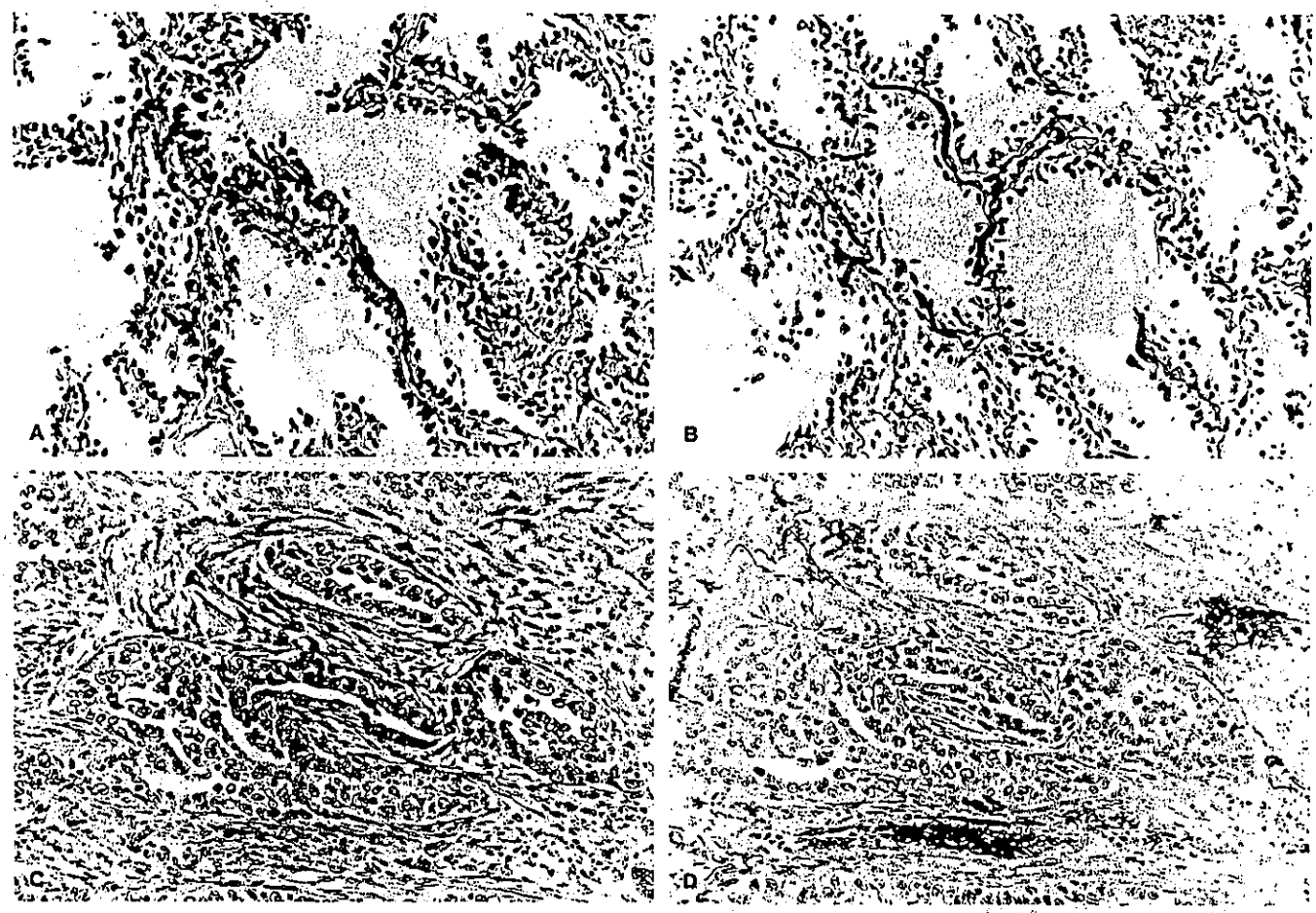

FIGURE 1. Features of histologic noninvasion (A, B) and invasion (C, D) in adenocarcinoma lesions. A and B, The tumor shows a pure bronchioloalveolar growth pattern and no evidence of stromal invasion. The elastic fiber framework is preserved. C and D: The tumor cells are arranged in acinic/papillotubular structures in a fibroblastic stroma, and the elastic framework is disrupted. The elastic stain highlights the elastic framework. Hematoxylin and eosin staining (A, C) and Elastica staining (B, D): original magnification ×200.

view as previously reported.[27] Pleural involvement was classified as positive when the tumor was exposed on the pleural surface or when the tumor invaded the parietal pleura or chest wall. Vascular and lymphatic permeation was evaluated based on the presence of identifiable tumor cells in the blood vessel lumen or lymphatic lumen, respectively.

TABLE 2. Histologic Grade of Invasion in Adenocarcinoma

Grade	Description
0	Pure bronchioloalveolar growth pattern and no evidence of stromal invasion
1	Stromal invasion in the area of bronchioloalveolar growth
2	Stromal invasion localized on the periphery of a fibrotic focus
3	Stromal invasion into the center of a fibrotic focus

Statistics

To compare the frequencies among different groups, a χ^2 test or Tukey's significant difference test was used. Survival curves were estimated by the Kaplan-Meier method using the date of resection as the starting point and the date of recurrence or last follow-up as the end point. Deaths by causes other than lung cancer were considered censored. Survival curves were compared by the log-rank test. A P value of less than 0.05 was considered statistically significant.

