Table 3. Results of immunohistochemistry and FISH analysis

Case no.	Immunophenotype of a low-grade (grade 1 or 2) FL component								FISH†		Immunophenotype of a DLBCL component‡								FISH		
	CD20	CD10	Bcl-2	Bcl-6	MUM1	CD30	CD5	CD138	IGH/BCL2	BCL6 translocation	CD20	CD10	Bcl-2	Bcl-6	MUM1	CD30	CD5	CD138	GC/non-GC	IGH/BCL2	BCL6 translocation
1	+	+	+	+	−	−	−	−	+		+	+	+	+	−	−	nt	−	GC	+	
2	+	+	+	−	−	nt	−	−	+		+	+	+	+	+	+	+	−	GC	+	
3	+	+	+	+	−	−	nt	−	nt		+	+	+	+	+	−	−	−	GC	+	
4	nt	nt	nt	nt	nt	nt	nt	nt	nt		+	+	+	+	−	−	−	−	GC	+	
5	+	+	+	+	−	−	nt	−	nt		+	nt	+	+	−	−	−	−	GC	nt	
6	+	+	+	+	−	−	−	−	+		+	−	+	−	+	+	−	−	nonGC	+	
7	+	+	+	+	−	−	nt	−	nt		+	−	+	+	+	−	−	−	nonGC	+	
8	+	+	+	+	−	nt	nt	−	+		+	+	+	+	−	−	−	−	GC	+	
9	+	+	+	+	−	−	−	−	+		+	−	nt	nt	nt	nt	nt	nt		+	
10	+	+	+	nt	nt	nt	nt	nt	nt		+	+	−	+	−	−	−	−	GC	+	
11	+	+	+	−	−	−	−	−	nt		+	+	+	−	−	−	−	−	GC	nt	
12	+	+	+	+	+	−	−	−	nt		+	+	+	+	+	−	−	−	GC	−	−
13	+	+	+	+	−	−	nt	−	+		+	+	+	+	−	−	−	−	GC	+	
14	+	nt	+	−	−	−	nt	−	nt		+	+	+	+	−	−	−	−	GC	+	
15	+	+	+	+	−	−	nt	−	+		+	+	+	+	−	nt	−	−	GC	+	
16	+	+	+	+	+	nt	−	nt	+		+	+	+	+	−	nt	−	nt	GC	+	
17	nt	nt	nt	nt	nt	nt	nt	nt	nt		+	+	+	nt	nt	nt	−	nt	GC	nt	
18	+	−	+	+	−	nt	−	nt	nt		+	−	+	+	+	nt	−	nt	nonGC	nt	
19	+	+	+	+	−	−	−	−	nt		+	−	+	nt	nt	nt	nt	nt		nt	
20	+	+	+	+	−	−	nt	−	nt		+	+	+	+	+	−	−	−	GC	+	
21	+	−	−	nt	nt	nt	nt	nt	−	nt	+	−	+	+	−	−	−	−	GC	−	
22	+	+	+	+	−	−	−	−	nt		+	+	+	+	−	−	−	−	GC	+	
23	+	−	+	+	−	−	nt	−	+		+	−	+	nt	nt	nt	−	nt		+	
24	+	+	+	+	−	−	−	−	nt		+	−	+	+	+	+	−	−	nonGC	+	
25	+	+	+	−	+	−	−	−	+		+	+	+	+	+	−	−	−	GC	+	
26	+	+	+	−	−	−	nt	−	+		+	+	+	+	−	nt	nt	nt	GC	+	
27	+	+	+	+	−	nt	nt	nt	+		+	nt	−	+	−	−	−	−	GC	+	
28	+	+	+	nt	nt	nt	nt	nt	+		+	+	+	nt	nt	nt	−	nt	GC	+	
29	+	nt	+	+	+	−	−	−	nt		+	−	+	+	+	+	−	−	nonGC	−	−
30	+	+	+	nt	nt	nt	−	nt	+		+	+	+	+	−	−	−	−	GC	+	
31	+	−	+	nt	nt	nt	nt	nt	nt		+	−	+	nt	−	nt	nt	nt		nt	
32	nt	nt	+	+	+	−	nt	−	nt		+	−	+	nt	+	+	−	nt	nonGC	+	
33	+	+	+	nt	nt	nt	−	nt	nt		+	+	+	nt	nt	−	−	nt	GC	nt	
34	+	+	−,+	+	−	−	−	−	nt		+	+	+	nt	−	nt	−	nt	GC	+	
35	+	+	+	+	−	−	−	−	nt		+	+	+	+	−	−	−	−	GC	+	
36	+	+	+	+	−	−	−	−	nt		+	+	−	+	−	−	−	−	GC	−	−
37	+	nt	+	nt	nt	−	nt	nt	−	nt	+	−	−	nt	nt	nt	−	nt		nt	
38	+	+	+	nt	nt	nt	−	nt	+		+	+	+	−	+	−	−	−	GC	+	
39	+	nt	+	nt	nt	−	nt	nt	nt		+	−	+	+	−	+	−	−	GC	−	+
40	+	+	+	+	−	−	nt	−	nt		+	+	+	−	−	−	−	−	GC	−	−
41	+	+	+	+	−	−	nt	−	nt		+	+	+	+	−	−	−	−	GC	+	
42	+	+	+	+	−	−	−	−	nt		+	−	+	+	−	−	−	nt	GC	nt	
43	+	−	+	nt	nt	nt	−	nt	+		+	+	+	nt	nt	nt	−	nt	GC	+	

†judged from the final biopsy specimen, ‡fluorescence *in situ* hybridization for IGH/BCL2 fusion.
DLBCL, diffuse large B-cell lymphoma; FISH, fluorescence *in situ* hybridization; FL, follicular lymphoma; GC, germinal center B-cell phenotype; nt, not tested.

