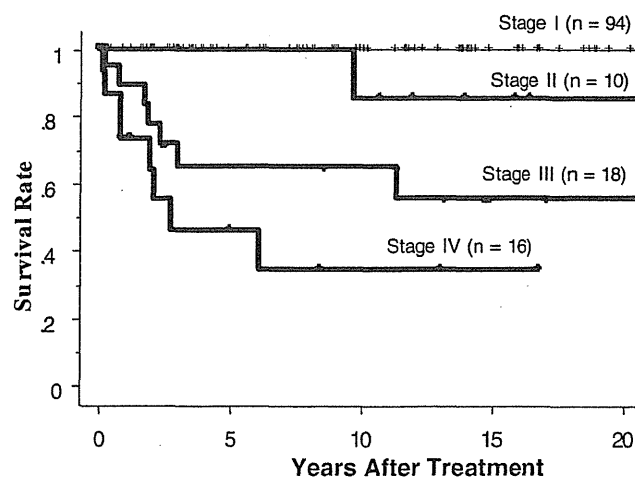


Fig. 2. Survival curves according to stage defined by Scheme 1. The 5- and 10-year survival rates were 100%, 100% (stage I), 100%, 86% (stage II), 64%, 64% (stage III), and 44%, 34% (stage IV). The differences in survival: between stages II and III ($P = 0.13$), stages II and IV ($P = 0.012$), and stages III and IV ($P = 0.18$).

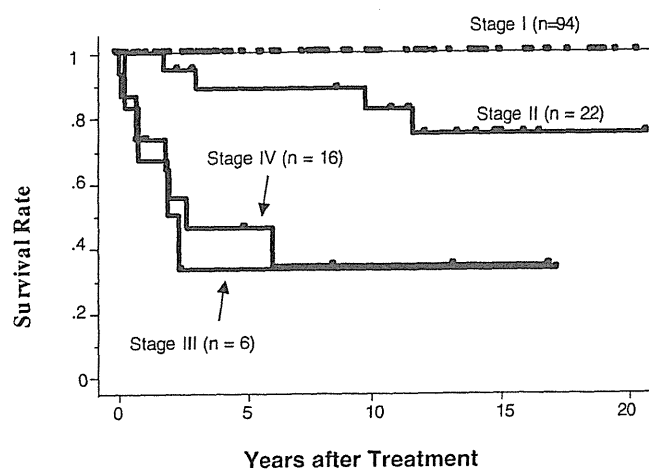


Fig. 3. Survival curves according to stage defined by Scheme 2. The 5- and 10-year survival rates were 100%, 100% (stage I), 90%, 83% (stage II), 34%, 34% (stage III), and 44%, 34% (stage IV). The differences in survival: between stages II and III ($P = 0.003$) and stages II and IV ($P = 0.003$).

lymphatic/hematogenous spread is quite rare even in the later phase of the disease. Indeed, nodal involvement was not seen in any of the present cases. This characteristic nature of the disease has been the main cause of the delay in establishing a TNM-type staging system. However, the recent multimodal approach for advanced thymoma necessitates the description of 'stage' to help decide upon a suitable therapeutic strategy [10].

With regard to the Masaoka system, several issues have been raised by other groups as well as our own, although the stage defined by the Masaoka system does reflect the prognosis to some degree. One of the most important issues is the definition of stage I and II thymomas. In the present study, the survival curves for these two stages were completely superimposed, with 5- and 10-year survival rates of 100%. A recent report by Okumura and associates on 273 thymomas also showed similar survival rates for stages I and II [11]. Although the 5- and 10-year survival rates varied in each report, minimal differences in survival between stages I and II have repeatedly been reported [6,11]. These facts clearly indicate that capsular invasion does not affect survival, and thus should not be used to define stage groups. Another issue is the heterogeneous prognosis of patients with stage III thymoma. By definition, thymomas with only slight invasion into the pericardium are considered stage III, and they can be resected easily with a potentially favorable prognosis. On the other hand, huge tumors with aggressive invasion into the lung, great vessels, etc. are also considered stage III, and they might be unresectable with an unfavorable prognosis. Therefore, although these two tumors are in the same stage group, the appropriate therapeutic strategies are considerably different. Due to the wide range in the extent of the disease and the prognosis, different treatment plans could be applied, which would contradict the original purpose of 'staging'. These two issues regarding stages I–III seem the most crucial defect of the Masaoka system, and should be addressed.