RESULTS

Pathologic Findings

The distribution of histologic grade of invasion (grade 0-3) was as follows: 85 (22%) in grade 0, 37 (10%) in grade 1, 46 (12%) in grade 2, and 212 (56%) in grade 3. The pathologic characteristics of each grade are summarized in Table 3. The histologic grade of invasion defined in this study was closely

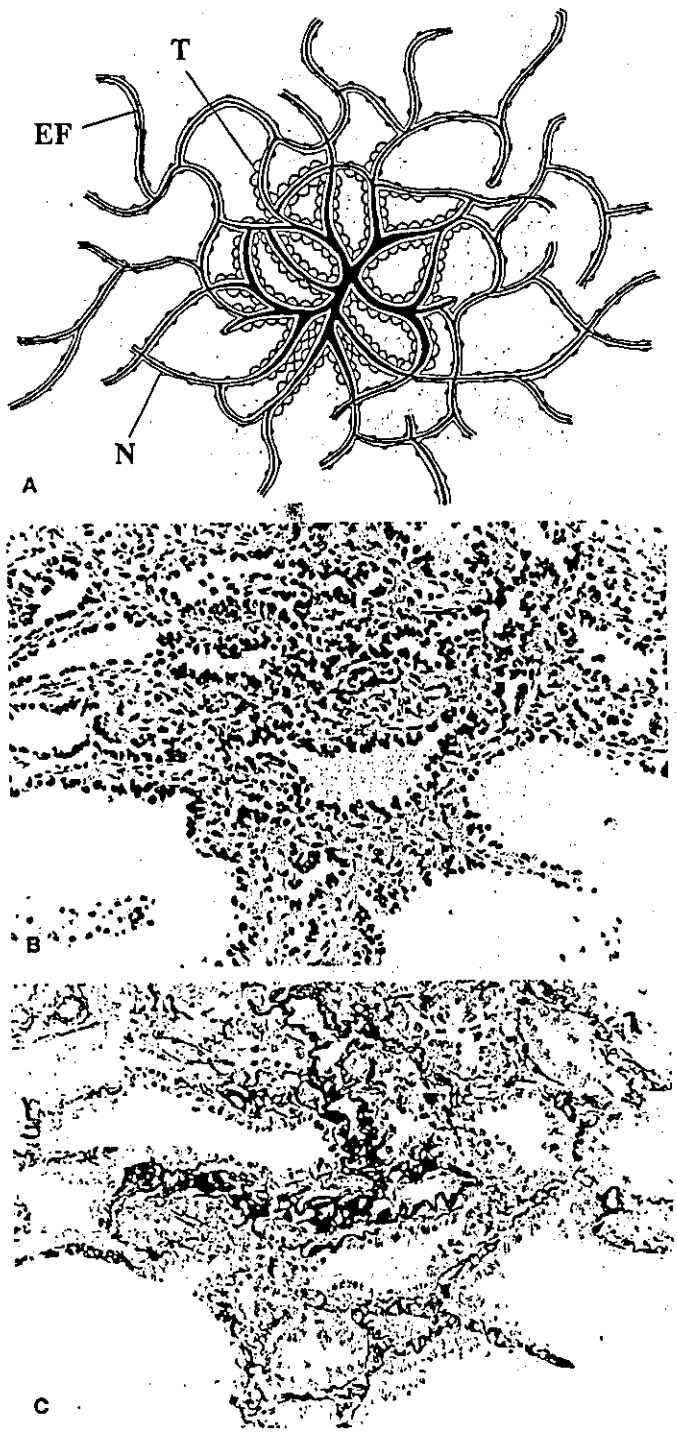

FIGURE 2. Schematic drawing (A) and microphotograph of grade 0 invasion (B, C). The tumor shows a bronchioloalveolar growth pattern with no stromal invasion. Hematoxylin and eosin staining (B) and Elastica staining (C): original magnification ×200. N, normal alveolar cells; T, tumor cells; EF, elastic fiber framework.

