

heterogeneity of the DLBCL component using morphological, immunohistochemical, and FISH analyses.

FL transformed most commonly to the DLBCL centroblastic subtype,⁽⁴⁾ but occasionally to the DLBCL anaplastic subtype with CD30 expression.⁽⁵⁾ We confirmed that most of the DLBCLs were of the centroblastic subtype, with two exceptional cases of the anaplastic subtype.

CD10 shows restricted expression in germinal center B-cells of reactive lymphoid tissue. Although the reported frequency of CD10 expression in FL varies, about 60% of FLs express CD10.⁽⁴⁾ However, no previous report has documented changes in CD10 expression through transformation from low-grade FL to DLBCL. In this study, 14 DLBCLs were negative for CD10, and among them, six showed loss of CD10 expression through transformation. Previous reports have indicated that CD10 expression is often stronger in follicles than in interfollicular neoplastic cells, and that the frequency of CD10 expression is lower in FL grade 3 than in low-grade FL.⁽²⁴⁾ It is suggested that loss of CD10 expression through FL transformation is possible in the process of escape from the follicular dendritic cell meshwork and diffusion, and tumor cell enlargement.

Bcl-2 is expressed on resting and B and T cells, but not on normal germinal center cells. Bcl-2 protein has an antiapoptotic function,⁽²⁵⁾ and is expressed in the majority of FLs ranging from nearly 100% in grade 1–75% in grade 3,⁽²⁶⁾ and in about 30–50% of de novo DLBCLs.⁽²³⁾ Bcl-6 is expressed in germinal center B-cells and a subset of CD4⁺ T cells.⁽²⁷⁾ Bcl-6 is expressed in 88% of FLs,⁽²⁸⁾ and 55–97% of de novo DLBCLs.⁽²⁹⁾ Most of the DLBCLs that had transformed from FL retained a high frequency of Bcl-2 and Bcl-6 expression, which tended to be higher than that in de novo DLBCL. Because Bcl-2 and Bcl-6 expression was retained during transformation in most cases, Bcl-2 and Bcl-6 positivity might be a precondition for transformation of DLBCL from FL.

MUM1 is a lymphoid-specific member of the interferon regulatory factor family of transcription factors.⁽³⁰⁾ MUM1 is normally expressed in plasma cells and a minor subset of germinal center B cells, and has been reported to be expressed in 50–77% of DLBCLs.^(31–33) In this study, MUM1 was positive in 16% of low-grade FLs and 34% of DLBCLs. This rate was lower than that in de novo DLBCL, but it was surprising that 34% of transformed FLs expressed MUM1. Twenty cases that were MUM1-positive in both the FL and DLBCL components were suggested to be derived from germinal center MUM1-positive B cells, and seven cases showing MUM1-gain indicated that this event was not infrequent during FL transformation.

CD30 was positive in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma, anaplastic large cell lymphoma, anaplastic variant of DLBCL, and a subset of non-neoplastic activated B and T cells.^(1,5) Most FLs contain a small number of CD30-positive cells, located mainly at the edge of the neoplastic follicles,⁽³⁴⁾ and we confirmed this feature. Transformation of FL into CD30-positive large B-cell lymphoma with anaplastic features has been reported.⁽⁵⁾ In this study, 20% of the cases gained CD30 expression in DLBCL, which included two cases of the DLBCL

anaplastic variant, indicating that CD30-positive large lymphoid cells tended to increase gradually during transformation.

CD5 is reported to be an unfavorable prognostic marker in de novo DLBCL.⁽³⁵⁾ Richter's syndrome, a transformant of chronic lymphocytic leukemia/small lymphocytic lymphoma, is a well-known secondary CD5⁺ DLBCL. Manazza *et al.* reported that CD5 and CD10-double-positive FL transformed to CD5⁺ DLBCL.⁽³⁶⁾ Our present study is the first to indicate that CD5⁺/CD10⁺ FL with IGH/BCL2 fusion can transform to secondary CD5⁺/CD10⁻ DLBCL with IGH/BCL2 fusion.

Notably, our series included one case of classical Hodgkin lymphoma that had transformed from FL via DLBCL. Previous reports have suggested that composite follicular lymphoma and Hodgkin lymphoma represent two morphologic manifestations of the same tumor clones.^(6,9) In the present case, IGH/BCL2 fusion was detected in both the FL and the Hodgkin/Reed-Sternberg cells by FISH analysis, strongly suggesting transformation from FL.

Although transformed FL is generally considered to have a GCB phenotype, we demonstrated that a proportion of FLs can show a dramatic change in immunophenotype through transformation. Davies *et al.*⁽¹⁸⁾ examined 35 cases of transformed FL, and found that 89% of them had a GCB phenotype and 9% had a non-GCB phenotype. In our study, six (16%) of the DLBCLs had a non-GCB phenotype. Some previous studies examining the difference in prognosis between patients with a GCB phenotype *versus* those with non-GCB-phenotype DLBCL revealed that the former group had a more favorable prognosis.^(15,16) However, Colomo *et al.* found no prognostic difference between them,⁽³⁷⁾ and recently therefore this issue has been controversial. In the present study, GCB *versus* non-GCB was not a significant prognostic factor. However, as the number of cases was small, further studies are necessary to clarify the prognostic difference between GCB and non-GCB in transformed FL.

We detected a high relative frequency (82%) of IGH/BCL2 fusion in transformed FL. Although the rate is higher than that in Japanese FL, it is almost equal to that in FL grade 1 (10/12, 83%).⁽²¹⁾ Because the present cases of DLBCL had transformed from low-grade FL, we were unable to conclude whether cases showing IGH/BCL2 fusion transformed more frequently than cases without it.

In conclusion, our study has clearly demonstrated heterogeneity of the immunophenotype in DLBCL transformed from low-grade FL, suggesting that various mechanisms may affect FL transformation. As many genetic changes including c-MYC amplification and p53 mutation have been detected in transformed FL, it will be necessary to analyze the relationship between these genetic abnormalities and morphological, immunohistochemical, and IGH/BCL2 fusion status in transformed FL.

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Follicular Lymphoma of the Duodenum: A Clinicopathologic Analysis of 26 Cases

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Objective: Follicular lymphomas (FLs) occur commonly in the lymph nodes, and duodenal FL (DFL) is reported to be rare.

Methods: We analysed the clinical, morphological, immunohistochemical and genetic features of 26 cases of DFL. Primary DFLs and systemic FLs that involved the duodenum at any point during the clinical course were included in the analysis.

Results: Typically, primary DFLs (14 cases) were found incidentally at routine medical check-ups, whereas involvement of the duodenum by systemic FLs (12 cases) was found through staging procedures. All cases involved the second portion of the duodenum. *Helicobacter pylori* infection was common (71%). In all cases, the histologic grade was low (either grade 1 or 2), and CD20, CD10 and Bcl-2 were positive by immunohistochemistry. Immunoglobulin heavy chain gene (IGH) and bcl-2 gene (BCL2) fusion was frequently shown by fluorescence *in situ* hybridization (FISH) analysis: nine of 12 cases (75%) of primary DFL and 10 of 12 cases (83%) of systemic DFL were positive. Treatment regimens employed were rituximab (R) plus chemotherapy (10), R (6), chemotherapy (3), irradiation (3) and the other three patients were subjected to observation. After a median follow-up duration of 40 months (ranging 11–96 months), 17 patients were alive without disease, seven were alive with disease and one had died of lymphoma.

Conclusions: Primary DFLs resemble systemic and nodal FLs, except that the former has high incidence of early stage and low-grade histology. The duodenum appears to be a frequently involved extranodal site of FL with IGH/BCL2.

Key words: duodenum – follicular lymphoma – immunohistochemistry – FISH

INTRODUCTION

Follicular lymphoma (FL) is a neoplasm of follicular centre B cells and is one of the most common subtypes of non-Hodgkin lymphoma (NHL) in Europe and the USA (1). The most common subtype was reported to be diffuse large B-cell lymphoma (33%), followed by FL (18%) in Japan (2). Most patients with FL present with nodal involvement, and extranodal presentation occurs at the advanced stage. Primary extranodal FL has been reported to occur in the skin (3), salivary gland (4), ocular adnexa (5) and female genital tract (6). The gastrointestinal (GI) tract is the most commonly involved extranodal site of NHL, accounting for ~40% of all

extranodal primary NHLs (7). However, FL of the GI tract represents only ~1–3.6% of all GI tract NHLs (8).

Duodenal FL (DFL) is a rare entity, with only a few reported cases (9–11). DFL is reported to be a characteristic clinicopathologic entity due to its localized nature and good prognosis (9,10). Yoshino et al. (9) reported that DFL was present around the Vater's papilla and showed multiple small-size polyps. Sato et al. (11) reported that DFL had intermediate characteristics of FL and mucosa-associated lymphoid tissue (MALT) lymphoma.