Thus, we proposed two staging systems, and assessed the distribution of the prognosis among the four stage groups in comparison with those in the Masaoka system. In the new systems, we merged Masaoka's stages I and II to create a new stage I, which was defined as tumors limited to within both the mediastinal pleurae regardless of capsular invasion. Furthermore, to create new stages II and III from Masaoka's stage III, we considered both the tumor size and number of involved structures/organs. The prognostic significance of tumor size has been previously demonstrated by two important studies. Blumberg and colleagues showed that patients with large thymomas (>11 cm) had a significantly decreased ($P = 0.0006$) survival, with a 5-year survival rate of only 58% compared with 84% for patients with smaller (5–11 cm) thymomas [12]. Similarly, Lewis and colleagues showed that patients with thymomas 15 cm or more in diameter had a significantly worse prognosis than those with smaller thymomas ($P < 0.0001$) [13]. Our previous multivariate analysis of 130 resected thymomas also indicated that the size of the tumor was a significant prognostic factor [8]. The number of involved structures/organs might affect the resectability and, therefore, can be expected to relate to the choice of treatment and survival.

Among the three staging systems assessed, Scheme 1 gave the most balanced distribution of survival according to stage, although the limited number of case in this study made the statistical demonstration of 'balanced distribution of survival curves' difficult. The 10-year survival rates in stages I–IV worsened in a stepwise manner: 100, 86, 64, 34%, respectively. According to the Scheme 1 system, for example, a thymoma of 9.6 cm in diameter invading the lung parenchyma would be considered stage II, and could be expected to have a favorable survival rate of around 86% at 10 years with curative resection. However, due to the possibility of local recurrence, postoperative radiation might be indicated. Thus, we think that this new staging system can provide more practical information regarding the treatment plan and prognosis.

TNM-type staging systems have previously been reported by Yamakawa [14] (for thymoma) and Tsuchiya [15] (for thymic carcinoma). Due to the large difference in pathobiological properties and prognosis, two different stage groupings are defined for thymoma and other thymic malignancies. Due to the rarity of thymic carcinoma and neuroendocrine tumors, further accumulation of data and prognostic simulation are indispensable for establishing an appropriate stage grouping.

Acknowledgements

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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/m² 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/m² 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/m² by eight consecutive weekly infusions. We also analyzed pre-treatment 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) $<4 \times$ upper limit of normal (ULN), total bilirubin $<2 \times$ ULN, serum

creatinine $<1.5 \times$ ULN and PaO₂ ≥ 65 mmHg. Absolute neutrophil count was $\geq 1200/\mu\text{l}$ and platelet count $\geq 75\,000/\mu\text{l}$.

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; $>1000/\mu\text{l}$ 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/m² 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 (⁶⁷Ga) 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 (AH SCT), as shown in Table 1. No patients had received monoclonal antibody therapy.

Central pathology review

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

Early termination of rituximab treatment

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

Table 1. Patient characteristics

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

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

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

ORR, PFS and TTP

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

Among 57 eligible patients, 11 patients had a washout period <4 weeks (21–26 days, eight cases; 18 days, one case; 17 days, one case; 15 days, one case). None of the 11 patients responded to

the last prior salvage chemotherapy (three SD and eight PD), and they all had massive tumor lesions immediately before rituximab treatment. Only one patient responded to rituximab (one CR, one SD, eight PD, and one not evaluable).

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

Table 2. Responses

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

Responses to rituximab were evaluated according to the International Workshop NHL response criteria. No patient showed CR/unconfirmed.

CI, confidence interval; CR, complete response; NE, not evaluable due to insufficient follow-up; ORR, overall response rate; PD, progressive disease; PR, partial response; SD, stable disease.