Table 4. Summary of results of immunohistochemistry and FISH analysis

Antibody	FL component	DLBCL component	Gain/loss/no change
CD20	100% (40/40)	100% (43/43)	
CD10	86% (31/36)*	66% (27/41)†	1/6/29
Bcl-2	96% (42/44)	91% (38/42)	1/4/37
Bcl-6	84% (26/31)	88% (28/32)	4/2/20
MUM1	16% (5/31)	34% (12/35)	7/1/20
CD30	0% (0/28)	20% (6/30)	5/0/16
CD138	0% (0/28)	0% (0/28)	
CD5	0% (0/21)	3% (1/38)	1/0/19
GCB, non-GCB		84% (31/37), 16% (6/37)	
FISH: IGH/BCL2	89% (16/18)	82% (28/34)	

†excluding bone marrow specimens.
DLBCL, diffuse large B-cell lymphoma; FISH, fluorescence *in situ* hybridization; FL, follicular lymphoma; GCB, germinal center B-cell phenotype.

of FLs and 66% (27/41) of DLBCLs, representing a gain in one case, loss in six cases, and no change in 29 cases (including 21 positive cases and eight negative cases). Bone marrow specimens were excluded because only these materials showed extremely low CD10 expression. Among six cases of DLBCL showing loss of CD10, two were diagnosed as DLBCL several times (nos. 6 and 7), and both cases showed loss of CD10 expression between the first and second occasions. Bcl-2 and Bcl-6 were frequently expressed in both FL and DLBCL. Bcl-2 was positive in 96% (42/44) of FLs and 91% (38/42) of DLBCLs, representing a gain in one case, loss in four cases, and no change in 37 cases through transformation. Bcl-6 was positive in 84% (26/31) of FLs and 88% (28/32) of DLBCLs, representing a gain in four cases, loss in two cases, and no change in 20 cases through transformation. Among 32 DLBCL cases for which both Bcl-2 and Bcl-6 immunohistochemistry could be performed, 25 were Bcl-2+/Bcl-6+, four were Bcl-2+/Bcl-6–, 3 were Bcl-2–/Bcl-6+, and no case was Bcl-2–/Bcl-6–.

The postgerminal center B-cell and plasma cell marker MUM 1 was positive in 16% (5/31) of FLs and in 34% (12/35) of DLBCLs, representing a gain in seven cases, loss in one case, and no change in 20 cases (including four positive cases and 16 negative cases). CD30 was negative in all FLs and positive in 20% (6/30) of DLBCLs. Two cases of DLBCL with anaplastic morphology were positive for CD30. CD30 showed scattered expression in marginally and sparsely distributed large lymphoid cells of low-grade FL and FL grade 3, but no case showed positivity in over 30% of the cells. CD5 was negative in all FLs and positive in only one case (no. 2) of DLBCL. This positive case was FL grade 2 in an abdominal lymph node with a $CD10^+$/Bcl-2+/Bcl-6–/$CD5^-$/cyclin D1-immunophenotype and IGH/BCL2 fusion by FISH analysis initially, and showed transformation to centroblastic monomorphous DLBCL in the tonsil, revealing a $CD10^-$/Bcl-2+/Bcl-6+/$CD5^+$/cyclin D1-immunophenotype and IGH/BCL2 fusion by FISH analysis. In the one case of classical Hodgkin lymphoma after transformation from FL via DLBCL (no. 38), FL in the stomach and esophagus and DLBCL in a cervical lymph node had a $CD20^+$/$CD30^-$/$CD10^+$ phenotype and IGH/BCL2 fusion by FISH analysis, but Hodgkin/Reed-Sternberg cells in an inguinal lymph node had a $CD20^-$/$CD30^+$/$CD15^+$/$CD10^-$ phenotype, and were negative for EBER-1 *in situ* hybridization and positive for IGH/BCL2 fusion by FISH (Fig. 2).

Thirty-one (84%) DLBCLs were classified as GCB, and six (16%) DLBCLs were classified as non-GCB. Two cases of DLBCL (nos. 6 and 7) for which several sequential biopsies were taken were judged from the final biopsy specimen: these were non-GCB in the final DLBCL specimens, but had been GCB in the initial specimens.

FISH analysis. Paraffin-embedded sections were available for 18 FL cases and 34 DLBCL cases. IGH/BCL2 fusion was detected in 89% (16/18) of FL cases and in 82% (28/34) of DLBCL cases. In all six DLBCL cases without IGH/BCL2 fusion, BCL6 translocation was detected in one case (17%). Two FL cases without IGH/BCL2 fusion were not available paraffin-embedded sections.