Most cases of nodal FL have a Bcl-2-positive immunophenotype and show immunoglobulin heavy chain gene (IGH) and bcl-2 gene (BCL2) fusion; up to 85% of tumours shows IGH/BCL2 fusion in Europe and the USA (12), whereas 60% does so in Japan (13). However, the situation might differ for cases originating from other sites. For example, FL

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of the skin, which is the most common site of extranodal FL, has a Bcl-2-negative immunophenotype and rarely shows IGH/BCL2 fusion (14).

In this study, we examined the clinical, morphological, immunohistochemical and genetic features of 26 cases of DFL at a single institution. We categorized the 26 DFLs for which staging data were available into two groups: 14 primary DFLs and 12 systemic DFLs, and compared them with previously described cases of nodal FL.

PATIENTS AND METHODS

PATIENTS

Twenty-six consecutive cases with a histologic diagnosis of DFL between April 1997 and October 2005 were retrieved from the archival pathology files of the National Cancer Center Hospital, Tokyo, Japan. All of the 26 DFLs were confirmed on the basis of morphologic and immunohistochemical features, and if available, the genetic features of FL. Clinical information was obtained from the medical records. We categorized the 26 DFLs into two groups on the basis of clinical stage: 14 primary DFLs and 12 systemic DFLs. For staging, the Ann Arbor staging system was used. The 26 patients were examined for staging by bone marrow aspiration or biopsy and computed tomography, and optionally, gallium scintigraphy. More recently, positron emission tomography and/or panendoscopy have been advocated as a tool for staging optionally, but were not performed in this series.

HISTOLOGIC EXAMINATION

Biopsy materials were fixed with 10% buffered formalin overnight, then 4 µm-thick sections were made from paraffin blocks and stained with haematoxylin and eosin (HE) for routine diagnosis. Each HE specimen was histologically reviewed by three of the authors (KS, AMM and YM). Following the World Health Organization classification (15), each case was graded based on the number of centroblasts/high-power field (HPF), and patterns of follicular and/or diffuse growth were judged semi-quantitatively. Specifically, FL grade 1 showed 1–5 centroblasts/HPF; grade 2, 6–15 centroblasts/HPF and grade 3, >15 centroblasts/HPF. The growth patterns were recorded as follicular when >75% of the tumour showed follicularity, follicular and diffuse when 25–75% of the tumour showed follicularity and focally follicular when <25% of the tumour showed follicularity. *Helicobacter (H.) pylori* infection was assessed by histologic examination using Giemsa staining, serologic testing or rapid urease test and was defined as positive if any of these tests gave a positive result.

IMMUNOHISTOCHEMICAL ANALYSIS

Immunohistochemical analysis was performed using a panel of antibodies. Sections 4 µm thick were cut from each paraffin block, deparaffinized and incubated at 121°C in pH 6.0

citrate buffer for 10 min for antigen retrieval. Antibodies included those against the following antigens: CD3 (PS1, Novocastra, Newcastle-upon-Tyne, UK: Polymer method), CD20 [L26, DAKO, Glostrup, Denmark: labelled streptavidin-biotin method (LSAB)], CD5 (4C7 Novocastra: Polymer method), CD10 (56C6, Novocastra: Polymer method), Bcl-2 (124, DAKO: LSAB) and cyclin D1 (DSC-6, Novocastra: Polymer method). Positive and negative controls were used for each antibody and for each case. CD20 and Bcl-2 were stained using a Biogenex autostainer™, and CD3, CD5, CD10 and cyclin D1 were stained using a DAKO autostainer plus™. Immunoreactivity was judged positive if 30% or more of the tumour cells were stained.

FLUORESCENCE *IN SITU* HYBRIDIZATION ANALYSIS

The tissue fluorescence *in situ* hybridization (FISH) procedure was performed on formalin-fixed paraffin sections in accordance with the procedure described by Sekiguchi et al. (13). A dual-colour LSI IgH Spectrum Green/LSI BCL2 Spectrum Orange Dual Fusion Translocation Probe (Vysis, Downers Grove, IL, USA) was used to detect t(14;18): IGH/BCL2 fusion. To analyse the hybridization, a total of 100–200 nuclei per case were judged, and if fusion signals were detected in 7% or more nuclei, IGH/BCL2 fusion was judged to be positive.

RESULTS

The characteristics of the patients are shown in Table 1. The median age was 54 years (range 36–72 years), and there were 13 males and 13 females. The distribution according to the Ann Arbor staging system was stage I, 10 cases (38%); II, four cases (15%); III, one case (4%); IV, 11 cases (42%). Fourteen cases (stage I or II) were classified as primary DFL and 12 cases (stage III or IV) as systemic DFL. Seventy-nine per cent (11 of 14) of primary DFLs were found at routine medical check-ups for gastric cancer screening, and the remaining 21% (three of 14) of patients complained of digestive symptoms. Seventy-five per cent (nine of 12) of patients with systemic DFL complained of lymph node enlargement, weight loss or digestive symptoms, and duodenal involvement was found during the staging procedure at initial presentation. The remaining 25% (three of 12) of systemic DFLs were found at routine medical check-ups.

All of the 25 cases for which endoscopic reports were available for review involved the second portion of the duodenum, and in 10 cases (40%), lesions were found in the vicinity of the Vater's papilla. Fourteen cases had multiple polypoid lesions and 11 cases had a single elevated lesion.

The results of histologic and immunohistochemical analyses are shown in Table 2. The histologic grades of DFLs were as follows: in primary DFL, grade 1, 13 cases (93%); grade 2, one case (7%), in systemic DFL, grade 1, nine cases (75%); grade 2, three cases (25%). Two of the primary DFLs had been diagnosed as MALT lymphoma initially, and

Table 1. Summary of clinical findings and outcome in 26 patients with duodenal follicular lymphoma

Case	Age/sex	Primary symptoms	Site in duodenum	Stage	Treatment	Relapse (month) ¹	Outcome (month) ²
1	68/F	Medical check-up	Second portion, multiple	I	Eradication+R		AWOD (75)
2	50/F	Epigastralgia	Near Vater's papilla, multiple	I	Irradiation	LN, submandibular (4)	AWOD (58)
3	49/F	Medical check-up	Near Vater's papilla, single	I	Irradiation		AWOD (62)
4	60/M	Medical check-up	Second portion, single	I	CHOP		AWOD (69)
5	66/M	Medical check-up	Second portion, multiple	I	Observation		AWD (11)
6	52/F	Medical check-up	Near Vater's papilla, single	I	Eradication		AWD (51)
7	60/F	Medical check-up	Near Vater's papilla, single	I	Observation		AWD (39)
8	58/F	Epigastralgia	Near Vater's papilla, single	I	R monotherapy		AWOD (32)
9	72/M	Medical check-up	Second portion, multiple	I	R monotherapy		AWOD (32)
10	53/M	Medical check-up	Second portion, multiple	I	R monotherapy		AWOD (32)
11	46/F	Medical check-up	Second-third portion, multiple	II	R-CHOP		AWD (31)
12	63/M	Medical check-up	Second portion, single	II	R monotherapy		AWD (22)
13	42/M	Left flank pain	Second portion, multiple	II	CHOP		AWOD (57)
14	45/M	Medical check-up	Second portion, multiple	II	R monotherapy		AWD (30)
15	58/M	Right cervical LN enlargement	Near Vater's papilla, single	III	R-CHOP		AWOD (21)
16	52/F	Systemic LN enlargement	Near Vater's papilla, single	IV	C-MOPP		AWOD (96)
17	58/M	Medical check-up	Near Vater's papilla, single	IV	R-C-MOPP		AWOD (77)
18	61/F	Malaise	Second portion, single	IV	R-CHOP	Leukaemic change (12)	DOD (23)
19	49/F	Medical check-up	Bulb-third portion, multiple	IV	R-CHOP		AWOD (49)
20	52/F	Appetite loss and diarrhea	Second portion, multiple	IV	R-CHOP		AWOD (23)
21	62/M	Medical check-up	Bulb-second portion, multiple	IV	R-CHOP		AWD (16)
22	36/F	Abdominal distension	Unknown	IV	R-CHOP		AWOD (42)
23	45/M	Subcutaneous nodule on right upper arm	Near Vater's papilla, single	IV	Irradiation		AWOD (20)
24	46/M	Epigastric discomfort	Second portion, multiple	IV	Unknown		Unknown
25	50/F	Left inguinal LN enlargement	Bulb-second portion, multiple	IV	R-CHOP		AWOD (24)
26	37/M	Weight loss	Near Vater's papilla, multiple	IV	R-CHOP		AWOD (17)

AWOD, alive without disease; AWD, alive with disease; DOD, died of disease; LN, lymph node; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; R, rituximab; C-MOPP, cyclophosphamide, vincristine, procarbazine and prednisone.

¹ Recurrent sites and months after treatment.

² Outcome at last follow-up and months after initial diagnosis.

their diagnoses were switched to FL during follow-up. Immunohistochemically, all of the DFLs were positive for CD20, CD10 and Bcl-2, and negative for CD3, CD5 and cyclin D1 (Figure 1). *H. pylori* infection was frequent in both primary and systemic DFLs: eight of 12 (67%) and seven of nine (78%), respectively.