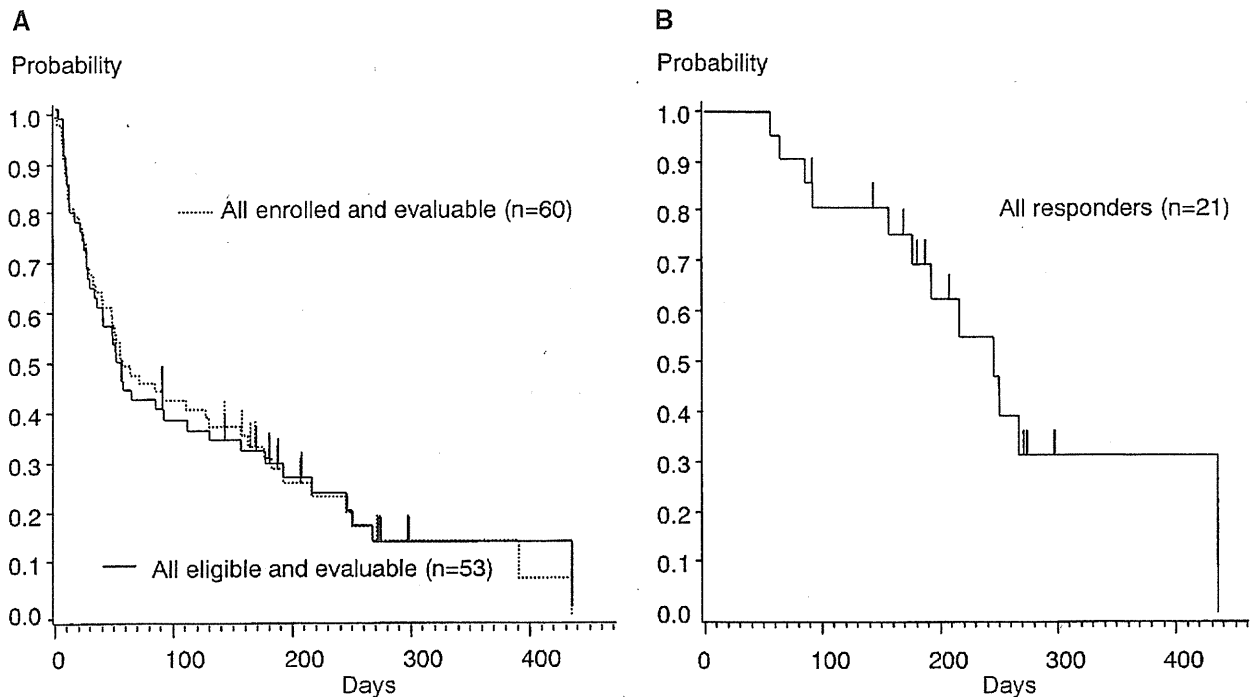


Figure 1. (A) Progression-free survival (PFS) and (B) time to progression (TTP). The median PFS values for all eligible and evaluable patients ($n = 53$) and for all enrolled and evaluable patients ($n = 60$) were 52 days [95% confidence interval (CI) 33–111] and 61 days (95% CI 41–156), respectively.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

Non-hematological toxicities

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

One patient developed a grade 3 upper-respiratory infection 3 months after completion of the planned rituximab treatment. Hematological testing indicated that the patient had also developed grade 4 neutropenia. Supportive care with antibiotics, G-CSF and

immunoglobulin preparations was performed under hospitalization, and he recovered 9 days after the onset of infection.

Hematological toxicities

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

Abnormal laboratory findings

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

Table 3. Adverse drug reactions (*n* = 67)

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

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

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

Infection

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

Early death

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

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

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

HACA development

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

Factors affecting ORR and PFS

Univariate and multivariate analyses of pretreatment factors affecting ORR and PFS were performed in 53 patients who were eligible and evaluable. As shown in Table 4, elevated LDH and

primary chemorefractoriness were found to be unfavorable factors significantly affecting ORR and PFS in the univariate and multivariate analyses. In the univariate analysis, PFS in patients in the low/low-intermediate risk group according to IPI was longer than that in patients in the high-intermediate/high risk group ($P = 0.034$). PFS in patients with a history of AHSCT was also longer than that in patients without it ($P = 0.045$).

Pharmacokinetic parameters and correlation with responses

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

Discussion

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

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

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

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

^a*P* value by Fisher's exact test.

^b*P* value by logistic regression model (stepwise procedure).

^c*P* value by log-rank test.

^d*P* value by Cox's proportional hazard model (stepwise procedure).

Statistically significant difference at **P* < 0.05 and ***P* < 0.01.

CI, confidence interval; LDH, lactate dehydrogenase; ORR, overall response rate.

Table 5. Pharmacokinetic parameters of responders and non-responders

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

^aStudent's *t*-test.

^bSignificant difference at *P* < 0.05.

AUC, area under the concentration–time curve; *C*_{max}, maximum concentration; SD, standard deviation; *t*_{1/2}, serum half-life.

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

One patient developed grade 4 elevations of AST (2564 IU/l) and ALT (3176 IU/l) concomitantly with grade 3 elevation of total bilirubin after completion of the planned infusion, and was diagnosed with acute hepatitis in the present study. Virus testing revealed that hepatitis viruses A, B and C were negative, but TT virus-DNA was present in his serum. TT virus has been reported to be a novel virus associated with elevation of hepatic transami-

nase in patients with post-transfusion as well as acute and chronic non-A to G hepatitis [28, 29]. Neither hepatomegaly nor space-occupying lesion was observed on CT films in this patient. Pre-treatment transaminase levels were all within normal ranges. Moreover, the acute hepatitis resolved without particular treatment, suggesting that TT virus might have been causative for the hepatitis.