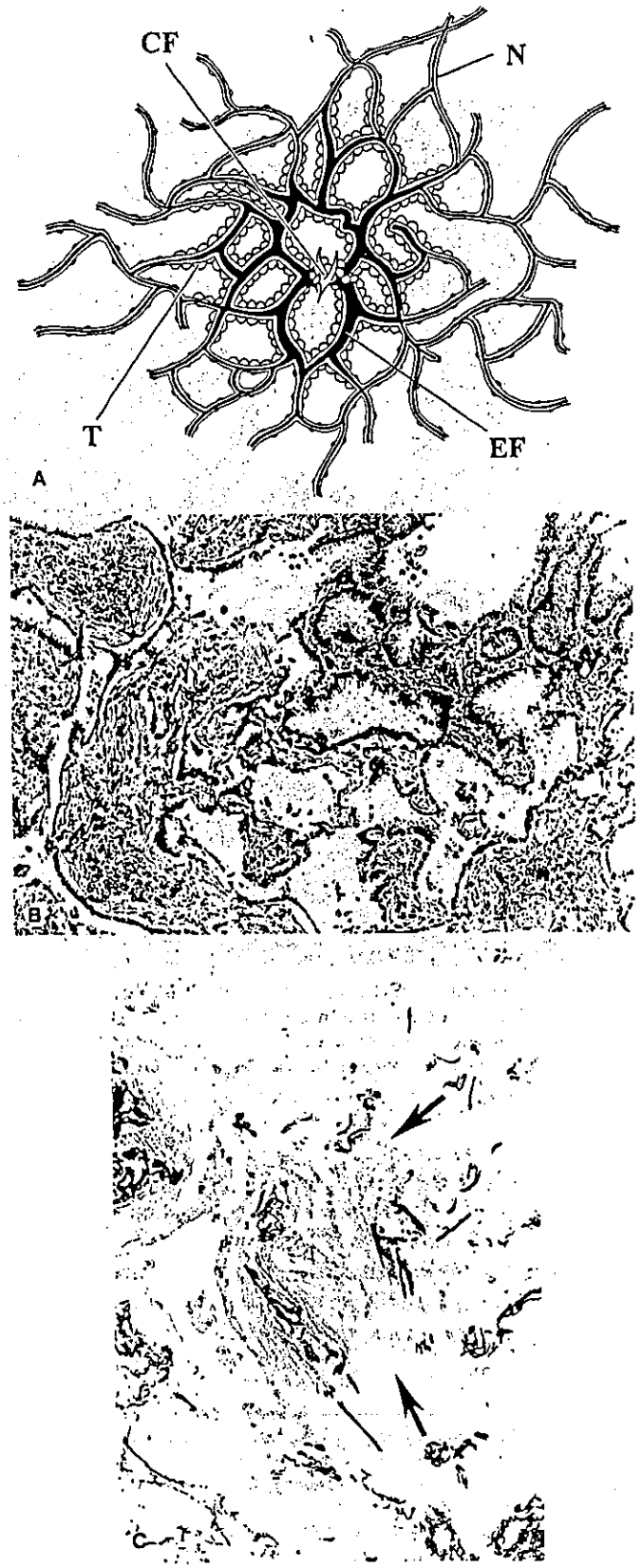

FIGURE 3. Schematic drawing (A) and microphotograph (B, C) of grade 1 invasion. The tumor shows features of histologic invasion in the area of bronchioloalveolar growth (arrows). Hematoxylin and eosin staining (B): original magnification ×40. Elastica staining (C): original magnification ×200. CF, collagen fiber (collagenization).

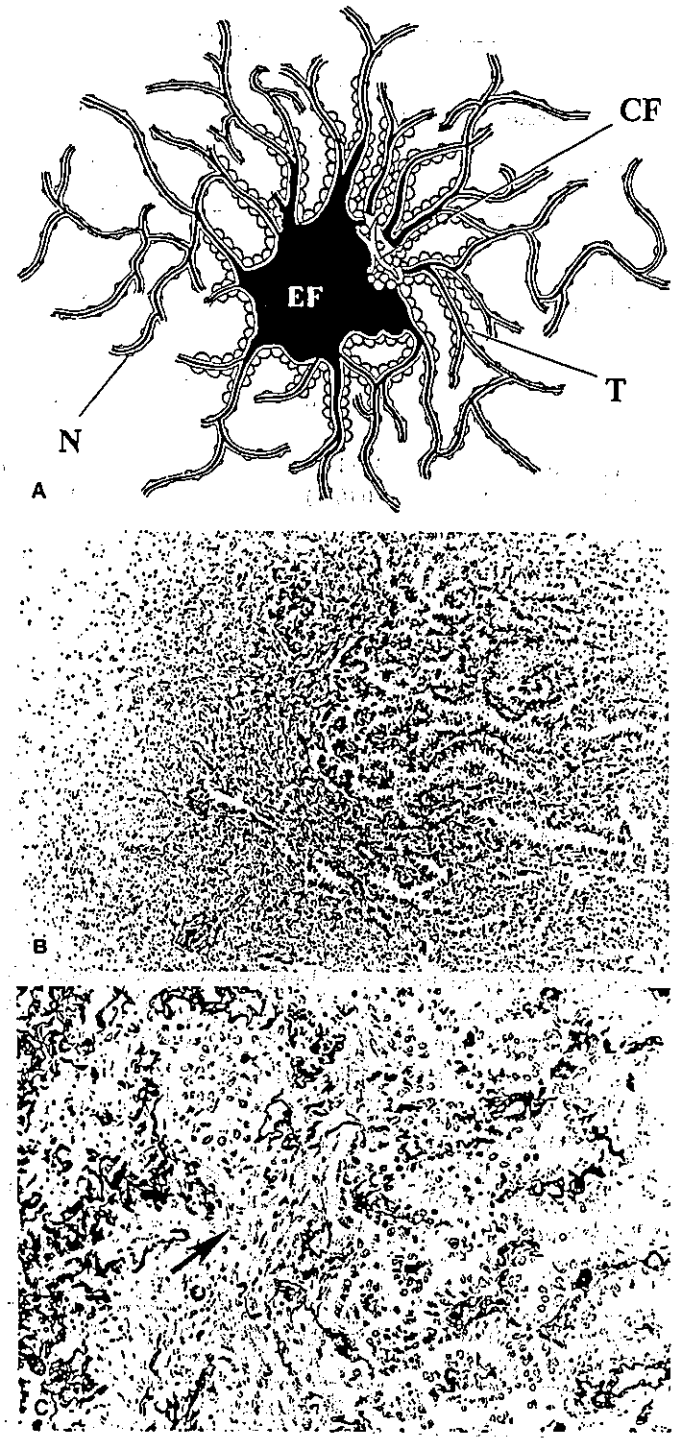

FIGURE 4. Schematic drawing (A) and microphotograph (B, C) of grade 2 invasion. The tumor shows features of histologic invasion localized on the periphery of a central fibrosis (arrow). The invasive foci are seen at the boundary between the central collapsed fibrosis and the surrounding tumor cells showing bronchioloalveolar growth. Hematoxylin and eosin staining (B): original magnification ×40. Elastica staining (C): original magnification ×200. EF is the black area.