FISH analysis revealed IGH/BCL2 fusion in nine of the 12 cases (75%) of primary DFL and 10 of the 12 cases (83%) of systemic DFL.

Initial therapies for patients with primary DFL included cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) (two cases), rituximab (R) plus CHOP (R-CHOP) (1), irradiation (2), R monotherapy (6) and observation (3). For patients with systemic DFL, initial therapies included R-CHOP (eight cases), cyclophosphamide, vincristine, procarbazine and prednisone (C-MOPP) (1), R-C-MOPP (1) and irradiation (1) (Table 1). Two of the primary DFLs were

initially treated by *H. pylori* eradication because their initial diagnosis was MALT lymphoma, and the clinical response to eradication was no change (NC) in both cases. After a median follow-up duration of 40 months (range 11–96 months), 17 of 25 patients were alive without disease, seven were alive with disease and one had died of lymphoma. The latter patient died due to leukemic change of FL 12 months after the diagnosis of systemic DFL. One of the patients alive with disease experienced transformation to diffuse large B-cell lymphoma in a submandibular lymph node 4 months after the diagnosis of primary DFL. The information on the outcome for one patient was not available.

DISCUSSION

Primary FL of the GI tract is rare. Misdrjaj et al. (16) reported the first case of primary DFL in 1997. Since then,

Table 2. Summary of morphologic, immunohistochemical and FISH analyses in 26 cases of duodenal follicular lymphoma

Case	Grade	Follicular/diffuse ¹	CD20	CD10	Bcl-2	CD3	CD5	Cyclin D1	FISH: IGH/BCL2	<i>H. pylori</i>
1	1	Follicular	+	+	+	-	-	-	+	+
2	1	Follicular	+	+	+	-	-	-	+	+
3	1	Follicular	+	+	+	-	-	-	ND	ND
4	1	Follicular	+	+	+	-	ND	ND	ND	ND
5	1	Follicular	+	+	+	-	-	-	-	-
6	2	Follicular	+	+	+	-	ND	ND	-	+
7	1	Follicular	+	+	+	-	ND	ND	+	-
8	1	Follicular	+	+	+	-	-	ND	+	+
9	1	Follicular and diffuse	+	+	+	-	-	ND	-	+
10	1	Follicular	+	+	+	-	-	-	+	-
11	1	Follicular	+	+	+	-	ND	ND	+	+
12	1	Follicular	+	+	+	-	-	ND	+	+
13	1	Follicular	+	+	+	-	ND	ND	+	+
14	1	Follicular	+	+	+	-	-	-	+	-
15	1	Follicular	+	+	+	-	ND	-	-	+
16	1	Follicular	+	+	+	-	ND	ND	+	ND
17	1	Follicular	+	+	+	-	-	-	+	+
18	1	Follicular	+	+	+	-	-	ND	+	+
19	1	Follicular	+	+	+	-	-	-	+	+
20	1	Follicular	+	+	+	-	-	-	+	-
21	2	Follicular	+	+	+	-	-	-	+	-
22	2	Follicular	+	+	+	-	ND	ND	-	+
23	1	Follicular	+	+	ND	-	ND	-	+	ND
24	1	Follicular	+	+	+	-	ND	-	+	ND
25	2	Follicular	+	+	+	-	-	-	+	+
26	1	Follicular	+	+	+	-	ND	ND	+	+

FISH, fluorescence *in situ* hybridization; *H. pylori*, *Helicobacter pylori*; ND, not done.

¹Categorization as follicular: >75% of lymphoma has follicular pattern; follicular and diffuse: 25–75% follicular pattern.

several other studies have suggested that DFL may be a characteristic clinicopathologic entity due to its localized nature and good prognosis (9,10). Patients usually present with symptoms related to bowel thickening. In some cases, the symptoms are mild and non-specific; patients often have relatively long-standing symptoms before seeking medical attention. In this series, 79% (11 of 14) of primary DFLs were detected at routine medical check-ups. However, most of the patients with systemic DFL complained of symptoms such as lymph node enlargement and weight loss, and DFL was detected through the staging procedure.

All cases involved the second portion of the duodenum, and in 40% of cases, the lesions were found in the vicinity of the Vater's papilla. Yoshino et al. (9) speculated that occurrence of primary DFL at this site might be related to bile duct diseases, as suggested by the female predilection of the disease. However, in the present study, both primary and systemic DFL involved the second portion of duodenum, the speculation was not acceptable for systemic DFL.

DFL is reported to have histologic and immunohistochemical features that are similar to those of nodal FL, and not those of cutaneous FL (10). In the present study, all 26 DFLs showed low-grade histology, a predominant follicular growth pattern and immunohistochemical expression of CD20, CD10 and Bcl-2. *H. pylori* infection was frequent in both primary and systemic DFLs. Toyoda et al. (17) reported regression of DFL after eradication of *H. pylori*. Antibiotic therapy may be effective for the treatment for some patients with DFL, but in two of our primary DFL, the clinical response to eradication was NC.

The majority of FLs arise in lymph nodes and possess a characteristic chromosomal translocation, t(14;18)(q32;q21), juxtaposing the BCL2 gene with an immunoglobulin gene. The translocation is present in up to 85% of cases in Europe and the USA (12), and in ~60% of cases in Japan (13). Among extranodal FLs, cutaneous FL (18) and salivary gland FL (19) have a low incidence of, or lack, t(14;18)(q32;q21). In GI tract FL, previous reports with small

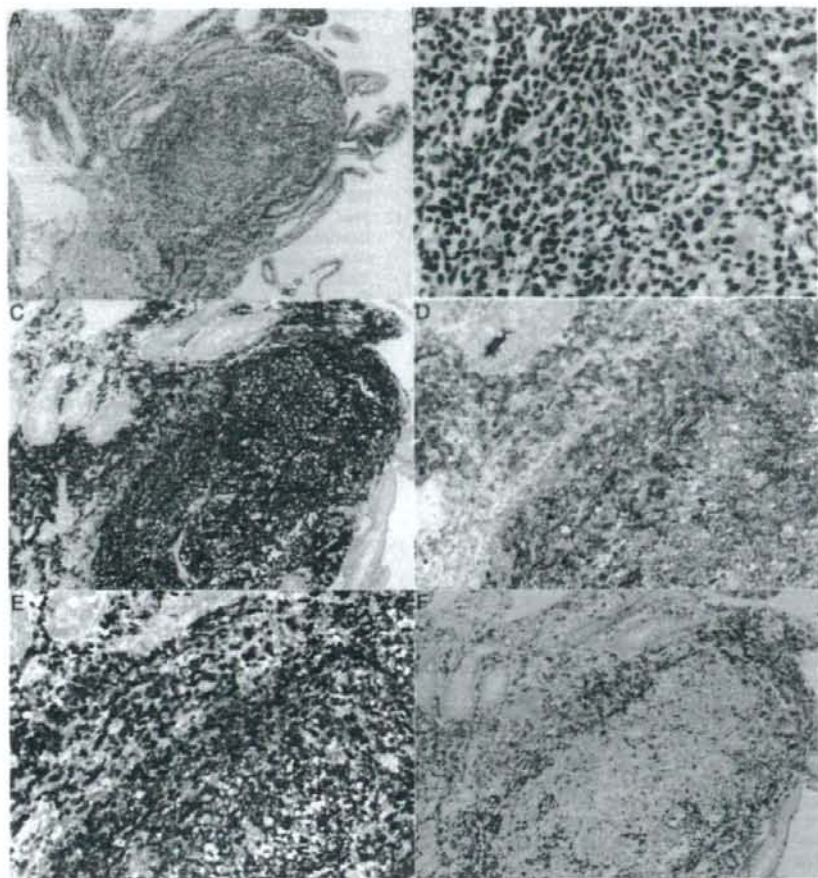


Figure 1. A case of primary duodenal follicular lymphoma, grade 1. The lesion shows well circumscribed follicles (A, HE, $\times 40$), composed of a uniform population of small cleaved cells (B, HE, $\times 400$). Immunohistochemically, CD20 (C, $\times 100$), CD10 (D, $\times 200$) and Bcl-2 (E, $\times 200$) are positive in the follicular area, but CD3 is negative (F, $\times 100$).

numbers of cases suggested occurrence of $t(14;18)(q32;q21)$ (9,10). Our FISH analysis indicated a high incidence of IGH/BCL2 fusion, which was detected in nine (75%) of the 12 primary DFLs, and 10 (83%) of the 12 systemic DFLs. DFL is suggested to resemble nodal FL more closely than FL at other extranodal sites. Moreover, in Japan, the frequency of $t(14;18)$ in DFL tends to be higher than that of nodal FL. It is speculated that IGH/BCL2 fusion-positive cells are likely to involve the duodenum. On the other hand, Sato et al. (11) recently reported that most cases of DFL are localized, and appear to have characteristics intermediate between MALT lymphoma and nodal FL according to IGH/BCL2 and VH usage analyses. They detected IGH/BCL2 fusion using the major break point by polymerase chain reaction (PCR) in 27% of DFLs, which was a lower frequency than our result obtained using FISH. The difference might be due to the low sensitivity of PCR using their primer sets, which detect only about half of the translocations.