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

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

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

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

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

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Primary Mediastinal Large B-Cell Lymphoma: A Single-Institution Clinical Study in Japan

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Abstract

Several clinicopathologic studies of primary mediastinal large B-cell lymphoma (Med-DLBCL) have been reported from Western countries; however, only a few series of at most 10 cases are available in Japan. To further clarify the Med-DLBCL occurring in Japan, we analyzed the clinical features of 28 patients with Med-DLBCL diagnoses who were treated at the National Cancer Center Hospital between 1982 and 2002. The median age was 37 years (range, 18-80 years). The ages of 16 male patients ranged widely from 18 to 80 years, whereas the 12 female patients appeared to show a single age peak at 20 to 40 years. Only 13 patients (46%) achieved a complete response with initial treatments, mostly by CHOP-like regimens (cyclophosphamide, doxorubicin, vincristine [Oncovin], and prednisolone) followed by radiotherapy. The estimated 3-year overall and failure-free survival rates were 32% and 33%, respectively, indicating the relatively unfavorable prognosis of the patients in our series. The following factors were found to be significantly associated with shortened survival prospects: age >60 years, serum lactate dehydrogenase level greater than normal, performance status >1, and presence of bulky mediastinal mass. In conclusion, the clinical features of Japanese patients with Med-DLBCL may be different from those with the disease in Western countries. Because this investigation was a single-institution study with a limited number of patients, however, multicenter confirmatory studies are needed.

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Key words: Primary mediastinal large B-cell lymphoma; Japan; Geographical variation; Treatment; Prognostic factor

1. Introduction

Primary mediastinal large B-cell lymphoma (Med-DLBCL) is a diffuse large B-cell lymphoma (DLBCL) that arises from thymic B-cells, and it has been recognized as a distinct entity in the revised European-American lymphoma classification [1] and in the World Health Organization (WHO) classification [2]. According to the reports from Western countries, patients with Med-DLBCL are typically young, of slight to moderate female preponderance compared with patients with other DLBCL, and with an anterior and superior mediastinal mass that frequently causes superior vena cava syndrome (SVCS), cough, chest pain, and hoarseness [2-5]. Many case series have been reported from

Western countries [3-13], although only a few series of at most 10 cases are available from Japan [14-16].

Recent investigations have revealed that the molecular signature of Med-DLBCL differs from that of other types of DLBCL and shares features with Hodgkin's lymphoma [17-19]. Considering the low incidence of Hodgkin's lymphoma in Japan and other East Asian countries [20], the incidence and clinical features of patients with Med-DLBCL in Japan may also be different from those of patients in Western countries. To further clarify the features of patients with Med-DLBCL in Japan, we analyzed the clinical features of 28 patients with Med-DLBCL diagnoses who were treated in the past 20 years at our institution. To our knowledge, this is the largest case series of Med-DLBCL in Asia.

2. Patients and Methods

2.1. Patients

The cases of 28 patients with Med-DLBCL diagnoses and who were treated at the National Cancer Center Hospital

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between September 1982 and September 2002 were analyzed. The diagnostic criteria of Med-DLBCL in the present study were (1) the presence of a mediastinal mass of at least 5 cm in the largest diameter and the absence of other larger masses in the mediastinum and (2) a histopathologic diagnosis of DLBCL, which is a diagnostic criterion of Med-DLBCL proposed by the Nebraska Lymphoma Study Group [13]. Clinical data were obtained from medical charts and included age, sex, clinical stage, serum lactate dehydrogenase (LDH) level, performance status (PS), any bulky disease, any pleural and pericardial effusions, site of involvement, number of extranodal sites, International Prognostic Index (IPI) [21], and treatment. Clinical stage was determined according to the revised American Joint Committee on Cancer staging system for lymphoid neoplasms [22], which is a modified version of the Ann Arbor staging classification [23]. In this study, bulky disease was defined as a mass greater than 10 cm in the largest diameter as detected by computed tomography. PS was determined according to the Eastern Cooperative Oncology Group scale [24].

2.2. Histopathologic Review

Diagnostic tissue materials were obtained by computed tomography-guided percutaneous needle biopsy in 18 patients (64%), open-thoracic biopsy or surgical resection in 7 patients (25%), and superficial lymph node biopsy in 3 patients (11%). To ensure the diagnosis of Med-DLBCL, we performed in all cases a histopathologic review on hematoxylin-eosin-stained sections as well as paraffin-section immunostains. Primary antibodies used for immunohistochemistry analyses included anti-CD3 (PS1; Novocastra Laboratories, Newcastle upon Tyne, UK) and anti-CD20 (L26; DakoCytomation, Glostrup, Denmark). Anti-CD30 antibody (BerH2; DakoCytomation) was also used in selected cases. Tumor phenotype was judged as B-cell when the tumor cells were stained positively for CD20.

2.3. Assessment of Response and Survival

Complete response (CR) was defined as a disappearance of all clinical evidence of lymphoma that lasted for a minimum of 4 weeks. Unconfirmed or uncertain CR (CRu) was defined as a greater than 50% decrease in the sum of the products of the greatest perpendicular diameters (SPD) of all measurable lesions with no clinical evidence of disease lasting for 3 months or longer without treatment, in accord with Cotswolds' response criteria for Hodgkin's lymphoma [25]. In this study, CRu was included in the category of CR. Partial response was defined as a greater than 50% decrease in the SPD of all measurable lesions lasting for at least 4 weeks. No change was defined as either a decrease of less than 50% or an increase of less than 25% in the SPD of any previously identified measurable lesions. Progressive disease was defined as any increase of greater than 25% in the SPD of any measurable lesions or the appearance of a new lesion.