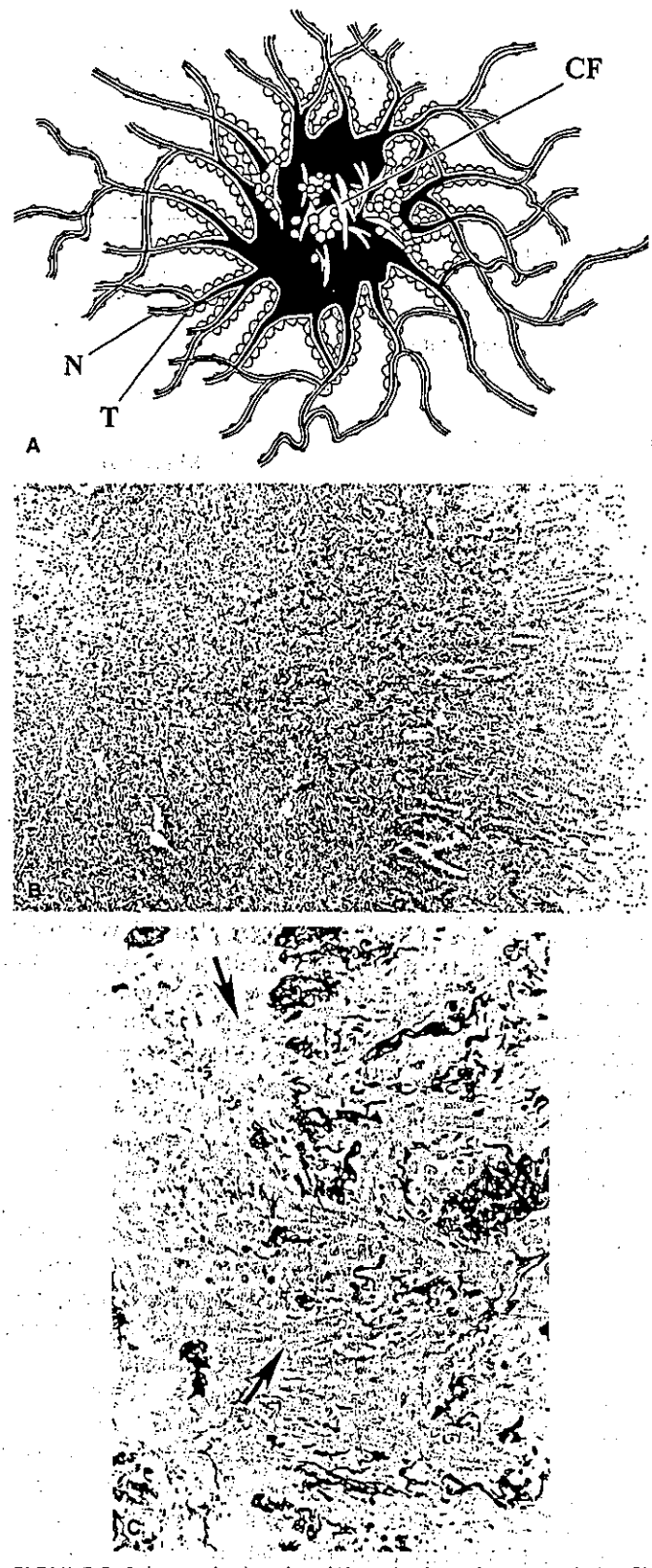

FIGURE 5. Schematic drawing (A) and microphotograph (B, C) of grade 3 invasion. The tumor shows features of histologic invasion into the center of a fibrotic focus (arrows). Hematoxylin and eosin staining (B): original magnification ×40. Elastica staining (C): original magnification ×200.

TABLE 3. Grade of Invasion and Pathologic Characteristics

	Grade of Invasion			
	Grade 0 (n = 85)	Grade 1 (n = 37)	Grade 2 (n = 46)	Grade 3 (n = 212)
Tumor size (cm)				
Mean	1.2	1.6	1.5	1.6
Range	0.4–2.0	0.7–2.0	0.7–2.0	0.8–2.0
Size of fibrotic focus (mm)				
Mean	2.0	3.8	6.7	7.9
Range	0–11	0–17	2–16	1–19
Pleural involvement				
Negative	85 (100%)	32 (100%)	46 (100%)	194 (92%)
Positive	0 (0%)	0 (0%)	0 (0%)	18 (8%)
Vascular/lymphatic permeation				
Negative	85 (100%)	36 (97%)	45 (98%)	68 (32%)
Positive	0 (0%)	1 (3%)	1 (2%)	144 (68%)
Nodal involvement				
N0	85 (100%)	37 (100%)	46 (100%)	155 (73%)
N1–N3	0 (0%)	0 (0%)	0 (0%)	57 (27%)
Pathologic stage				
I	85 (100%)	37 (100%)	46 (100%)	150 (71%)
II	0 (0%)	0 (0%)	0 (0%)	26 (12%)
III–IV	0 (0%)	0 (0%)	0 (0%)	36 (17%)