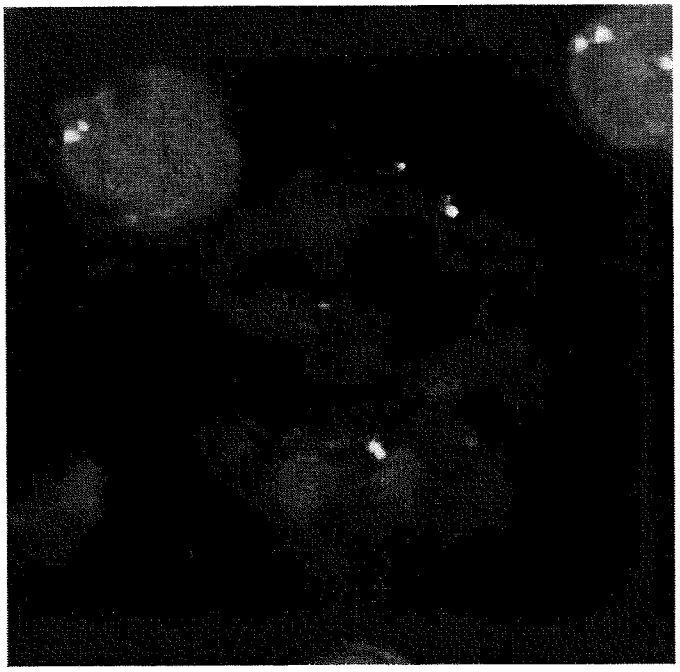

Fig. 2. The result of fluorescence *in situ* hybridization of classical Hodgkin lymphoma transformed from follicular lymphoma (FL). IGH and BCL2 fusion pattern with the LSI IGH Spectrum Green/LSI BCL2 Spectrum Orange Dual Fusion Translocation Probe (Vysis, Downers Grove, IL, USA). Two fusion IGH/BCL2 signals are present.

Statistical analysis. Only the initial treatment regimen was a significant prognostic factor: patients who received CHOP or R-CHOP showed a better outcome than patients who received other treatments ($P < 0.001$). No other significant prognostic factors were detected, including GCB *versus* non-GCB. However, patients with DLBCL showing CD30-positivity (six cases) did not die as a result of disease progression.

Discussion

As transformation of FL to DLBCL is currently the focus of widespread clinical and pathological interests, we studied the

doi: 10.1111/j.1349-7006.2008.00873.x

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] Richiter'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.[8,9] 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.

Acknowledgments

The authors would like to thank C. Kina and S. Miura for technical assistance with immunohistochemistry. This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor, and Welfare of Japan.

References

1 A clinical evaluation of the International Lymphoma Study Group Classification of Non-Hodgkin's Lymphoma. The non-Hodgkin's Lymphoma Classification Project. *Blood* 1997; **89**: 3909–18.
2 Horning SJ, Rosenberg SA. The natural history of initially untreated low-grade non-Hodgkin's lymphoma. *N Eng J Med* 1984; **311**: 1471–5.
3 Gallagher CJ, Gregory WM, Jones AE *et al.* Follicular lymphoma: prognostic factors for response and survival. *J Clin Oncol* 1986; **4**: 1470–80.
4 Harris NL, Ferry JA. Follicular lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*, 2nd edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2001; 823–53.
5 Alsabeh R, Medeiros LJ, Glackin C *et al.* Transformation of follicular lymphoma into CD30-large cell lymphoma with anaplastic cytologic features. *Am J Surg Pathol* 1997; **21**: 528–36.
6 Yano T, Jaffe ES, Longo DJ *et al.* MYC rearrangements in histologically progressed follicular lymphomas. *Blood* 1992; **80**: 758–67.
7 de Jong D, Voetdujk B, Bavestock G *et al.* Activation of the c-myc oncogene in a precursor B-cell blast crisis of follicular lymphoma, presenting as composite lymphoma. *N Eng J Med* 1998; **318**: 1373.
8 Brauninger A, Hansmann ML, Strickler JG *et al.* Identification of common germinal-center B-cell precursors in two patients with both Hodgkin's disease and non-Hodgkin's lymphoma. *N Eng J Med* 1999; **340**: 1239–47.
9 Marafioti T, Hummel M, Anagnostopoulos I *et al.* Classical Hodgkin's disease and follicular lymphoma originating from the same germinal center B cell. *J Clin Oncol* 1999; **17**: 3804–9.