Overall survival (OS) was measured from the beginning of treatment until death from any cause, and surviving patients were censored at the last contact date. Failure-free survival was defined as the time from the beginning of treat-

ment until the first recognition of disease progression, change of chemotherapy regimen, or death from any cause. Disease-free survival for patients who achieved a CR or CRu was defined as the time from the day of achieving a CR or CRu until relapse or death related to therapy.

2.4. Initial Treatments

Twenty-two (79%) of the 28 patients received chemotherapy initially. Surgical resection was performed in 4 patients (14%), and all of them received chemotherapy after diagnosis. Thus, these 26 patients (93%) were categorized as the chemotherapy group in initial treatments. The remaining 2 patients (7%) received radiotherapy of 40 Gy and 46.8 Gy, which was followed by combination chemotherapy. Of the 26 patients who received chemotherapy as initial treatments, 18 (69%) received CHOP-like regimens (cyclophosphamide [CPA], doxorubicin [DOX], vincristine [VCR], and prednisolone [PSL]). Seventeen of the 18 patients received standard-dose CHOP therapy, and the remaining patient received VEPA therapy (VCR, CPA, PSL, and DOX) [26]. Two patients (8%) were treated with a second-generation regimen (LSG4) consisting of VEPA-B (VEPA plus bleomycin [BLM]), M-FEPA, (methotrexate [MTX], vindesine [VDS], CPA, PSL, and DOX), and VEPP-B (VCR, etoposide [ETP], procarbazine [PCZ], PSL, and BLM) [27]. Four patients (15%) received a dose-intensified third-generation regimen (LSG9) consisting of VEPA-B, M-FEPA, and FEPP-AB (VDS, ETP, PCZ, PSL, DOX, and BLM) [28], and 2 patients (8%) were treated with other chemotherapy regimens.

2.5. Statistical Analysis

To compare the mean ages of male and female patients, we performed a 2-sample Student *t* test with the Welch correction after the confirmation of each variance and the histograms of the 2 groups. All survival curves were evaluated by means of the method of Kaplan and Meier. The log-rank test was used in univariate analyses to identify factors affecting OS. The following factors were analyzed: age, clinical stage, serum LDH level, PS, number of extranodal lesions, bulky disease, pleural effusion, pericardial effusion, initial chemotherapy regimen, additional radiotherapy, and IPI. *P* values of .05 or less were considered indicative of statistical significance.

3. Results

3.1. Histopathologic Review

Histopathologic review confirmed the filed diagnosis of DLBCL in all cases. However, there were 2 cases in the 1980s in which an incorrect diagnosis was given at the time of biopsy. One was called a thymoma, and the other was called a germ cell tumor.

In all cases, there were aggregations of atypical large lymphoid cells to some extent. Occasionally, they formed sheet-like nests compartmentalized by sclerotic fibrous stroma, mimicking epithelial tumors. The extent of sclerosis or

Table 1.

Characteristics of Patients with Primary Mediastinal Large B-Cell Lymphoma at Initial Diagnosis

Median age (range), y	37 (18-80)
M/F sex, n (%)	16 (57)/12 (43)
Lactate dehydrogenase >1× normal, n (%)	22 (79)
Clinical stage >II, n (%)	13 (46)
Performance status >1, n (%)	11 (39)
Extranodal lesions >1, n (%)	8 (29)
IPI high-intermediate or high, n (%)*	11 (39)
Bulky mediastinal mass (>10 cm), n (%)	17 (61)
Chest symptoms, n (%)	20 (71)
Superior vena cava syndrome, n (%)	5 (18)
B symptoms, n (%)	5 (18)
Pleural effusion, n (%)	9 (32)
Pericardial effusion, n (%)	10 (36)

*IPI indicates International Prognostic Index.

hyalinization of fibrous stroma varied among cases. Extensive coagulation necrosis was seen in 1 of the patients who underwent surgical resection.

The tumor cells stained positively for CD20 and negatively for CD3 in all cases, confirming the determination of B-cell lymphoma. Although the amount of CD30⁺ neoplastic B-cells varied, these cells could readily be differentiated from nodular sclerosis Hodgkin's lymphoma by their morphologic characteristics, such as the confluent growth of tumor cells, the loss of typical Reed-Sternberg cells or lacunar cells, and the loss of granulomatous background accompanied by granulocytes.