related to the progression of adenocarcinoma, as represented by tumor size, size of the fibrotic focus, pleural involvement, vascular/lymphatic permeation, nodal involvement, and pathologic stage. The tumor size was smaller in grade 0 than in other grades of invasion. This difference was statistically significant between grade 0 and grade 1 (Tukey's significant difference test, $P < 0.0001$), between grade 0 and grade 2 (Tukey's significant difference test, $P = 0.0005$), and between grade 0 and grade 3 (Tukey's significant difference test, $P < 0.0001$), but there were no significant differences among grades of invasion other than grade 0. The size of the fibrotic focus within the lesion tended to increase in tumors with a more advanced grade of invasion. Although this difference was only marginally significant between grade 2 and grade 3 (Tukey's significant difference test, $P = 0.056$), it was significant between grade 0 and grade 1 (Tukey's significant difference test, $P = 0.0009$) and between grade 1 and grade 2 (Tukey's significant difference test, $P = 0.0009$). Pleural involvement was only seen in tumors with grade 3 invasion, whereas no pleural involvement was seen in tumors with grade 0, grade 1, or grade 2 invasion. Vascular/lymphatic permeation was seen for one lesion (3%) of grade 1, one (2%) of grade 2, and 144 (68%) of grade 3, but not for grade 0. Lymph node involvement was seen for 57 tumors (27%) with grade 3 invasion: 24 in N1 stations, 32 in N2 stations, and one in N3 stations. However, there was no lymph node involvement for tumors with grade 0, grade 1, or grade 2 invasion. The pathologic stage was IA in all of the lesions in grade 0, grade 1, and grade 2. On the other hand, there were 144 lesions (68%) of stage IA in grade 3. The relationship between the grade of invasion and the histologic subtype as adenocarcinoma defined by the WHO classification is shown in Table 4. All of the acinar, papillary, and solid adenocarcinomas had grade 3 invasion. On the other hand, adenocarcinomas with mixed subtypes (n = 257) had various grades of invasion: 37 lesions (14%) with grade 1 invasion, 46 (18%) with grade 2 invasion, and 174 (68%) with grade 3 invasion.

TABLE 4. Relationship Between Grade of Invasion and WHO Classification

	WHO Classification (n = 380)				
Grade	BAC	Acinar	Papillary	Solid	Mixed Subtypes
0	85	0	0	0	0
1	0	0	0	0	37
2	0	0	0	0	46
3	0	4	7	27	174
Total	85	4	7	27	257

WHO, World Health Organization; BAC, bronchioloalveolar carcinoma.

Prognosis

The postoperative median follow-up period was 4.5 years. There were no operative deaths. The 3- and 5-year disease-free survival rates of all 380 patients with adenocarcinoma ≤2.0 cm in diameter were 82.3% and 76.4%, respectively (Fig. 6). The disease-free survival curves, according to the histologic subtype by the WHO classification, are shown in Figure 7. The 5-year disease-free survival rates were 100% (BAC), 72.4% (mixed subtypes), 55.6% (solid), and 42.9% (papillary), respectively. No significant difference in disease-free survival was found among these histologic subtypes. The disease-free survival curves according to the histologic grade of invasion (grade 0-3) are shown in Figure 8. The 5-year disease-free survival rates were 100%, 100%, 100%, and 59.6% for grade 0, grade 1, grade 2, and grade 3, respectively. Tumors with grade 0, grade 1, or grade 2 invasion all had a very excellent prognosis, which indicated that tumors with grade 1 and grade 2 invasion could be considered minimally invasive lesions.

DISCUSSION

In 1999, the WHO histologic classification of lung and pleural tumors was revised. With regard to adenocarcinoma, BAC is described as a form of adenocarcinoma with a pure bronchioloalveolar growth pattern and no evidence of stromal, vascular, or pleural invasion. If there is histologic evidence of invasive growth, it was considered "adenocarcinoma with mixed subtypes." In recent reports, the pattern of recurrence and survival in patients with resected stage I BAC were investigated.[4,9,24,30] The 5-year disease-free survival rate was reported to be 73% by Volpino et al,[30] 74% by Breathnach et al,[4] and 81% by Rena et al.[24] Despite the clear definition of a "noninvasive" morphology for BACs, these reports included BACs

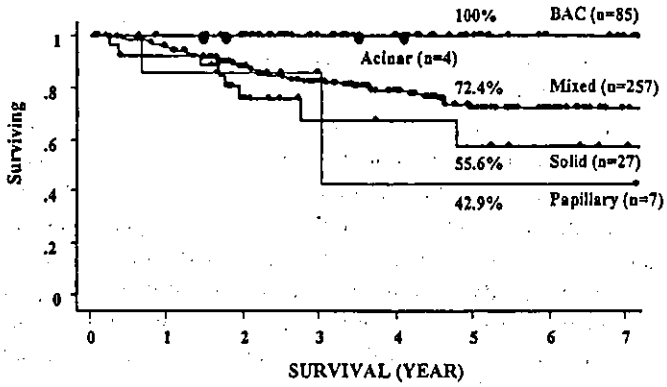

FIGURE 7. Survival curves according to the WHO classification. The 5-year disease-free survival rates are 100% (BAC), 72.4% (mixed subtypes), 55.6% (solid), and 42.9% (papillary), respectively.

with both local and distant recurrence. These results suggested that tumors with postoperative recurrence must have had invasive features and therefore should not be diagnosed as BAC without invasive growth. The difficulties of unequivocally recognizing invasive features by morphology must be addressed.