10 Sander CA, Yano T, Clark HM *et al.* p53 mutation is associated with progression in follicular lymphomas. *Blood* 1993; **82**: 1994–2004.
11 Lo Coco F, Gaidano G, Louie DC *et al.* p53 mutations are associated with histologic transformation of follicular lymphoma. *Blood* 1993; **82**: 2289–95.
12 Pinyol M, Cobo F, Bea S *et al.* p16 (INF4a) gene inactivation by deletions, mutations, and hypermethylation is associated with transformed and aggressive variants of non-Hodgkin's lymphomas. *Blood* 1998; **91**: 2977–84.
13 Elenitoba-Johnson KS, Gascoyne RD, Lim MS *et al.* Homozygous deletions at chromosome 9p21 involving p16 and p15 are associated with histologic progression in follicle center lymphoma. *Blood* 1998; **91**: 4677–85.
14 Tilly H, Rossi A, Stamatoullas A *et al.* Prognostic value of chromosomal abnormalities in follicular lymphoma. *Blood* 1994; **84**: 1043–9.
15 Alizadeh AA, Eisen MB, Davis RE *et al.* Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000; **403**: 503–11.
16 Rosenwald A, Wright G, Chan WC *et al.* The use of molecular profiling to predict survival after chemotherapy for diffuse large B-cell lymphoma. *N Eng J Med* 2002; **346**: 1937–47.
17 Hans SP, Weisenburger DD, Greiner TC *et al.* Conformation of molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004; **103**: 275–82.
18 Davies AJ, Rosenwald A, Wright G *et al.* Transformation of follicular lymphoma to diffuse large B-cell lymphoma proceeds by distinct oncogenic mechanisms. *Br J Haematol* 2007; **136**: 286–93.
19 Godon A, Moreau A, Talmant P *et al.* Is t(14;18) (q32;q21) a constant finding in follicular lymphoma? An interphase FISH study on 63 patients. *Leukemia* 2003; **17**: 255–9.
20 Biagi JJ, Suymour JF. Insights into the molecular pathogenesis of follicular lymphoma arising from analysis of geographic variation. *Blood* 2002; **99**: 4265–75.
21 Sekiguchi N, Kobayashi Y, Yokota Y *et al.* Follicular lymphoma subgrouping by fluorescence in situ hybridization analysis. *Cancer Sci* 2005; **96**: 77–82.
22 Leoncini L, Delsol G, Gascoyne RD *et al.* Aggressive B-cell lymphomas: a review based on the workshop of the XI meeting of the European association for haematopathology. *Histopathology* 2005; **46**: 241–55.
23 Jaffe ES, Harris NL, Stein H *et al. World Health Organization Classification of Tumors, Pathology and Genetics, Tumours of Haematopoietic and Lymphoid Tissues.* Lyon: IARC Press, 2001; **162–167**: 171–4.
24 Dogan AMQ, Aiello A *et al.* Follicular lymphomas contain a clonally linked but phenotypically distinct neoplastic B-cell population in the interfollicular zone. *Blood* 1998; **91**: 4708–14.
25 Nunez G, London L, Hockenbery D *et al.* Deregulated Bcl-2 gene expression selectively prolongs survival of growth factor-deprived hemoopietic cell lines. *J Immunol* 1990; **144**: 3602–10.
26 Lai R, Arber DA, Chang KL *et al.* Frequency of bcl-2 expression in non-Hodtkin lymphoma: a study of 778 cases with comparison of marginal zone lymphoma and monocytoid B-cell hyperplasia. *Mod Pathol* 1998; **11**: 864–9.
27 Falini B, Fizzotti M, Pileri S *et al.* Bcl-6 protein expression in normal and neoplastic lymphoid tissues. *Ann Oncol* 1997; **8**: 101–4.
28 Peh SC, Shaminie J, Tai YC *et al.* The pattern and frequency of t(14;18) translocation and immunophenotype in Asian follicular lymphoma. *Histopathology* 2004; **45**: 501–10.
29 Anagnostopoulos I, Dallenbach F, Stein H. Diffuse large cell lymphomas. In: Knowles DM, ed. *Neoplastic Hematopathology*, 2nd edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2001; 860.
30 Mamane Y, Heylbroeck C, Genin P *et al.* Interferon regulatory factors: the next generation. *Gene* 1999; **237**: 1–14.
31 Natkunam Y, Warnke RA, Montgomery K *et al.* Analysis of MUM1/IRF4 protein expression using tissue microarrays and immunohistochemistry. *Mod Pathol* 2001; **14**: 686–94.
32 Falini B, Fizzotti M, Pucciarini A *et al.* A monoclonal antibody (MUM1p) detects expression of the MUM1/IRF4 protein in a subset of germinal center B cells, plasma cells, and activated T cells. *Blood* 2000; **95**: 2084–92.
33 Tsuboi K, Iida S, Inagaki H *et al.* MUM1/IRF4 expression as a frequent event in mature lymphoid malignancies. *Leukemia* 2000; **14**: 449–56.
34 Piris M, Gatter KC, Mason DY. CD30 expression in follicular lymphoma. *Histopathology* 1991; **18**: 25–9.
35 Yamaguchi M, Seto M, Okamoto M *et al.* De novo CD5$^+$ diffuse large B-cell lymphoma: a clinicopathologic study of 109 patients. *Blood* 2002; **99**: 815–21.
36 Manazza AD, Bonello L, Pagano M *et al.* Follicular origin of a subset of CD5$^+$ diffuse large B-cell lymphomas. *Am J Clin Pathol* 2005; **124**: 182–90.
37 Colomo L, Lopez-Guillermo A, Perales M *et al.* Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. *Blood* 2003; **101**: 78–84.

doi: 10.1111/j.1349-7006.2008.00873.x

Reprinted from
Jpn J Clin Oncol 2008;38(8)547–552
doi:10.1093/jjco/hyn069

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

Kazuhiro Sentani[1], Akiko Miyagi Maeshima[1], Junko Nomoto[2], Dai Maruyama[2], Sung-Won Kim[2], Takashi Watanabe[2], Yukio Kobayashi[2], Kensei Tobinai[2] and Yoshihiro Matsuno[1,3]

[1]Clinical Laboratory, [2]Hematology and Stem Cell Transplantation Divisions, National Cancer Center Hospital, Tokyo and [3]Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, Japan

Jpn J Clin Oncol 2008;38(8)547–552
doi:10.1093/jjco/hyn069

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

Kazuhiro Sentani[1], Akiko Miyagi Maeshima[1], Junko Nomoto[2], Dai Maruyama[2], Sung-Won Kim[2], Takashi Watanabe[2], Yukio Kobayashi[2], Kensei Tobinai[2] and Yoshihiro Matsuno[1,3]

[1]Clinical Laboratory, [2]Hematology and Stem Cell Transplantation Divisions, National Cancer Center Hospital, Tokyo and [3]Department of Surgical Pathology, Hokkaido University Hospital, Sapporo, Japan

Received March 31, 2008; accepted July 7, 2008; published online August 7, 2008

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

For reprints and all correspondence: Yoshihiro Matsuno, Department of Surgical Pathology, Hokkaido University Hospital, Kita 14 Nishi 5, Kita-ku, Sapporo 060-8648, Japan. E-mail: ymatsuno@med.hokudai.ac.jp

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^{TM}, and CD3, CD5, CD10 and cyclin D1 were stained using a DAKO autostainer plus^{TM}. 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. Misdraji 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	*H. pylori*
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.
[1]Categorization 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