3.2. Clinical Features

The clinical characteristics of the 28 patients at diagnosis are summarized in Table 1. The median age was 37 years (range, 18-80 years). There were 16 male patients with a median age of 39 years and 12 female patients with a median age of 31 years (Figure 1). The 16 male patients ranged widely in age from 18 to 80 years, whereas the 12 female patients appeared to show a single age peak at 20 to 40 years. The female patients were younger than the male patients (mean, 31.8 years versus 43.5 years; $P = .04$). Bulky mediastinal mass with the largest diameter greater than

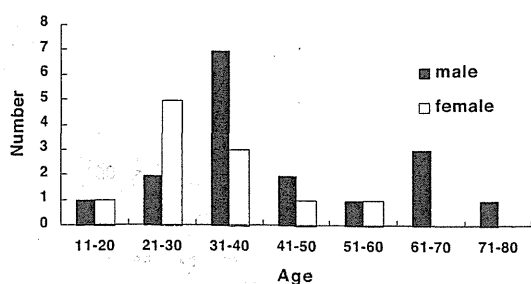


Figure 1. Age distribution of 28 patients with Med-DLBCL. Male patients showed a bimodal age distribution with 1 peak at 30 to 50 years of age and another peak in the 60s, whereas female patients showed a single peak at 20 to 40 years.

10 cm was found in 17 patients (61%). At diagnosis, 20 patients (71%) presented with chest symptoms caused by a mediastinal mass. SVCS and B symptoms were present in 5 patients (18%) each. Pleural effusion was found in 9 patients (32%), including 6 patients with positive cytologic findings, and pericardial effusion was found in 10 patients (36%), all with negative or unknown cytologic findings. In 21 patients (75%), we recognized direct extension to intrathoracic or extrathoracic extranodal sites adjacent to the mediastinal mass (lung parenchyma in 11 patients, chest wall in 9, pleura in 8, and thyroid in 1). Six patients (21%) showed extrathoracic involvement at diagnosis (para-aortic abdominal lymph nodes in 4 patients, ovary in 2, stomach in 2, spleen in 1, and adrenal gland in 1). No patient had bone marrow involvement or central nervous system involvement at initial diagnosis. Four patients (14%) had stage I disease, 11 (39%) had stage II disease, and the remaining 13 (46%) had stage IV disease. Eleven patients (39%) were categorized in a group of high or high-intermediate risk according to the IPI.

A diagnosis different from Med-DLBCL (malignant thymoma, germ cell tumor) was initially made in 5 patients (18%), 3 of whom had clinical their diagnoses determined by computed tomography or magnetic resonance imaging without histopathologic diagnosis because of the rapid progression of disease. Two of these 3 patients received 1 course of a DOX-containing chemotherapy regimen under the clinical diagnosis of malignant thymoma. They received CHOP therapy immediately after the accurate diagnosis of Med-DLBCL was obtained by a needle biopsy within 1 month of the initial diagnosis. Thus, these patients were included in the CHOP-like regimen group in this study to reflect the initial treatment. The remaining patient with an initial clinical diagnosis of germ cell tumor received radiotherapy, and the accurate Med-DLBCL diagnosis by needle biopsy was made 14 months after the initial diagnosis. One patient received surgical resection after an erroneous diagnosis of thymoma was made after a needle biopsy; after the accurate diagnosis was obtained, the patient received CHOP therapy. One patient received an erroneous diagnosis of germ cell tumor in 1982 and received vindesine, cisplatin, and radiation therapy.

3.3. Responses and Survival

All 28 patients were assessable for response to initial treatment, OS, and failure-free survival. The median follow-up duration for all 28 patients was 21 months and that for the 11 surviving patients was 35.7 months. Nine patients achieved a CR, and 4 achieved a CRu; thus, the CR rate was 46% (13/28). Nine patients (32%) achieved a partial response. Three patients (11%) showed progressive disease during initial treatment, and the remaining 3 (11%) showed no change. The overall response rate was 79% (22/28).

Ten patients (36%), including the 9 in continuous first CR, were alive at the time of the last follow-up. One treatment-related death was caused by interstitial pneumonia related to thoracic irradiation during the initial treatment. All 3 patients who relapsed after achieving a CR experienced the relapse within 1 year of the beginning of treatment. The estimated 3-year disease-free survival rate of the 13 patients who

achieved a CR or CRu with initial treatment was 66% (Figure 2). One patient was alive in CR with second-line chemotherapy that included high-dose chemotherapy with autologous hematopoietic stem cell transplantation (AHST). All 14 patients who did not achieve a CR or CRu with any therapy died. One patient who underwent high-dose chemotherapy with AHST died from treatment-related causes. The remaining 13 patients died of disease progression. The estimated 3-year failure-free survival and OS rates for all 28 patients were 33% and 32%, respectively (Figure 2). Five of the 12 patients who received radiation as 1 of the initial treatments did not achieve a CR.

3.4. Factors Affecting OS

The results of univariate analyses of various factors affecting OS are shown in Table 2. The following factors were found to be significantly associated with shortened survival times: age >60 years, serum LDH level higher than normal, PS >1, and presence of a bulky mediastinal mass. The OS curves according to the responses to initial treatment are shown in Figure 3. There were significant differences among the groups ($P < .001$).