Patients with DFL underwent chemotherapy, R-containing chemotherapy, irradiation or observation. Although the follow-up period was short, all patients with DFL except one of systemic DFL were alive at the last follow-up, suggesting that DFL is an indolent disease with a favourable outcome, irrespective of whether it is primary or systemic. As R monotherapy was reported to be an effective treatment for a stage I DFL (20), we have a schedule to evaluate the issue in patients with primary DFL in our institute in the future.

In conclusion, we have revealed the characteristics of DFL; primary DFL is found incidentally at medical check-ups, occurs in the second portion of the duodenum in the vicinity of the Vater's papilla, and appears to have a favourable outcome. It is histologically low grade, and phenotypically and genotypically similar to nodal FL. The characteristics of systemic DFL are similar to those of primary DFL histologically and genotypically. It is found at the time of systemic survey for pre-treatment staging.

Primary DFLs resemble systemic and nodal FLs, except that the former has a high incidence of early stage disease and low-grade histology. Primary and systemic DFLs may constitute a continuous spectrum. The duodenum may be a frequently involved extranodal site of FL with IGH/BCL2.

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Conflict of interest statement

None declared.

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Progressive multifocal leukoencephalopathy in a patient with B-cell lymphoma during rituximab-containing chemotherapy: case report and review of the literature

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Abstract Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system caused by the JC polyomavirus. We describe a rare case of PML in a 48-year-old female patient with diffuse large B-cell lymphoma who received rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) therapy. While she was undergoing five cycles of R-CHOP, she noticed gradually progressive neurological symptoms, such as slurred speech and gait disturbance, and she eventually developed high-grade fever. She also developed *Pneumocystis jirovecii* pneumonia. The neurological symptoms deteriorated thereafter, and she developed spastic quadriparesis and bulbar palsy. Magnetic resonance imaging showed hyperintensity within the right cerebellar hemisphere on T2-weighted images. Polymerase chain reaction-based tests of the cerebrospinal fluid revealed the presence of the JC virus. Despite intravenous and intrathecal cytarabine treatment, the patient died of PML 5 months after it was diagnosed. Retrospective analysis of her laboratory data showed that her CD4⁺ T-cell count before R-CHOP therapy had decreased to 68 μL^{-1} . Thus, when administering rituximab-containing chemotherapy, even to patients with no prior history of opportunistic infections, attention should be paid to the potential occurrence of PML, particularly in patients with low CD4⁺ T-cell counts.

Keywords Progressive multifocal leukoencephalopathy · Lymphoma · Rituximab · CD4⁺ T-cell counts · JC virus

1 Introduction

Progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the central nervous system (CNS), is associated with high rates of morbidity and mortality, and occurs almost exclusively in immunocompromised patients [1]. The JC virus (JCV), a human polyomavirus, is the etiologic agent of PML [2]. It accumulates to high concentrations in oligodendrocytes, causing their destruction by cytolysis [3], and accumulates in astrocytes as well. PML was previously considered a very rare disease; however, its prevalence in association with acquired immunodeficiency syndrome (AIDS) has increased steadily in recent years. However, there have been several cases of PML reported in patients with hematological malignancies not associated with AIDS but related to fludarabine therapy, mainly those diagnosed with chronic lymphocytic leukemia [4, 5]. The newer forms of treatment, such as purine analogs for hematological malignancies, have augmented the incidence of PML. We herein describe an extremely rare case of PML that developed in a patient with diffuse large B-cell lymphoma (DLBCL) during cyclophosphamide (CPA), doxorubicin (DXR), vincristine (VCR), and prednisolone combined with rituximab (R-CHOP) therapy, and review the relevant literature.

2 Case report

In June 2006, a previously healthy 48-year-old Japanese female complained of severe pain in her buttocks and was

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referred to our hospital. A computerized tomography (CT) scan revealed an extensive osteolytic mass in her right iliac region and para-aortic lymphadenopathy. An open biopsy and subsequent histological examination of the right iliac mass revealed it to be DLBCL. The patient was diagnosed with stage II disease, and classified as being in a low-risk group by the International Prognostic Index criteria. She was treated with the CHOP regimen (day 1, intravenous: CPA 750 mg m⁻², DXR 50 mg m⁻², and VCR 1.4 mg m⁻²; days 1–5, oral: prednisolone 100 mg) and concurrent rituximab at a dose of 375 mg m⁻²; she tolerated the treatment well.

In October 2006, while she was undergoing five cycles of chemotherapy and achieved a complete response (CR), she noticed gradually progressive neurological symptoms, such as double vision, slurred speech, clumsiness of the right hand, and gait disturbance. Her neurological symptoms continued to worsen, and she eventually developed high-grade fever and was admitted to our hospital. On physical examination, all vital signs except the body temperature were normal. Breath sounds were clear and oxygen saturation was normal. She appeared fully alert and oriented, although neurological examination revealed cerebellar dysmetria by finger-to-nose and heel-to-knee tests, which was worse on the right side. The patient was fully ambulatory, although her gait appeared relatively wide-based. There were no signs of sensory disturbance or unilateral pyramidal tract lesions.

Laboratory studies revealed a leukocyte count of 9,200 μL^{-1} (neutrophils, 8,400 μL^{-1} ; lymphocytes, 460 μL^{-1}), hemoglobin of 10.6 g μL^{-1} , platelet count of $37.5 \times 10^4 \mu\text{L}^{-1}$, LDH of 281 IU L⁻¹ [normal range (NR), <229 IU L⁻¹], and C-reactive protein of 12.5 mg dL⁻¹ (NR <0.1 mg L⁻¹). Lymphocyte subset analysis revealed an absolute CD4⁺ T-cell count of 68 cells μL^{-1} . Serum immunoglobulin (Ig) concentrations were 720 mg dL⁻¹ IgG (after that, the level of IgG was reduced to 391 mg dL⁻¹), 256 mg dL⁻¹ IgA, and 74 mg dL⁻¹ IgM. Her serum level of soluble interleukin-2 receptor was elevated to 1,490 U mL⁻¹ (NR <531 U mL⁻¹). Her serology was negative for hepatitis B, hepatitis C, syphilis, human immunodeficiency virus (HIV), and human T-cell leukemia virus type I. Routine cultures for bacteria and fungi were negative. The total cell count in the cerebrospinal fluid (CSF) was 1 cell μL^{-1} , with normal levels of glucose and protein, and a negative Gram's stain. Herpes simplex virus DNA was not detected in the patient's CSF by the polymerase chain reaction (PCR).

The patient was suspected to have CNS involvement by lymphoma cells. However, a total body CT scan did not reveal any signs of recurrent or systemic disease, and gadolinium-enhanced cranial magnetic resonance imaging (MRI) did not identify mass lesions. Because she was

suspected to have suffered from encephalitis and/or meningitis, treatment with ceftriaxone, ampicillin, and acyclovir was initiated; however, her fever did not subside. A CT scan of the brain showed a nonenhancing hypodense lesion in the right cerebellar hemisphere. Cranial MRI T2-weighted, fluid-attenuated inversion recovery, and diffusion-weighted images (DWI) showed high-intensity lesions in the right cerebellar hemisphere (Fig. 1). The lesions on the CT scan were not accompanied by a mass effect, and gadolinium did not enhance the lesions. The etiology of the neurological disorders could not be determined.

After 7 days the patient's respiratory status suddenly deteriorated. A chest X-ray and CT scan showed bilateral infiltrates expanding diffusely. The DNA sequence specific to *Pneumocystis jiroveci* was detected in the specimen obtained from the bronchoalveolar lavage fluid by PCR. Bacterial culture was negative and microscopic examination did not show any lymphoma cells. Antigens of cytomegalovirus, *Candida*, *Aspergillus*, and *Cryptococcus* were not detected. She was started on sulfamethoxazole and trimethoprim. Prednisolone at a dosage of 40 mg, twice daily, was also prescribed. Her respiratory symptoms improved with the treatment of *Pneumocystis jiroveci* pneumonia (PCP), and the abnormal findings on the chest X-ray and CT scan were normalized. Moreover, she developed adenovirus-, JC virus-, and BK virus-related hemorrhagic cystitis.