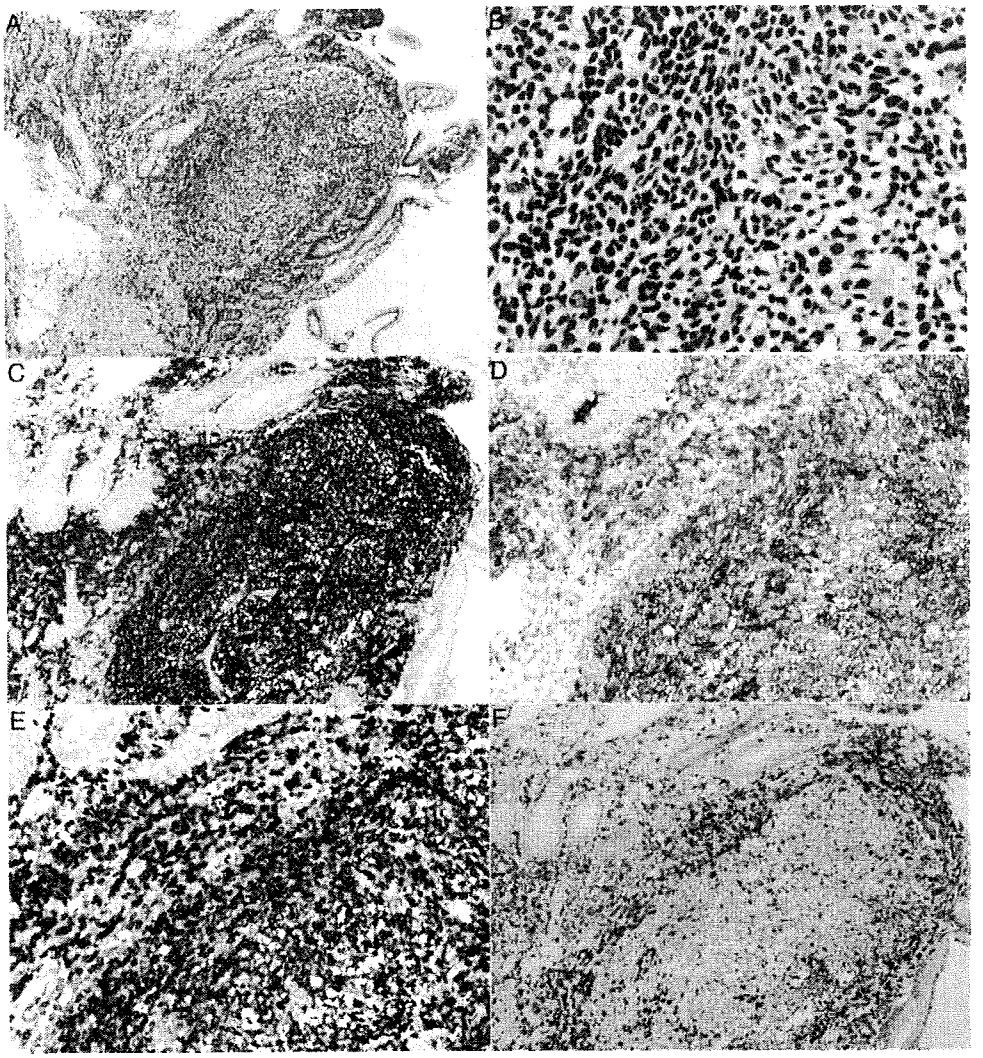

Figure 1. A case of primary duodenal follicular lymphoma, grade 1. The lesion shows well circumscribed follicles (A, HE, ×40), composed of a uniform population of small cleaved cells (B, HE, ×400). Immunohistochemically, CD20 (C, ×100), CD10 (D, ×200) and Bcl-2 (E, ×200) are positive in the follicular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

Funding

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health, Labor, and Welfare of Japan.

Conflict of interest statement

None declared.