4. Discussion

One of the unique findings in the present study is the lack of a female preponderance, although a slight to moderate preponderance of female patients was observed in most reported series, as shown Table 3. Another unique finding is that the age distributions of male patients and female patients may be different. Most previous studies did not show such a difference, although a sex-related difference in age distribution was also recognized in the series reported from the Massachusetts General Hospital in the United States [29].

In a clinical evaluation by the International Lymphoma Study Group that examined 1403 patients with non-Hodgkin's lymphoma (NHL) from 8 countries [30,31], the relative frequency of Med-DLBCL among NHL cases was 2.4%, and an increased relative incidence (9%) of Med-DLBCL in Locarno/Bellinzona in southern Switzerland, which is adjacent to Italy, has been reported [32]. Many reports on Med-DLBCL from Italy are available [3,6-8]. In contrast, only a few reports are available from Japan [14-16] and Southeast Asia [33]. Of the 3025 cases of NHL accord-

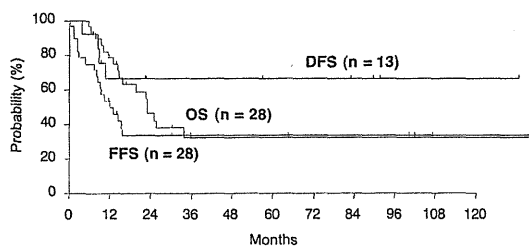


Figure 2. Survival curves. Overall survival (OS) and failure-free survival (FFS) of the 28 patients and disease-free survival (DFS) of the 13 patients who achieved a complete response with initial treatments.

Table 2.

Univariate Analysis of Factors Affecting Overall Survival (OS)*

Characteristic	3-Year OS, %	Median OS, mo	P
Age			.001†
>60 y (n = 4)	0	6	
≤60 y (n = 24)	38	25	
Clinical stage			.115
I, II (n = 15)	47	34	
III, IV (n = 13)	18	23	
Serum LDH level			.028†
>1× normal (n = 22)	18	19	
≤1× normal (n = 6)	80	Not reached	
Performance status			.01†
>1 (n = 11)	0	19	
0 or 1 (n = 17)	53	Not reached	
Extranodal lesions			.958
>1 (n = 8)	30	23	
≤1 (n = 20)	33	23	
Bulky mediastinal mass			.004†
Yes (n = 17)	8	16	
No (n = 11)	71	Not reached	
Pleural effusion			.465
Yes (n = 9)	28	23	
No (n = 19)	36	23	
Pericardial effusion			.944
Yes (n = 10)	30	23	
No (n = 18)	35	23	
Chemotherapy regimen			.291
CHOP-like (n = 18)	36	23	
2nd or 3rd generation (n = 6)	65	Not reached	
Radiotherapy (after initial chemotherapy)			.646
Yes (n = 10)	46	23	
No (n = 8)	57	Not reached	
International Prognostic Index			.071
Low or low-intermediate risk (n = 17)	46	34	
High-intermediate or high risk (n = 11)	11	23	

*LDH indicates lactate dehydrogenase; CHOP, regimen of cyclophosphamide, doxorubicin, vincristine [Oncovin], and prednisolone.

†P values <.05 are statistically significant.

ing to the WHO classification that were examined in a Japanese multicenter clinicopathologic study, only 8 patients (0.26%) were categorized as having Med-DLBCL

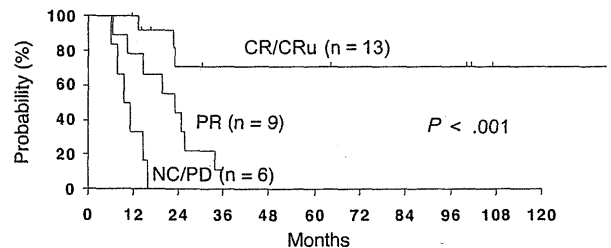


Figure 3. Overall survival according to response to initial treatment. The survival rates of the 3 groups were significantly different ($P < .001$). CR indicates complete response; CRu, unconfirmed or uncertain CR; PR, partial response; NC, no change; PD, progressive disease.

Table 3.