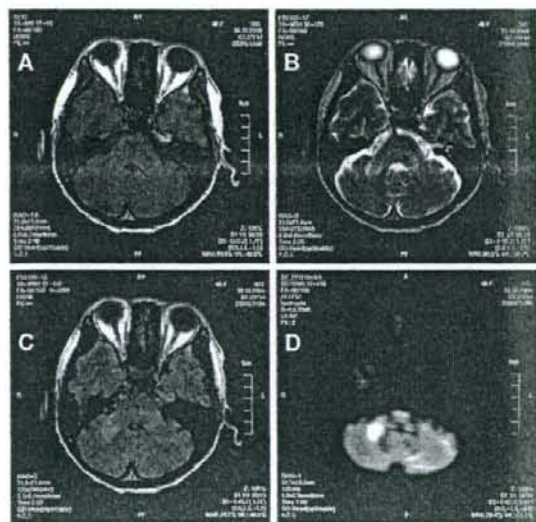


Fig. 1 October 2006: magnetic resonance imaging showing mild cerebellar atrophy in a T1-weighted image (a), white matter abnormality in a T2-weighted image (b), white matter abnormality in fluid-attenuated inversion recovery (c), and intracellular edema in a diffusion-weighted image (d)

Her neurologic status deteriorated rapidly with the development of decreased attention, marked gaze-evoked nystagmus, asymmetric spastic quadriparesis, and bulbar paralysis. MRI showed the progression of cerebellar atrophy (Fig. 2) compared with the initial MRI, as well as new scattered areas of signal hyperintensity within the right cerebellar hemisphere and adjacent brainstem. MRI revealed areas of white matter disease consistent with a demyelinating process, and PCR of the CSF showed JCV DNA.

PML was diagnosed, and intravenous injections of cytarabine were given accordingly at a dosage of 2 mg kg^{-1} for five consecutive days. Despite the treatment, the patient's neurological condition continued to worsen. A repeat MRI showed progressive worsening of the radiological findings. The patient subsequently received an intrathecal injection of cytarabine at a dose of 20 mg per day. However, the therapy was unsuccessful, and she died 8 months after the diagnosis of DLBCL, i.e., 5 months after the onset of PML.

3 Discussion

Progressive multifocal leukoencephalopathy is an uncommon demyelinating disease of the CNS caused by lytic infection of oligodendrocytes with JCV [3]. Up to 64% of

healthy adults shed JCV in the urine in the absence of any clinical symptoms, which suggests that asymptomatic, active JCV infection is common in immunocompromised persons [6]. The clinical manifestations of PML are hypothesized to occur when B cells infected with JCV are activated under an immunosuppressed condition; subsequently, JCV enters the brain, where astrocytes and oligodendrocytes support JCV replication, resulting in neurological damage [7]. The disease is characterized by the development of disseminated demyelinating plaques in the cerebral white matter and adjacent areas [3]. Death within 6 months is common.

JCV reactivation is typically observed among patients with severe immunodeficiency. The cause of the immunologic deficit is mostly a long-standing hematological disorder, HIV infection, hematological malignancies, immunosuppressive medications, or immunosuppressive treatment after organ transplantation [3]. In the HIV-infected population, PML is strongly correlated with depressed CD4^+ T-cell counts [8]. Rare cases of PML have been reported among patients with lymphoma [4]. Our report details the clinical features of a patient with DLBCL who developed both PML and PCP. Because both PML and PCP are opportunistic infections, we suspected a preceding immunosuppressed state, and identified a decrease in absolute CD4^+ T-cell counts at the initial presentation of DLBCL. The patient had no underlying diseases that might cause immunodeficiency, such as additional malignancies or AIDS. The definition of idiopathic CD4^+ T-lymphocytopenia is consistent with this condition [9], but it is not certain if this is an abnormality directly related to malignant lymphoma. On the other hand, a reduction in the CD4^+ T-cell count is noted at the initial examination in some patients with malignant lymphoma, suggesting a possibility for the development of an opportunistic infection. The present patient developed PCP and viral hemorrhagic cystitis in addition to PML. We believe that we should be cautious regarding the development of an opportunistic infection in patients with a low CD4^+ T-cell count at the initial examination, and the R-CHOP regimen should be accompanied by supportive care such as trimethoprim-sulfamethoxazole to prevent PCP under close observation. For patients with a low CD4^+ T-cell count before the initiation of treatment, both the risk of developing an opportunistic infection following the R-CHOP regimen and the prognosis of malignant lymphoma should be assessed: if the latter is relatively favorable, one should consider the possibility of omitting either rituximab or glucocorticoids in the drug combination treatment of the R-CHOP regimen. In recipients of bone marrow transplants, PML has also been associated with rituximab treatment [5, 10]. To our knowledge, however, initial R-CHOP immunochemotherapy for B-cell lymphoma has

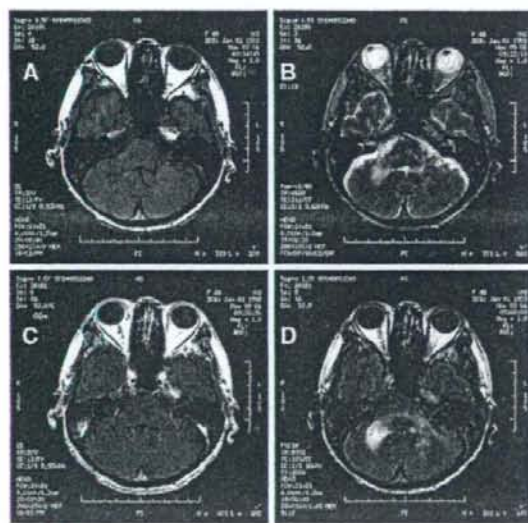


Fig. 2 December 2006: magnetic resonance imaging showing cerebellar atrophy in a T1-weighted image (a), white matter abnormality and expansion into the region of the adjacent brainstem in a T2-weighted image (b), cerebellar atrophy without areas of enhancement in a T1-weighted image (c), and white matter abnormality and expansion of the region of the adjacent brainstem in fluid-attenuated inversion recovery (d)

not previously been identified as a treatment with an increased risk of PML.

A definitive diagnosis of PML can be based on the concomitant presence of a compatible clinical and neuroimaging picture and characteristic histopathologic features with JCV detection in the brain tissue [11]. However, brain biopsy is an invasive method with considerable risks. In addition, the severity and extent of this disease are so marked that patients with PML are often in a poor clinical condition with an impaired hemostatic system, making noninvasive diagnostic methods highly desirable. Therefore, some studies have developed and validated less-invasive diagnostic methods based on the amplification of the JCV-target DNA from CSF by PCR. Fong et al. [12] showed that the sensitivity and specificity of JCV DNA by PCR were 74 and 96%, respectively, and the positive and negative predictive values were 89.5 and 88.5%, respectively, before the highly active antiretroviral therapy (HAART) era.

The MRI findings in patients with PML present characteristic images. There should be areas of decreased signal intensity on T1-weighted images and increased signal intensity on T2-weighted images [13]. The images are dominated by T2-signal abnormalities of the white matter [3, 14]. This is reflected by a slight tissue swelling in acute lesions and atrophy in end-stage areas during the demyelination process. Most lesions remain hyperintense on T2-weighted images because the tissue water content increases after the replacement of oligodendrocytes by astrocytes. Tissue destruction continues in clinically progressive patients. Generally, the lesions in PML are not contrast-enhanced by gadolinium and do not show a substantial mass effect. DWI reliably distinguishes intracellular edema from interstitial water accumulation. Intracellular edema is seen in acute cell damage, which is usually followed by cell death. Cell death as observed in the periphery of the cerebral lesions is explained as oligodendrocyte necrosis in the areas of demyelination [7].

In a randomized controlled trial involving 57 patients with HIV infection and biopsy-confirmed PML, cytarabine administered either intravenously or intrathecally did not improve the prognosis compared with antiretroviral therapy alone [15]. For PML associated with AIDS, HAART is relatively effective [16]. Although there have been no large-scale clinical trials on treatment for PML in the absence of AIDS, there is evidence that cytarabine decreases JCV replication and multiplication *in vitro* [17]. There have also been studies on improvement in non-HIV-related PML when cytarabine is given intravenously, intrathecally, or both [18–20]. The most successful of them, an open-label study involving 19 patients who had PML without HIV infection, showed that intravenous cytarabine at a dosage of 2 mg kg⁻¹ daily for five consecutive

days appeared to stabilize the neurological and functional status in seven patients (36%) at 2–4.5 years of follow-up, despite significant bone marrow toxicity complications [21]. Based on these studies, we chose cytarabine for the treatment of PML in the present case.

A recent study reported that approximately 80% of PML patients have AIDS, 13% have hematological malignancies, 5% are transplant recipients, and 2% have chronic inflammatory diseases [22]. Since 1990, when purine analogs first became available, the incidence of PML has increased [5]. The Food and Drug Administration alert in December 2006 reported that two patients with systemic lupus erythematosus died after treatment with rituximab. Thus, this new class of medication, such as purine analogs and rituximab used in treating hematological malignancies, may increase the number of patients at risk of developing PML [5, 12]. CD4⁺ T-cell counts of less than 200 μL^{-1} are known to be a risk factor for the development of PML [5]. For those patients with reduced CD4 T-cell counts and T-cell deviations (compromised cellular immunity), the administration of rituximab or the application of the CHOP regimen may impair humoral immunity, which in turn may lead to the onset of PML. For the present patient, the CHOP regimen that preceded rituximab caused a decrease in the IgG level, and the subsequent rituximab administration resulted in a further reduction in IgG (the level ultimately reaching 391 mg/dL). It was suggested that the R-CHOP regimen may compromise humoral immunity in patients with a reduced CD4⁺ T-cell count and expose them to a risk of developing PML or other opportunistic infections. This was true in our case: our patient's CD4⁺ T-cell count before R-CHOP therapy was 68 μL^{-1} . Thus, when a variegated neurological symptom is noted during rituximab-containing chemotherapy, one must consider the possibility of PML while making a differential diagnosis.