References

1. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The Non-Hodgkin's Lymphoma Classification Project. *Blood* 1997;89:3909–18.
2. Aoki R, Karube K, Sugita Y, Nomura Y, Shimizu K, Kimura Y, et al. Distribution of malignant lymphoma in Japan. *Pathol Int* 2008;58: 174–82.
3. Mirza I, Macpherson N, Paproski S, Gascoyne RD, Yang B, Finn WG, et al. Primary cutaneous follicular lymphoma: an assessment of clinical, histopathologic, immunophenotypic, and molecular features. *J Clin Oncol* 2002;20:647–55.
4. Kojima M, Nakamura S, Ichimura K, Shimizu K, Itoh H, Masawa N. Follicular lymphoma of the salivary gland: a clinicopathological and molecular study of six cases. *Int J Surg Pathol* 2001;9:287–93.
5. Ferry JA, Fung CY, Zukerberg L, Lucarelli MJ, Hasserjian RP, Preffer FI, et al. Lymphoma of the ocular adnexa: a study of 353 cases. *Am J Surg Pathol* 2007;31:170–84.
6. Kosari F, Daneshbod Y, Parwaresch R, Krams M, Wacker HH. Lymphomas of the female genital tract: a study of 186 cases and review of the literature. *Am J Surg Pathol* 2005;29:1512–20.
7. Cirillo M, Federico M, Curci G, Tamborrino E, Piccinini L, Silingardi V. Primary gastrointestinal lymphoma: a clinicopathological study of 58 cases. *Haematologica* 1992;77:156–61.
8. Otter R, Bieger R, Kluin PM, Hermans J, Willemze R. Primary gastrointestinal non-Hodgkin's lymphoma in a population-based registry. *Br J Cancer* 1989;60:745–50.
9. Yoshino T, Miyake K, Ichimura K, Mannami T, Ohara N, Hamazaki S, et al. Increased incidence of follicular lymphoma in the duodenum. *Am J Surg Pathol* 2000;24:688–93.
10. Shia J, Teruya-Feldstein J, Pan D, Hegde A, Klimstra DS, Chaganti RS, et al. Primary follicular lymphoma of the gastrointestinal tract: a clinical and pathologic study of 26 cases. *Am J Surg Pathol* 2002;26:216–24.
11. Sato Y, Ichimura K, Tanaka T, Takata K, Morito T, Sato H, et al. Duodenal follicular lymphomas share common characteristics with mucosa-associated lymphoid tissue lymphomas. *J Clin Pathol* 2008;61:377–81.
12. Knutsen T. Cytogenetic mechanisms in the pathogenesis and progression of follicular lymphoma. *Cancer Surv* 1997;30:163–92.
13. Sekiguchi N, Kobayashi Y, Yokota Y, Kusumoto S, Tanimoto K, Watanabe T, et al. Follicular lymphoma subgrouping by fluorescence *in situ* hybridization analysis. *Cancer Sci* 2005;96:77–82.
14. Goodlad JR, Krajewski AS, Batstone PJ, McKay P, White JM, Benton EC, et al. Primary cutaneous follicular lymphoma: a clinicopathologic and molecular study of 16 cases in support of a distinct entity. *Am J Surg Pathol* 2002;26:733–41.
15. Jaffe ES, Harris NL, Stein H, Vardiman JW. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press 2001.
16. Misdraji J, Fernandez del Castillo C, Ferry JA. Follicle center lymphoma of the ampulla of Vater presenting with jaundice: report of a case. *Am J Surg Pathol* 1997;21:484–8.
17. Toyoda H, Yamaguchi M, Nakamura S, Nakamura T, Kimura M, Suzuki H, et al. Regression of primary lymphoma of the ampulla of Vater after eradication of *Helicobacter pylori*. *Gastrointest Endosc* 2001;54:92–6.
18. Kim BK, Surti U, Pandya A, Cohen J, Rabkin MS, Swerdlow SH. Clinicopathologic, immunophenotypic, and molecular cytogenetic fluorescence *in situ* hybridization analysis of primary and secondary cutaneous follicular lymphomas. *Am J Surg Pathol* 2005;29:69–82.
19. Goodlad JR, MacPherson S, Jackson R, Batstone P, White J. Extranodal follicular lymphoma: a clinicopathological and genetic analysis of 15 cases arising at non-cutaneous extranodal sites. *Histopathology* 2004;44:268–76.
20. Aguiar-Bujanda D, Quinones-Morales I, Camacho-Galan R, Llorca-Martinez I, Rivero-Vera JC, Bohn-Sarmiento U, et al. Primary duodenal follicular lymphoma successfully treated with rituximab. *Clin Transl Oncol* 2007;9:471–2.

Int J Hematol (2008) 88:443–447
DOI 10.1007/s12185-008-0168-2

CASE REPORT

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

Hiroki Yokoyama · Takashi Watanabe · Dai Maruyama · Sung-Won Kim · Yukio Kobayashi · Kensei Tobinai

Received: 4 October 2007 / Revised: 23 June 2008 / Accepted: 6 September 2008 / Published online: 15 October 2008

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 jiroveci* 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.

H. Yokoyama · T. Watanabe (✉) · D. Maruyama · S.-W. Kim · Y. Kobayashi · K. Tobinai
Hematology and Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: takawata@ncc.go.jp

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

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-stanced. 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 × 10^4 μL^{-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.

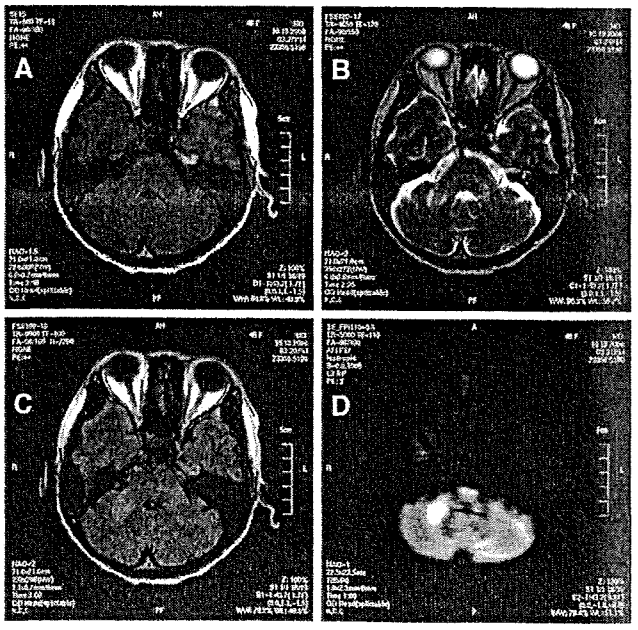

Fig. 1 October 2006: magnetic resonance imaging showing mild cerebellar atrophy in a T1-weighted image (**a**), white matter abnormality in a T2-weighted image (**b**), white matter abnormality in fluid-attenuated inversion recovery (**c**), and intracellular edema in a diffusion-weighted image (**d**)

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.