Main Features and Clinical Outcomes of Patients with Primary Mediastinal Large B-Cell Lymphoma in Reported Studies and the Present Study*

First Author	Country	Patients, n	B-Cell		Median Age (Range), y	Stage I or II, %	Regimen (No. of Patients)	Radiation	ORR, %/	
			Phenotype, %	M/F, n					CR, %	OS, %
Falini, 1995 [6]	Italy	18	100	5/13	31 (18-44)	94	MACOP-B (7) F-MACHOP (11)	No	100/57 91/18	NA
Lazzarino, 1997 [3]	Italy	106	100	49/57	30 (14-73)	86	CHOP-like (70), 3rd generation (29)	Yes	65/23	50 (3 y)
Zinzani, 1999 [8]	Italy	50	100	20/30	31 (21-55)	84	MACOP-B (50)	Yes	86/86	82 (8 y)
Bieri, 1999 [9]	Switzerland	27	100	10/17	45 (21-87)	67	CHOP (11), 3rd generation (12)	Yes	70/56	50 (10 y)
Haioun, 1989 [10]	France	20	82	3/17	33 (17-52)	90	DOX-containing	Yes	100/45	33 (7 y)
Cazals-Hatem, 1996 [4]	France	141	100	58/83	37 (19-70)	77	DOX-containing	No	NA/79	66 (3 y)
Zinzani, 2002 [7]	Europe	426	NA	165/261	32 (13-87)	74	1st Generation (105) 3rd Generation (277) HDC/AHSCT (44)	Yes Yes Yes	81/49 87/51 88/53	44 (10 y) 71 (10 y) 77 (10 y)
Jacobson, 1988 [11]	USA	30	NA	10/20	34 (15-71)	76	CHOP (22)	Yes	80/80	65 (5 y)
Kirn, 1993 [12]	USA	57	80	30/27	30 (NA)	NA	CHOP (10), m/M-BACOD (38)	Yes	93/53	50 (5 y)
Abou-Elella, 1999 [13]	USA	43	100	20/23	42 (15-92)	58	DOX/MIT-containing	No	NA/63	39 (5 y)
Toh, 1998 [33]	Singapore	7	NA	3/4	22	NA	DOX-containing	Yes	NA/43	NA
Yonetani, 2001 [14]	Japan	10	100	5/5	25.5 (19-63)	90	CHOP-like (7), MACOP-B (3)	Yes	90/90	70 (2 y)
Present study	Japan	28	100	16/12	37 (18-80)	54	CHOP-like (18)	Yes	79/46	32 (3 y)

*ORR indicates overall response rate; CR, complete response; OS, overall survival; MACOP-B, regimen of methotrexate (MTX), doxorubicin (Adriamycin) (DOX), cyclophosphamide (CPA), vincristine (Oncovin) (VCR), prednisolone (PSL), and bleomycin (BLM); F-MACHOP, regimen of 5-fluorouracil, MTX, DOX, CPA, cytarabine, VCR, and PSL; NA, not applicable; CHOP, regimen of CPA, DOX, VCR, and PSL; HDC/AHSCT, high-dose chemotherapy with autologous hematopoietic stem cell transplantation; m/M-BACOD, regimen of moderate/high-dose MTX and BLM, DOX, CPA, VCR, and dexamethasone; MIT, mitoxantrone.

[34]. These results suggest that the incidence of Med-DLBCL has some geographical variation, although there may also be some differences in the recognition of this disease entity [32].

The conventional treatment strategy for other DLBCLs, such as a CHOP-like regimen followed by involved-field radiotherapy, was successful in fewer than half of the patients in the present study, although the researchers at the Massachusetts General Hospital reported superior results (65% 5-year OS) with primarily CHOP and radiotherapy [11]. On the other hand, several investigators have concluded that the survival prospects of patients treated with the third-generation regimens may be superior to those of patients treated with CHOP therapy [5,7], although different outcomes among patients who were treated with different third-generation regimens have been reported [6]. Zinzani et al reported the results of a phase II study of a MACOP-B regimen (MTX, DOX, CPA, VCR, PSL, and BLM) [35] and mediastinal radiotherapy that was conducted at 2 institutions in Italy [8]. Forty-three (86%) of 50 previously untreated patients achieved a CR, the OS was 82% at 8 years, and the relapse-free survival of the 43 patients who achieved a CR

was 93% at 8 years. In their study, a negative test result of gallium Ga 67 uptake in the residual mass was a favorable factor. Recently, Zinzani and the members of the International Extranodal Lymphoma Study Group conducted a retrospective European multinational study of 426 previously untreated patients with Med-DLBCL and reported that the long-term survival of patients treated with MACOP-B-type third-generation regimens was superior to that of patients treated with CHOP therapy [7]. These retrospective data need to be confirmed in prospective studies. Radiotherapy for the mediastinal mass is commonly used after the completion of chemotherapy [5,16], although its benefit has not been established in randomized studies, and excellent results have been obtained without radiotherapy in the Groupe d'Etude des Lymphomes de l'Adulte studies [4], as shown in Table 3. The usefulness of high-dose chemotherapy as consolidation and salvage therapy has also been suggested [5,7,36,37].

In the present study, there were significant differences in survival according to the responses to initial treatment, as shown in Figure 3. Approximately two thirds of the patients who achieved a CR or CRu appeared to achieve a cure, as shown in Figure 2, whereas only 1 of the remaining 15 patients