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Phase I and II pharmacokinetic and pharmacodynamic study of the proteasome inhibitor bortezomib in Japanese patients with relapsed or refractory multiple myeloma

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The purpose of this phase I and II study was to evaluate the safety, pharmacokinetics, pharmacodynamics, and efficacy of bortezomib in Japanese patients with relapsed or refractory multiple myeloma. This was a dose-escalation study designed to determine the recommended dose for Japanese patients (phase I) and to investigate the antitumor activity and safety (phase II) of bortezomib administered on days 1, 4, 8, and 11 every 21 days. Thirty-four patients were enrolled. A dose-limiting toxicity was febrile neutropenia, which occurred in one of six patients in the highest-dose cohort in phase I and led to the selection of 1.3 mg/m² as the recommended dose. Adverse events \geq grade 3 were rare except for hematological toxicities, although there was one fatal case of interstitial lung disease. The overall response rate was 30% (95% confidence interval, 16–49%). Pharmacokinetic evaluation showed a biexponential decline, characterized by a rapid distribution followed by a longer elimination, after dose administration, whereas the area under the concentration–time curve increased proportionately with the dose. Bortezomib was effective in Japanese patients with relapsed or refractory multiple myeloma. A favorable tolerability profile was also seen, although the potential for pulmonary toxicity should be monitored closely. The pharmacokinetic and pharmacodynamic profiles of bortezomib in the present study warrant further investigations, including more relevant administration schedules. (*Cancer Sci* 2008; 99: 140–144)

Multiple myeloma, one of the B-cell lymphatic tumors, is a malignant hematopoietic tumor with poor prognosis for which a cure cannot ever be expected. The peak age of onset is high at 65–70 years, and its onset in patients younger than 40 years is rare. The median survival of patients with multiple myeloma is approximately 6–12 months if untreated, but it is prolonged to approximately 3 years with the administration of chemotherapy; the 5-year survival rate has been reported to be approximately 25% and the 10-year survival rate is $<$ 5%.^(1,2) As initial therapy for multiple myeloma, melphalan + prednisolone therapy and vincristine + doxorubicin + dexamethasone therapy have been used as global standards.^(3,4) High-dose chemotherapy combined with autologous hematopoietic stem-cell transplantation is reported to be significantly superior to multiagent chemotherapy in terms of response rate and progression-free survival,⁽⁵⁾ and is considered to be a standard therapy primarily for patients who are 65 years old or younger. However, no consensus has been reached on the standard therapy for relapsed or chemotherapy-refractory multiple myeloma patients.^(6–8) Multiple myeloma is

an intractable disease with poor prognosis that continues to relapse, and the duration to relapse becomes shorter in patients who repeatedly receive treatment. There are no available treatment options in which durable efficacy can be expected after relapse, and therefore effective therapeutic choices with new mechanisms of action have been long awaited.

Bortezomib is a novel small molecule that is a potent selective and reversible inhibitor of the proteasome, and has been approved for the treatment of recurrent or refractory multiple myeloma in the USA and Europe. The pharmacokinetics (PK) of bortezomib were reported in a phase I study in which it was administered in combination with gemcitabine twice weekly for 2 weeks followed by a 10-day rest period,⁽⁹⁾ and in another phase I study in which it was administered once weekly for 4 weeks followed by a 13-day rest period.⁽¹⁰⁾ Both studies were conducted in patients with advanced solid tumors and not patients with multiple myeloma. Therefore, the present phase I and II study was designed to assess the PK and pharmacodynamic (PD) effects of bortezomib in multiple myeloma patients, particularly in a Japanese population. In addition, efficacy and safety were evaluated to determine the recommended dose (RD).

Patients and Methods

Eligibility. The main eligibility criteria were: confirmed multiple myeloma according to the South-west Oncology Group diagnostic criteria;⁽¹¹⁾ had received at least previous standard front-line therapy (including melphalan and prednisolone, vincristine, doxorubicin, and dexamethasone chemotherapy, and high-dose chemotherapy with autologous stem cell transplantation); had documentation of relapse or refractoriness to the last line of therapy and required therapy because of progressive disease at enrolment. Progressive disease was defined as at least one of the following: more than 25% increase in monoclonal immunoglobulin in the serum or urine; development of new osteolytic lesions or soft tissue tumors, or worsening of existing lesions; hypercalcemia (corrected serum calcium value of $>$ 11.5 mg/dL); relapse from complete response (CR); the presence of measurable disease lesions; Karnofsky performance status \geq 60; 20–74 years of age; adequate bone marrow function (absolute neutrophil count \geq 1000/mm³, platelets \geq 75 000/mm³, and hemoglobin \geq 8 g/dL).

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hepatic function (aspartate aminotransferase and alanine aminotransferase levels ≤ 2.5 times the upper limit of institutional normal range, total bilirubin ≤ 1.5 times the upper limit of institutional normal range), renal function (creatinine clearance ≥ 30 mL/min), and cardiac function (left ventricular ejection fraction $\geq 55\%$ by echocardiography without New York Heart Association class III to IV congestive heart failure) in the previous 2 weeks; and had received no systemic chemotherapy or radiotherapy in the previous 4 weeks. This study was approved by the Institutional Review Board of each participating hospital. All patients gave written informed consent and the study was conducted in accordance with Good Clinical Practice for Trials of Drugs and the Declaration of Helsinki.

Study design. The RD was determined based on the occurrence of dose-limiting toxicity (DLT) in Japanese patients and in the dose-escalating phase I of the study. The safety and efficacy of bortezomib at the RD were assessed in phase II. In phase I, three patients were enrolled in the 0.7 mg/m²-dose group, and six patients each in the 1.0 and 1.3 mg/m²-dose groups. DLT was defined as \geq grade 3 non-hematological toxicity or grade 4 hematological toxicity for which the relation to bortezomib could not be ruled out. The RD was defined as a dose level with a DLT incidence closest to but lower than the estimated (expected) value of 30%. Bortezomib was administered for up to six cycles.

Drug administration. Bortezomib, supplied by Janssen Pharmaceutical (Tokyo, Japan) in vials containing 3.5 mg, was administered by intravenous push over 3–5 s on days 1, 4, 8, and 11, followed by a 10-day rest period, with this 3-week period comprising one cycle. There was an interval of at least 72 h between doses.

Response and safety assessments. Patients were monitored for response after every two treatment cycles by quantitation of serum immunoglobulins, serum protein electrophoresis and immunofixation (IF), and collection of a 24-h urine specimen for total protein, electrophoresis, and IF. Response was evaluated using the European Group for Blood and Marrow Transplantation criteria,⁽¹²⁾ after cycles 2, 4, and 6.

Adverse events were assessed and graded according to the National Cancer Institute Common Toxicity Criteria version 2.0 from the first dose until 28 days after the last dose of bortezomib.

Pharmacokinetic and pharmacodynamic analysis. Plasma bortezomib concentrations and blood 20S proteasome activity were measured in phase I. Blood samples were collected before each dose, at 5, 15, and 30 min, and 1, 2, 4, 6, 8, 12, 24, and 48 h after treatment on days 1 and 11. The measurement of plasma bortezomib concentration was conducted at Advion BioSciences (Ithaca, NY, USA) using liquid chromatography/tandem mass spectrometry (LC/MS/MS).⁽¹³⁾ The measurement of blood 20S proteasome activity was conducted at Millennium Pharmaceuticals (Cambridge, MA, USA) using the synthetic fluorescence substrate method validated for the chymotrypsin-like activity/trypsin-like activity ratio.⁽¹⁴⁾

Results

Patients and dose escalation. The study was conducted from May 2004 to January 2006, and 34 patients were enrolled. Patient characteristics are shown in Table 1. All patients had secretory-type myeloma, and the breakdown was 20 patients (59%) with IgG type, eight patients (24%) with IgA type, three patients (9%) with light-chain type, and three patients (9%) with IgA and light-chain type. Most patients had received prior therapy with steroids, alkylating agents, and/or vinca alkaloids. Ten patients (29%) had received stem cell transplantation including high-dose therapy. The median number of lines of prior therapy was two (range: one to eight). Osteolytic lesions were observed in 30 patients (88%) and soft-tissue tumors were observed in seven (21%). The median number of treatment

Table 1. Patient characteristics

Patient characteristic	n	%
Patients	34	
Sex		
Female	12	35
Male	22	65
Age (years)		
Median	60	
Range	34–72	
Durie-Salmon stage		
I	0	
II	15	44
III	19	56
Time since diagnosis (years)		
Median	3.4	
Range	1.0–13.7	
Karnofsky performance status		
100	15	44
90–80	18	53
70–60	1	3
Serum interleukin-6 (pg/mL)		
Mean	4.2	
Range	0.5–30.2	
Cytogenetics		
Karyotype abnormal	4	12
del(13)(q14)	7	21
t(11; 14)	4	12
Prior therapy		
Chemotherapy	34	100
Steroids	34	100
Alkylating agents	33	97
Vinca alkaloids	27	79
Anthracyclines	22	65
Thalidomide	8	24
Interferon	7	21
Radiation therapy	6	18
Autologous hematopoietic stem cell transplantation	10	29

cycles was four (range: one to six), and the median duration of treatment was 79 days (range: 1–152 days). Ten patients (29%) completed all six cycles. The reasons for discontinuation of therapy in 25 patients were progressive disease in 11 patients, patient's own request in six patients, serious adverse events in four patients, DLT in two patients, and others in three patients. Three patients were enrolled in the 0.7 mg/m² group and six in the 1.0 mg/m² group, and no DLT were observed at any dose level. In the 1.3 mg/m² group, DLT (grade 3 febrile neutropenia) occurred in one of the six patients. Therefore, 1.3 mg/m² was determined to be the RD in subsequent phase II, in which 18 patients were enrolled.