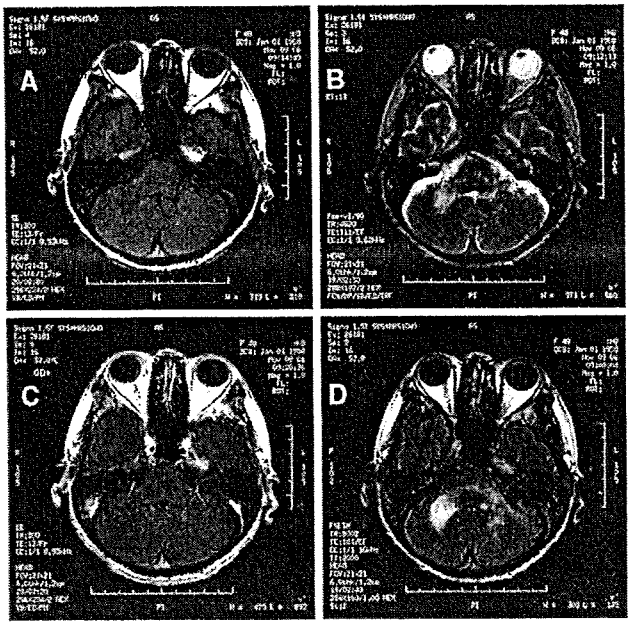

Fig. 2 December 2006: magnetic resonance imaging showing cerebellar atrophy in a T1-weighted image (**a**), white matter abnormality and expansion into the region of the adjacent brainstem in a T2-weighted image (**b**), cerebellar atrophy without areas of enhancement in a T1-weighted image (**c**),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

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 possiblity 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

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.

Acknowledgment This study was supported in part by grants from the Ministry of Health, Labor, and Welfare, Japan.

References

1. Koralnik IJ. New insights into progressive multifocal leukoencephalopathy. Curr Opin Neurol. 2004;17:365–70.
2. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. Lancet. 1971;1:1257–60.
3. Berger JR, Major EO. Progressive multifocal leukoencephalopathy. Semin Neurol. 1999;19:193–200.
4. Vidarsson B, Mosher DF, Salamat MS, Isaksson HJ, Onundarson PT. Progressive multifocal leukoencephalopathy after fludarabine therapy for low-grade lymphoproliferative disease. Am J Hematol. 2002;70:51–4.
5. García-Suárez J, de Miquel D, Krsnik I, Banas H, Arribas I, Burqaleta C. Changes in the natural history of progressive

multifocal leukoencephalopathy in HIV-negative lymphoproliferative disorders: Impact of novel therapies. Am J Hematol. 2005;80:271–81.
6. Gasnault J, Kahraman M, de Herve MG, Durali D, Delfraissy JF, Taoufik Y. Critical role of JC virus-specific CD4 T-cell responses in preventing progressive multifocal leukoencephalopathy. AIDS. 2003;17:1443–9.
7. Major EO, Amemiya K, Tormatore CS, Houff SA, Berger JR. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. Clin Microbiol Rev. 1992;5:49–73.
8. Fong IW, E Toma. The natural history of progressive multifocal leukoencephalopathy in patients with AIDS Canadian PML Study Group. Clin Infect Dis. 1995;2:1305–10.
9. Smith DK, Neal JJ, Holmberg SD. Unexplained opportunistic infections and $CD4^+$ T lymphocytopenia without HIV infection: an investigation of cases in the United States. The Centers for Disease Control Idiopathic $CD4^+$ T lymphocytopenia Task Force. N Engl J Med. 1993;328:373–9.
10. Goldberg SL, Pecora AL, Alter RS, et al. Unusual viral infections (progressive multifocal leukoencephalopathy and cytomegalovirus disease) after high-dose chemotherapy with autologous blood stem cell rescue and peritransplantation rituximab. Blood. 2002;99:1486–8.
11. Gibson PE, Gardner SD, Field AM. Use of a molecular probe for detecting JCV DNA directly in human brain material. J Med Virol. 1986;18:87–95.
12. Fong IW, Britton CB, Luinstra KE, Toma E, Mahony JB. Diagnostic value of detecting JC virus DNA in cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. J Clin Microbiol. 1995;33:484–6.
13. Whiteman ML, Post MJ, Berger LG, Tate LG, Bell MD, Limonte LP. Progressive multifocal leukoencephalopathy in 47 HIV-seropositive patients: Neuroimaging with clinical and pathologic correlation. Radiology. 1993;187:233–40.
14. Post MJ, Yiannoutsos C, Simpson D, et al. Progressive multifocal leukoencephalopathy in AIDS: are there any MR findings useful to patient management and predictive of patient survival? AIDS Clinical Trials Group, 243 Team. AJNR Am J Neuroradiol. 1999;20:1896–906.
15. Hall CD, Dafni U, Simpson D, et al. Failure of cytarabine in progressive multifocal leukoencephalopathy associated with human immunodeficiency virus infection. AIDS Clinical Trials Group 243 Team. N Engl J Med. 1998;338:1345–51.
16. Dworkin MS. A review of progressive multifocal leukoencephalopathy in persons with and without AIDS. Curr Clin Top Infect Dis. 2002;22:181–95.
17. Hou J, Major EO. The efficacy of nucleoside analogs against JC virus multiplication in a persistently infected human fetal brain cell line. J Neurovirol. 1998;4:451–6.
18. Bauer WR, Turel AP Jr, Johnson KP. Progressive multifocal leukoencephalopathy and cytarabine: remission with treatment. JAMA. 1973;226:174–6.
19. Marriott PJ, O'Brien MD, Mackenzie IC, Janota I. Progressive multifocal leucoencephalopathy: remission with cytarabine. J Neurol Neurosurg Psychiatry. 1975;38:205–9.
20. O'Riordan T, Daly PA, Hutchinson M, Shattock AG, Gardner SD. Progressive multifocal leukoencephalopathy—remission with cytarabine. J Infect. 1990;20:51–4.
21. Aksamit AJ. Treatment of non-AIDS progressive multifocal leukoencephalopathy with cytosine arabinoside. J Neurovirol. 2001;7:386–90.
22. Koralnik IJ, Schellingerhout D, Frosch MP. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 14–2004. A 66-year-old man with progressive neurologic deficits. N Engl J Med. 2004;350:1882–93.