Several studies have examined the morphologic features related to "tumor development or invasion" in adenocarcinoma.[10,19–21,25,27,28,31,32] Shimosato et al focused on "scar" formation, which is a characteristic histologic feature in adenocarcinoma of peripheral lung, and demonstrated that the degree of collagenization in the fibrotic focus was closely correlated with tumor growth and prognosis.[25] They proposed that tumors with little or no collagenization could be considered to be in an "early stage" of development. Yamashiro et al reported that histologic invasion could be suggested by tumor cells accompanied by a stromal desmoplastic reaction in ad-

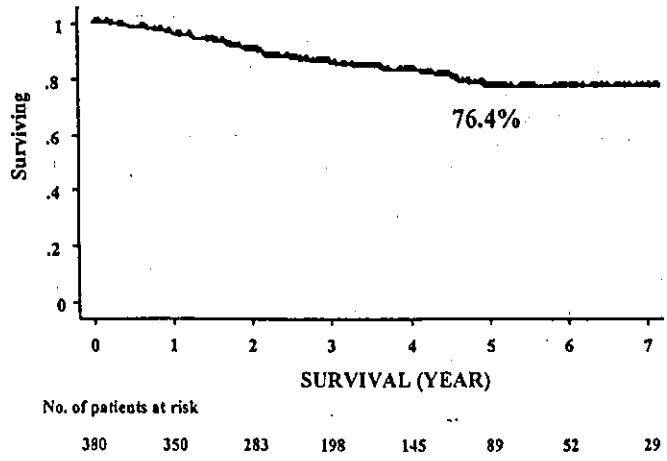

FIGURE 6. Survival curve for all 380 patients with pulmonary adenocarcinoma ≤2.0 cm in diameter. The 3- and 5-year disease-free survival rates are 82.3% and 76.4%, respectively.

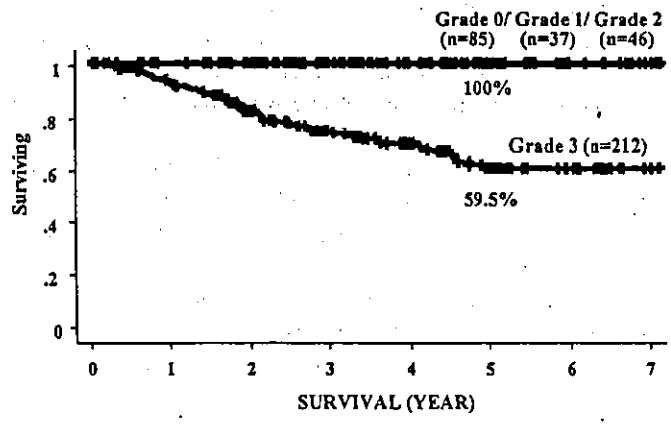

FIGURE 8. Survival curves according to histologic grade of invasion (grade 0–3). The 5-year disease-free survival rates are 100% (grade 0), 100% (grade 1), 100% (grade 2), and 59.6% (grade 3), respectively.

enocarcinoma, and a greater proportion of invasion to the fibrotic focus was correlated with a worse prognosis.[31] Noguchi et al suggested that active fibroblast proliferation in adenocarcinoma was related to the invasive growth of tumors.[21] They thought that localized bronchioloalveolar carcinomas without active fibroblastic proliferation could be considered in situ adenocarcinomas with an excellent prognosis (5-year survival rate, 100%). Eto et al analyzed the change in the stromal elastic framework in adenocarcinoma and concluded that the elastic framework was preserved in the early development of the tumor but was disrupted as the tumor grew, indicating stromal invasion.[10] Suzuki et al reported that adenocarcinomas with a fibrotic focus of ≤5 mm in size had an excellent prognosis (5-year survival rate, 100%), and the size of the fibrotic focus within the tumor was shown to be a significant prognostic factor.[27] In the present study, among 91 patients of adenocarcinoma with fibrotic focus ≤5 mm in size, there were 3 (3.3%) patients with recurrence. Terasaki et al measured the size of invasive foci, which were considered by fibroblastic proliferation and architectural distortion of tumor cells, and adenocarcinoma with invasive foci of ≤5 mm in size showed low prevalence of vascular, lymphatic, and pleural involvement.[28] Indeed, these histologic features were likely to be closely related to the tumor invasion and development. However, practically, a diagnosis of "invasion" often relies on the discretion of the pathologist because the definition of morphologic "invasion" is equivocal.

In the present study, histologic "invasion" was considered cellular arrangement in acinic/papillotubular structures or solid nests in a fibroblastic stroma accompanied by collagenization, as in the 1999 WHO classification. In addition, the structural deformity of the stromal elastic fiber framework was also evaluated. By highlighting the elastic fiber framework using elastic stain, we were able to more precisely analyze the morphologic details. Indeed, vascular/pleural involvement could not be demonstrated in any of the 85 patients without disruption of the stromal framework. As a result, a histologic diagnosis of BAC could be established precisely, and no postoperative recurrence was observed in these patients.

According to our definitions for the grade of invasion in adenocarcinoma, tumors with grade 1 or grade 2 invasion had neither lymph node metastasis nor postoperative recurrence, even though lymphatic permeation was seen in one case each in grades 1 and 2. The prognosis of tumors with grade 1 or grade 2 invasion, like that of BAC, was excellent. Therefore, despite stromal invasion, adenocarcinomas with grade 1 or grade 2 invasion should be considered "minimally invasive lesion" with the same prognosis as BAC.

In summary, the prognosis of BACs was excellent and the 5-year disease-free survival rate was 100%. In addition, adenocarcinomas with grade 1 or grade 2 invasion, ie, "stromal invasion in the area of bronchioloalveolar growth" and "stromal invasion localized on the periphery of a fibrotic focus," also had an excellent prognosis. Adenocarcinomas with grade 1 or grade 2 invasion can be considered "minimally invasive adenocarcinomas" or "early adenocarcinomas."

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Institutional report - Thoracic general

Thymoma needs a new staging system

Hisao Asamura[a,*], Kazuo Nakagawa[a], Yoshihiro Matsuno[b], Kenji Suzuki[a], Shun-ichi Watanabe[a], Ryosuke Tsuchiya[a]

[a]*Division of Thoracic Surgery, National Cancer Center Hospital, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan*
[b]*Division of Pathology, National Cancer Center Hospital, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104-0045, Japan*

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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.