Adverse events. The safety analysis dataset consisted of all patients who received at least one dose of bortezomib (34 patients). Adverse events observed in $\geq 20\%$ of patients are shown in Table 2. The events observed at a high frequency ($\geq 50\%$) were lymphopenia, neutropenia, leukopenia, thrombocytopenia, anemia, asthenia, diarrhea, constipation, nausea, anorexia, and pyrexia. At least one \geq grade 3 adverse event was observed in 88% of the patients. Major \geq grade 3 adverse events were hematological toxicities including lymphopenia, neutropenia, leukopenia, thrombocytopenia, and anemia. Grade 4 hematological toxicities included neutropenia in six patients (18%), three of which experienced this adverse event during cycle 1. At least grade 3 non-hematological toxicities occurred in fewer than 10%, and no DLT during cycle 1 were observed. Grade 4 non-hematological toxicities included hematuria, blood amylase

Table 2. All adverse events occurring in at least 20% of patients (n = 34)

Dose (mg/m ²)	0.7		1.0		1.3		All		Total	%
	(n = 3)		(n = 6)		(n = 25)		(n = 34)			
No. of Patients	1/2	3/4	1/2	3/4	1/2	3/4	1/2	3/4		
Adverse event										
Hematologic										
Lymphopenia	3	0	4	2	8	17	15	19	34	100
Neutropenia	1	1	2	4	7	16	10	21	31	91
Leukopenia	2	0	6	0	11	12	19	12	31	91
Thrombocytopenia	1	0	4	0	12	11	17	11	28	82
Anemia	2	0	2	3	10	8	14	11	25	74
Nonhematological										
Asthenia [†]	3	0	3	0	15	0	21	0	21	62
Diarrhea	1	0	2	0	15	1	18	1	19	56
Constipation	2	0	3	0	14	0	19	0	19	56
Nausea	2	0	2	0	14	0	18	0	18	53
Anorexia	3	0	2	0	14	0	18	0	18	53
Pyrexia	0	0	4	0	14	0	18	0	18	53
Peripheral neuropathy [‡]	0	0	3	0	12	1	15	1	16	47
AST increased	1	0	1	0	11	2	13	2	15	44
LDH increased	1	0	1	0	12	1	14	1	15	44
Vomiting	1	0	0	0	9	1	10	1	11	32
Rash	0	0	1	0	10	0	11	0	11	32
ALP increased	0	0	2	0	8	0	10	0	10	29
Headache	0	0	1	0	8	0	9	0	9	27
ALT increased	1	0	1	0	7	0	9	0	9	27
Hyperglycaemia	0	0	2	0	5	0	7	0	7	21
Hyponatremia	1	0	0	1	5	0	6	1	7	21
Renal impairment	1	0	1	0	5	0	7	0	7	21
CRP increased	0	0	1	0	6	0	7	0	7	21
Weight decreased	0	0	0	0	7	0	7	0	7	21

[†]Including fatigue and malaise. [‡]Including peripheral sensory neuropathy, peripheral motor neuropathy, and hypoesthesia. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; LDH, lactate dehydrogenase; NCI-CTC, National Cancer Institute Common Toxicity Criteria.

increase, and blood uric acid increase in one patient (3%) each. Hematuria was attributed to prostate cancer and judged as not related to bortezomib. The underlying disease was considered to be involved in the blood uric acid increase; this event was judged unlikely to be related to bortezomib. At the occurrence of grade 4 blood amylase increase, blood amylase isozymes were pancreatic-type in 86% and salivary-type in 14%. There were no gastrointestinal symptoms, such as abdominal pain, associated with amylase increase. Abdominal echography revealed no finding suggesting pancreatitis or pancreolithiasis, and the relevant events recovered 5 days after the onset. The causality of the grade 4 blood amylase increase with bortezomib was evaluated as 'probable', and therefore treatment was continued at a reduced dose from 1.3 to 1.0 mg/m².

One case of interstitial lung disease (ILD) that resulted in a fatal outcome was observed in phase II. The patient with grade 5 ILD had developed the event on day 10 in cycle 2 after receiving seven doses of bortezomib in total. Pyrexia, non-productive cough, hypoxia, and dyspnea were observed as early symptoms, and antibiotics, antimicrobials, steroid pulse therapy, and oxygen inhalation were initiated to treat it. However, respiratory failure worsened, so the patient was put on a ventilator, and the study was discontinued. After the onset of ILD, bronchoalveolar lavage was conducted, but the causative pathogen could not be identified. The available examinations for β -D-glucan, cytomegalovirus antigenemia, influenza virus, and urinary antigen of *Legionella* were found to be negative. The diagnosis from the pathological findings was diffuse alveolar damage. A retrospective

analysis of the pretreatment computed tomography (CT) images indicated that the patient had subtle interstitial shadows in the basal region of both lungs. In response, the protocol was amended to exclude patients with abnormal pretreatment bilateral interstitial shadows on CT. No cases of fatal pulmonary toxicity were observed thereafter.

Efficacy. Thirty-three patients were evaluable for efficacy, excluding one ineligible patient who had another malignancy (prostate cancer). Objective responses were observed in 10 of 33 patients (30%; 95% confidence interval 16–49%), including five IF-positive complete responses (CR^{IF+}) and five partial responses. Of the 10 responders, five patients had one line of prior therapy, two patients had three lines of prior therapy, and three patients had four or more lines of prior therapy. It is noteworthy that one patient who had received eight lines of prior therapy, including high-dose chemotherapy with autologous stem-cell transplantation, showed CR^{IF+}. Of the 10 patients who had received prior autologous hematopoietic stem cell transplantation, two patients showed CR^{IF+}, and three patients showed PR. With respect to osteolytic lesions, which is one of the efficacy endpoints, partial regression in five patients, partial disappearance in one patient, and regression of soft-tissue tumors in two patients were observed.

Pharmacokinetics and pharmacodynamics. The mean plasma bortezomib concentration–time profiles on days 1 and 11 obtained from 16 patients enrolled in phase I are shown in Fig. 1a. PK parameters obtained using non-compartmental analysis are shown in Table 3. The plasma bortezomib concentration–time

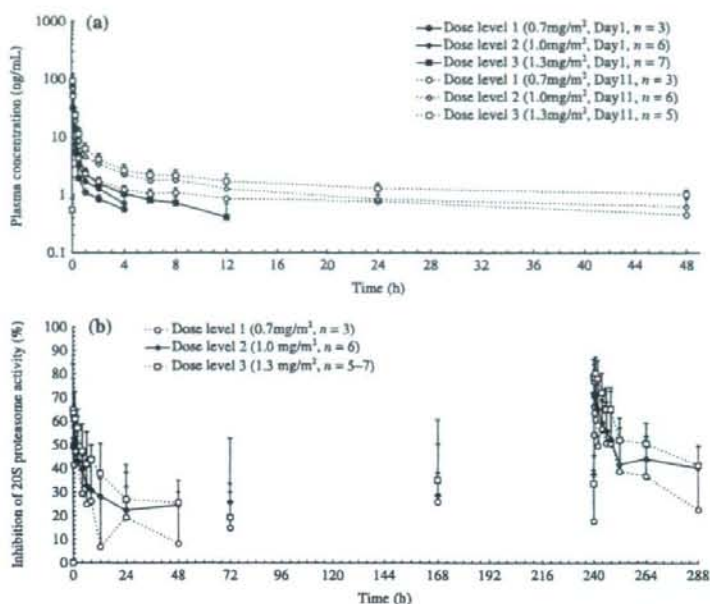


Fig. 1. (a) Plasma bortezomib concentrations (mean + SD). (b) Inhibition of blood 20S proteasome activity (mean + SD).