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

Yoshiaki Ogawa,[1,4] Kensei Tobinai,[2] Michinori Ogura,[3] Kiyoshi Ando,[1] Takahide Tsuchiya,[1] Yukio Kobayashi,[2] Takashi Watanabe,[2] Dai Maruyama,[2] Yasuo Morishima,[3] Yoshitoyo Kagami,[3] Hirofumi Taji,[3] Hironobu Minami,[4] Kuniaki Itoh,[4] Masanobu Nakata[4] and Tomomitsu Hotta[1]

[1]Department of Hematology and Oncology, Tokai University School of Medicine, 143, Shimokasuya, Isehara, Kanagawa, 259-1193; [2]Hematology and Stem Cell Transplantation Division, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-ku, Tokyo, 104-0045; [3]Department of Hematology and Cell Therapy, Aichi Cancer Center, 1-1, Kanokoden, Chikusa-ku, Nagoya, Aichi, 464-8681; [4]Division of Oncology and Hematology, National Cancer Center Hospital East, 6-5-1, Kashiwanoha, Kashiwa, Chiba, 277-8577, Japan

(Received July 22, 2007/Revised September 6, 2007/Accepted September 9, 2007/Online publication October 29, 2007)

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 ≥ 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 predonisone, 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 ≥ 60; 20–74 years of age; adequate bone marrow function (absolute neutrophil count ≥ 1000/mm³, platelets ≥ 75 000/mm³, and hemoglobin ≥ 8 g/dL),

*To whom correspondence should be addressed.
E-mail: yoshioga@is.icc.u-tokai.ac.jp

doi: 10.1111/j.1349-7006.2007.00638.x

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 ≥ 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/m^2-dose group, and six patients each in the 1.0 and 1.3 mg/m^2-dose groups. DLT was defined as ≥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 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/m^2 group and six in the 1.0 mg/m^2 group, and no DLT were observed at any dose level. In the 1.3 mg/m^2 group, DLT (grade 3 febrile neutropenia) occurred in one of the six patients. Therefore, 1.3 mg/m^2 was determined to be the RD in subsequent phase II, in which 18 patients were enrolled.

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

Adverse events. The safety analysis dataset consisted of all patients who received at least one dose of bortezomib (34 patients). Adverse events observed in ≥20% of patients are shown in Table 2. The events observed at a high frequency (≥50%) were lymphopenia, neutropenia, leukopenia, thrombocytopenia, anemia, asthenia, diarrhea, constipation, nausea, anorexia, and pyrexia. At least one ≥grade 3 adverse event was observed in 88% of the patients. Major ≥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/m²)	0.7		1.0		1.3		All			
No. of Patients	($n = 3$)		($n = 6$)		($n = 25$)		($n = 34$)		Total	%
NCI-CTC grade	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 pancreatolithiasis, 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

doi: 10.1111/j.1349-7006.2007.00638.x

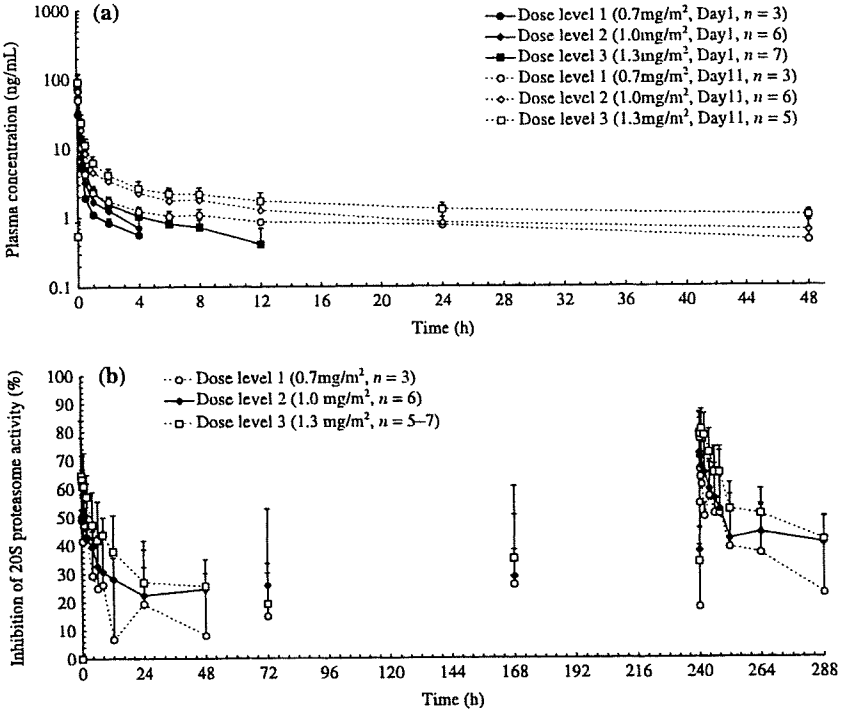

Fig. 1. (a) Plasma bortezomib concentrations (mean + SD). (b) Inhibition of blood 20S proteasome activity (mean + SD).

Table 3. Pharmacokinetic parameters (non-compartmental analysis)

Parameter	Day	Dose (mg/m²) 0.7 ($n = 3$)	1.0 ($n = 6$)	1.3 ($n = 5$–7)†
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	1	14.04 ± 0.70	28.58 ± 24.86	46.50 ± 19.89
(ng · h/mL)	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	1	83.35 ± 10.52	105.41 ± 75.66	51.97 ± 18.99
(L/h)	11	11.77 ± 4.67	19.63 ± 14.50	12.10 ± 3.73
V_z (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_{ss} (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

†Day 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; C_0, plasma concentration at the end of administration; C_0 ratio, C_0 on day 11/C_0 on day 1; V_z, the apparent volume of distribution during the terminal phase; V_{ss}, 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 (C_0, 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 C_0 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