Keywords: Thymoma; Thymectomy; Mediastinal tumor; Surgery; Pathology

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.

* Corresponding author. Tel.: +81-3-3542-2511; fax: +81-3-3542-3815.
E-mail address: hasamura@ncc.go.jp (H. Asamura).

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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 1
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 strctures/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 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 ($P = 0.003$).

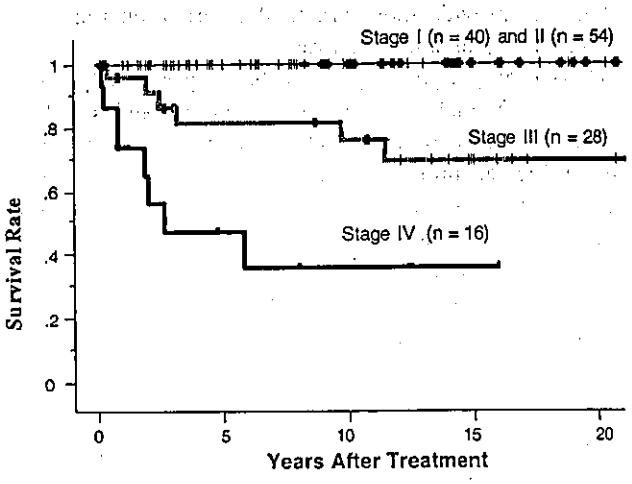

Fig. 1. Survival curves according to stage defined by the Masaoka system. The 5- and 10-year survival rates were 100%, 100% (stage I), 100%, 100% (stage II), 75%, 70% (stage III), and 44%, 34% (stage IV). The difference in survival: between stages III and IV ($P = 0.027$).

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,

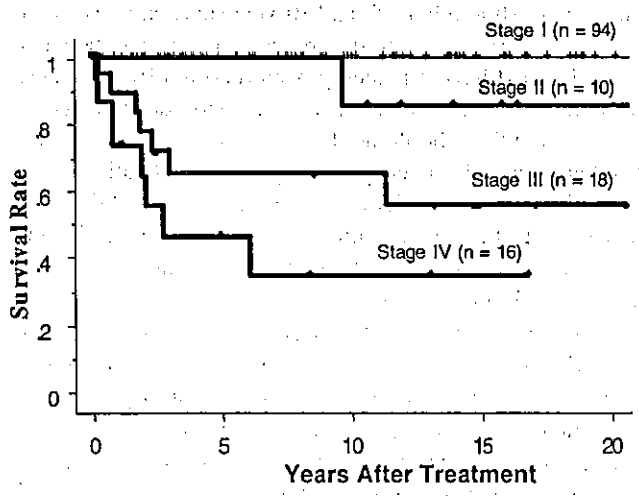

Fig. 2. Survival curves according to stage defined by Scheme 1. The 5- and 10-year survival rates were 100%, 100% (stage I), 100%, 86% (stage II), 64%, 64% (stage III), and 44%, 34% (stage IV). The differences in survival: between stages II and III ($P = 0.13$), stages II and IV ($P = 0.012$), and stages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

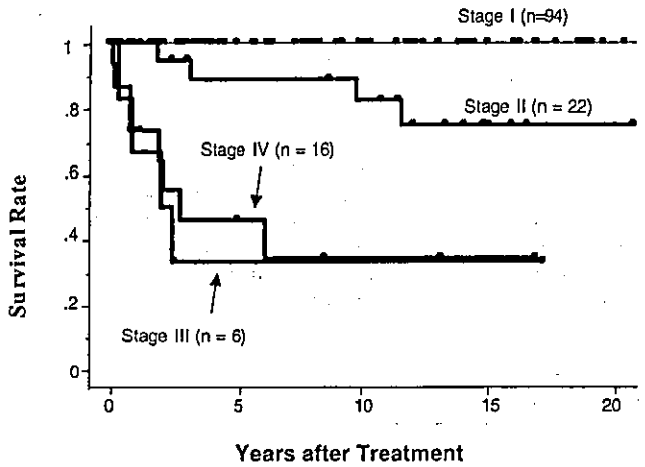

Fig. 3. Survival curves according to stage defined by Scheme 2. The 5- and 10-year survival rates were 100%, 100% (stage I), 90%, 83% (stage II), 34%, 34% (stage III), and 44%, 34% (stage IV). The differences in survival between stages II and III ($P = 0.003$) and stages II and IV ($P = 0.003$).

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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Original article

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

K. Tobinai[1]*, T. Igarashi[2], K. Itoh[2], Y. Kobayashi[1], M. Taniwaki[3], M. Ogura[4], T. Kinoshita[5], T. Hotta[6], K. Aikawa[7], K. Tsushita[8], A. Hiraoka[9], Y. Matsuno[1], S. Nakamura[4], S. Mori[10] & Y. Ohashi[10]
On behalf of the IDEC-C2B8 Japan Study Group

[1]Hematology and Clinical Laboratory Divisions, National Cancer Center Hospital, Tokyo; [2]Hematology and Oncology Division, National Cancer Center Hospital East, Kashiwa; [3]Third Department of Internal Medicine, Kyoto Prefectural University of Medicine, Kyoto; [4]Department of Hematology and Chemotherapy and Department of Pathology, Aichi Cancer Center Hospital, Nagoya; [5]First Department of Internal Medicine, Nagoya University School of Medicine, Nagoya; [6]Department of Hematology and Rheumatology, Tokai University School of Medicine, Isehara; [7]Department of Internal Medicine, Sapporo National Hospital, Sapporo; [8]Department of Internal Medicine, Nagoya National Hospital, Nagoya; [9]Department of Hematology/Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka; [10]Department of Pathology, Institute of Medical Science and Biostatistical Sciences, School of Health Science and Nursing Biostatistics, University of Tokyo, Tokyo, Japan