Table 3. Pharmacokinetic parameters (non-compartmental analysis)

Parameter	Day	Dose (mg/m ²)		
		0.7 (n = 3)	1.0 (n = 6)	1.3 (n = 5-7) ¹
C ₀ (ng/mL)	1	73.75 ± 7.89	144.92 ± 179.31	185.84 ± 57.65
	11	130.68 ± 71.97	147.19 ± 72.33	187.03 ± 54.31
AUC (ng · h/mL)	1	14.04 ± 0.70	28.58 ± 24.86	46.50 ± 19.89
	11	112.01 ± 47.74	108.39 ± 52.32	186.60 ± 49.79
Half life (h)	1	3.31 ± 0.88	6.81 ± 8.81	16.11 ± 20.75
	11	64.59 ± 30.29	32.46 ± 12.91	57.39 ± 24.92
Clearance (L/h)	1	83.35 ± 10.52	105.41 ± 75.66	51.97 ± 18.99
	11	11.77 ± 4.67	19.63 ± 14.50	12.10 ± 3.73
V _d (L)	1	406.92 ± 154.03	520.08 ± 349.87	894.41 ± 682.35
	11	978.51 ± 263.13	731.69 ± 242.35	957.81 ± 350.40
V _d (L)	1	186.46 ± 85.02	288.90 ± 260.74	507.75 ± 558.30
	11	812.60 ± 202.03	540.03 ± 218.72	763.81 ± 271.64
C ₀ ratio	11/1	1.789 ± 0.973	1.848 ± 1.133	1.103 ± 0.249
AUC ratio	11/1	7.940 ± 3.247	5.363 ± 2.970	5.142 ± 0.543

¹Day 1, n = 7; day 11, n = 5. Values are mean ± SD. AUC, area under the concentration-time curve from time zero to infinity; AUC ratio, AUC on day 11/AUC on day 1; C₀, plasma concentration at the end of administration; C₀ ratio, C₀ on day 11/C₀ on day 1; V_d, the apparent volume of distribution during the terminal phase; V_d, the apparent volume of distribution at steady state.

profiles showed a biphasic elimination profile, characterized by rapid distribution followed by a longer elimination at all dose levels. At any dose level, the elimination half-life (t_{1/2}) on day 11 was prolonged, and systemic clearance (CL) was lower compared with day 1. Therefore, delayed elimination of bortezomib from plasma associated with repeated administrations was observed, and the plasma bortezomib concentration after administration (C₀, estimated value) and area under the plasma concentration-time curve (AUC) showed higher values on day 11 compared with day 1. AUC showed dose dependency, whereas C₀ did not.

The inhibition of blood 20S proteasome activity is shown in Fig. 1b. The 20S proteasome inhibition recovered over time at all dose levels, but was prolonged compared with the temporal decrease in plasma bortezomib concentration, and the inhibition was still observed before treatment on days 4, 8, and 11.

Discussion

In the present study, bortezomib was generally well tolerated in the 25 Japanese patients whose treatments were started at the RD of 1.3 mg/m². Hematological toxicities, gastrointestinal toxicities, and peripheral neuropathies observed in our patients were similar to those reported for patients in clinical studies from the USA and Europe.^(15,16) Most could be managed without interventions or with the usual symptomatic therapy. Grade 4 neutropenia was observed in 18% of patients, but treatment could be continued with dose reduction. The response rate obtained in the present study was comparable to that reported by Richardson *et al.* in a pivotal phase III study.⁽¹⁶⁾ In addition, patients who had received heavy prior therapy also showed a consistent response. Therefore, 1.3 mg/m² is considered appropriate as an initial dose of bortezomib in Japanese patients. There was a fatal pulmonary disorder event (ILD) in one patient treated with the 1.3 mg/m² dose in which a causal relationship with bortezomib could not be ruled out. Hence, special care should be taken prior to initiating treatment with bortezomib to evaluate patients (e.g. chest X-ray or chest CT scan) and during and after bortezomib treatment if they develop subjective symptoms such as dyspnea, cough, and fever.

The assessment of PK and PD in multiple myeloma patients treated with bortezomib twice weekly for 2 weeks was conducted for the first time in Japanese patients. A decrease in CL associated with increased exposures and subsequently longer t_{1/2} values were observed after repeated administration and dose escalation. The relatively large volume of distribution suggests that bortezomib may be distributed extensively into the extravascular tissues. It can be postulated that CL values on day 1 are apparent values observed due to rapid tissue distribution, whereas

saturation of proteasome binding sites and tissue distribution occur after multiple dosing, and the CL value on day 11 may be a better representation of the true value.

It was also found that the blood 20S proteasome inhibition at each dose level recovered over time, but was prolonged compared with the temporal decrease in plasma bortezomib concentration. Similarly to CL, this could be due to the large distribution volume of bortezomib and its slow return from tissues to plasma.

Delayed elimination and enhanced proteasome inhibition were observed with repeated administration and dose increase, but no clear tendency in the incidence or degree of adverse reactions was observed. However, the PD results of the present study in Japanese patients demonstrate that the inhibition of 20S proteasome activity does not recover even after 72 h, which is specified as a minimum interval for bortezomib dosing.

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ONCOGENOMICS

Identification of the novel *AML1* fusion partner gene, *LAF4*, a fusion partner of *MLL*, in childhood T-cell acute lymphoblastic leukemia with t(2;21)(q11;q22) by bubble PCR method for cDNA

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The *AML1* gene is frequently rearranged by chromosomal translocations in acute leukemia. We identified that the *LAF4* gene on 2q11.2–12 was fused to the *AML1* gene on 21q22 in a pediatric patient having T-cell acute lymphoblastic leukemia (T-ALL) with t(2;21)(q11;q22) using the bubble PCR method for cDNA. The genomic break points were within intron 7 of *AML1* and of *LAF4*, resulting in the in-frame fusion of exon 7 of *AML1* and exon 8 of *LAF4*. The *LAF4* gene is a member of the *AF4/FMR2* family and was previously identified as a fusion partner of *MLL* in B-precursor ALL with t(2;11)(q11;q23), although *AML1-LAF4* was in T-ALL. *LAF4* is the first gene fused with both *AML1* and *MLL* in acute leukemia. Almost all *AML1* translocations except for *TEL-AML1* are associated with myeloid leukemia; however, *AML1-LAF4* was associated with T-ALL as well as *AML1-FGA7* in t(4;21)(q28;q22). These findings provide new insight into the common mechanism of *AML1* and *MLL* fusion proteins in the pathogenesis of ALL. Furthermore, we successfully applied bubble PCR to clone the novel *AML1-LAF4* fusion transcript. Bubble PCR is a powerful tool for detecting unknown fusion transcripts as well as genomic fusion points.

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Keywords: *AML1/RUNX1*; *LAF4*; T-cell acute lymphoblastic leukemia; *MLL*

Introduction

A large number of leukemias have been found to be associated with specific chromosomal aberrations. Recent studies have demonstrated that several chromosomal rearrangements and molecular abnormalities are strongly associated with distinct clinical subgroups and can predict clinical features and therapeutic responses (Rowley, 1999; Taki and Taniwaki, 2006). Some genes have been associated with recurrent rearrangements and have many fusion partner genes, such as *MLL* at 11q23, *TEL (ETV6)* at 12p13 and *NUP98* at 11p15; *AML1 (RUNX1, CBFA2)* at 21q22 is one of the most frequent targets of these chromosomal rearrangements in both acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) (Miyoshi *et al.*, 1991; Hayashi, 2000; Kurokawa and Hirai, 2003). To date, a number of in-frame fusion partners of *AML1* have been cloned: *YTHDF2* at 1p35 (Nguyen *et al.*, 2006), *ZNF687* at 1q21.2 (Nguyen *et al.*, 2006), *MDS1/EVI1* at 3q26 (Mitani *et al.*, 1994), *FGA7* at 4q28 (Mikhail *et al.*, 2004), *SH3D19* at 4q31.3 (Nguyen *et al.*, 2006), *USP42* at 7p22 (Paulsson *et al.*, 2006), *MTG8 (ETO, CBFA2T1)* at 8q22 (Erickson *et al.*, 1992; Miyoshi *et al.*, 1993), *FOG2* at 8q23 (Chan *et al.*, 2005), *TRPS1* at 8q24 (Asou *et al.*, 2007), *TEL (ETV6)* at 12p13 (Golub *et al.*, 1995), *MTG16* at 16q24 (Gamou *et al.*, 1998) and *PRDX4* at Xp22 (Zhang *et al.*, 2004). Most *AML1* translocations, except for *TEL-AML1*, are associated with AML, involving the N-terminus Runt domain and lacking the C-terminus transactivation domain (Kurokawa and Hirai, 2003). *AML1* fusion proteins are associated with leukemogenesis by dominantly interfering with normal *AML1*-mediated transcription and acting as a transcriptional repressor (Okuda *et al.*, 1998; Wang *et al.*, 1998). Clinically, patients with AML harboring t(8;21) in both children and adults show a high rate of complete remission, and its prognosis is considered better than that of patients with a normal karyotype or other chromosomal aberrations (Grimwade *et al.*, 1998).

In the present study, we analysed pediatric T-ALL with t(2;21)(q11;q22) and identified the *LAF4* gene,

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