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

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

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

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

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

Introduction

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

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

Correspondence to: Dr K. Tobinai, Hematology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Tel: +81-3-3542-2511; Fax: +81-3-3542-3815; E-mail: ktobinai@ncc.go.jp

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

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

Patients and methods

Study design and end points

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

Eligibility criteria

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

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

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

Central review of pathology

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

Rituximab administration and premedication

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

Monitoring of patients

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

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

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

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

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

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

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

Central review of CT films

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

Statistical methods

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

Results

Patients' characteristics

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

Central pathology review

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

Early termination of rituximab treatment

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

Table 1. Patient characteristics

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

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

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

ORR, PFS and TTP

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

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

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

Table 2. Responses

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

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

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

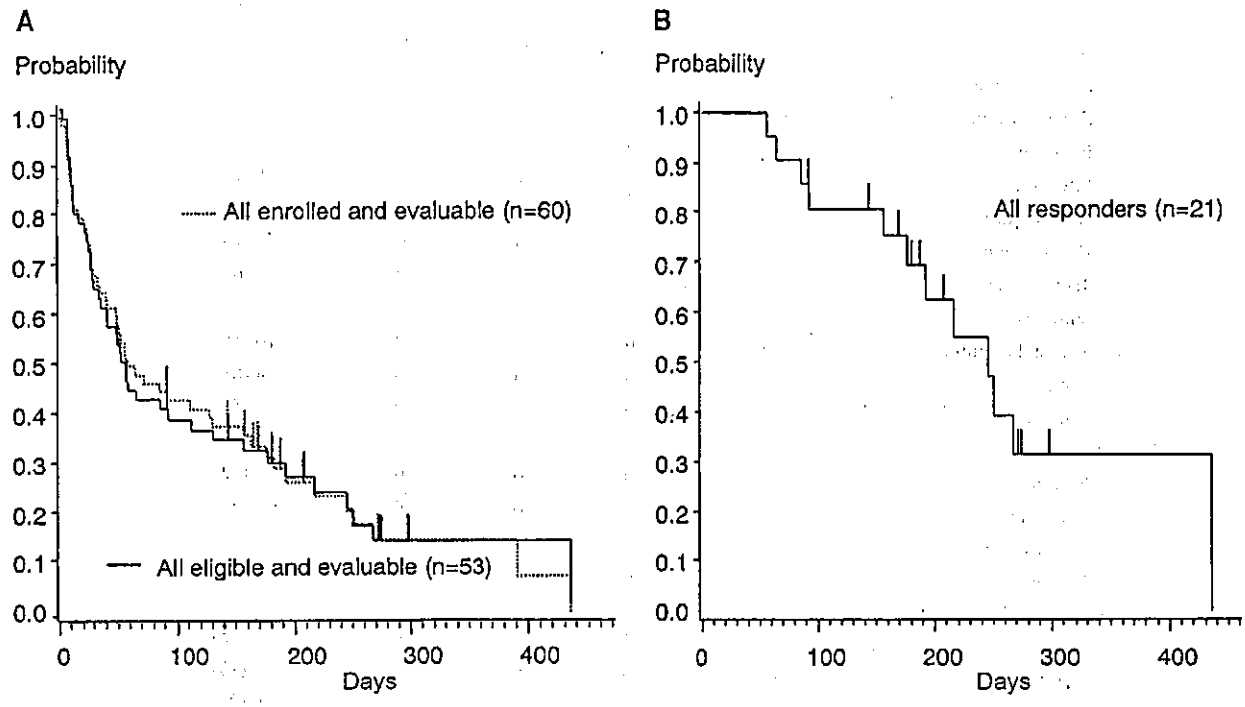

Figure 1. (**A**) Progression-free survival (PFS) and (**B**) time to progression (TTP). The median PFS values for all eligible and evaluable patients ($n = 53$) and for all enrolled and evaluable patients ($n = 60$) were 52 days [95% confidence interval (CI) 33–111] and 61 days (95% CI 41–156), respectively.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

Non-hematological toxicities

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

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

Hematological toxicities

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

Abnormal laboratory findings

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

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

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

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

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

Infection

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

Early death

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

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

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

HACA development

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

Factors affecting ORR and PFS

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

Pharmacokinetic parameters and correlation with responses

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

Discussion

We report here the findings of a multicenter phase II study in Japan to evaluate the efficacy and feasibility of eight consecutive weekly administrations of rituximab for relapsed or refractory patients with aggressive B-cell lymphoma. The first clinical study of rituximab for aggressive B-cell lymphoma was conducted in Europe by Coiffier et al. [10]. The study evaluated rituximab monotherapy in 54 relapsed or elderly untreated patients with aggressive B-cell lymphoma that mainly consisted of DLBCL. Rituximab was given as two dosing schedules: eight consecutive weekly infusions at 375 mg/m² (arm A; $n = 28$), or one infusion at 375 mg/m² followed by seven consecutive weekly infusions at 500 mg/m² (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/m² than at 375 mg/m² (three versus six cases).

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

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

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

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

Table 5. Pharmacokinetic parameters of responders and non-responders

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

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

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

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

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

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