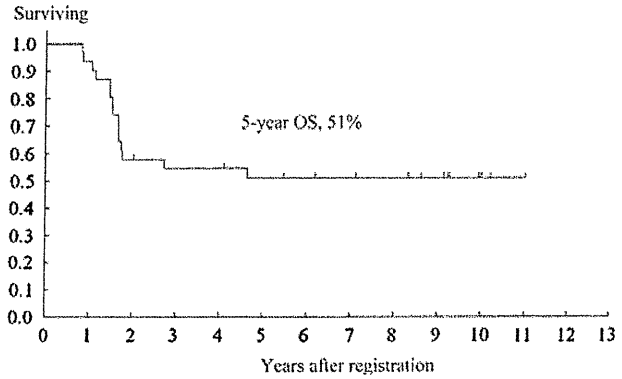

Figure 4. OS of the 31 patients who received stem cell transplantation in first complete remission. Estimated probability of OS at 5 years was 51% (95% CI, 32–67%).

Table 4. Univariate analysis of factors affecting overall survival

Variables	Category	Distribution (*n*)	*P* value[a]	HR[b] (95% CI)
Age	40/>40	60/48	0.02	1.702 (1.088–2.663)
Gender	M/F	54/54	0.488	0.853 (0.545–1.335)
Disease	ALL/LBL	96/12	0.434	0.747 (0.359–1.553)
PS	0, 1/2–4	84/24	0.554	0.847 (0.488–1.469)
WBC count ($\times 10^4/\mu l$)	<1.0/1.0	49/56	0.092	1.481 (0.938–2.338)
Immunophenotype	Non-T/T	85/23	0.132	0.64 (0.358–1.143)
CRP (mg/dl)	0.3/>0.3	25/77	0.078	1.719 (0.942–3.139)
Ph or t(4;11)	−/+	82/26	0	2.567 (1.568–4.203)

HR, hazard ratio; CRP, C-reactive protein; Ph, Philadelphia chromosome.
[a]*P* value by log-rank test.
[b]Hazard ratio by the Cox proportional hazards regression analysis.

(CRP: ≤0.3 versus >0.3 mg/dl) and chromosome anomaly of Ph chromosome or t(4;11) (not detected versus detected); small *P* values in univariate analysis using the log-rank test were obtained in the following subsets: age ≤40 years versus older and chromosome anomaly of Ph chromosome and t(4;11) in not detected versus detected (Table 4). Multivariate analysis showed that only the detection of Ph chromosome or t(4;11) was significantly associated with shortened survival ($P = 0.0197$), although age >40 years ($P = 0.0654$) and male sex ($P = 0.0724$) showed tendencies toward shorter survival. As shown in Figs 5 and 6, the 5-year OS of patients with Ph chromosome-negative ALL and AYA patients aged 25 years or younger was 37% (95% CI; 26–48%) and 41% (95% CI; 26–55%), respectively.

Toxicity

The number of patients with treatment-related death (TRD) was six; three patients died in the induction phase and the

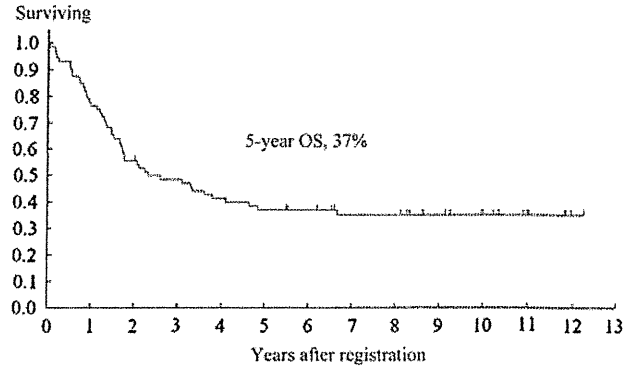

Figure 5. OS of patients with Philadelphia chromosome-negative ALL. Estimated probability at 5 years was 37% (95% CI, 26–48%).

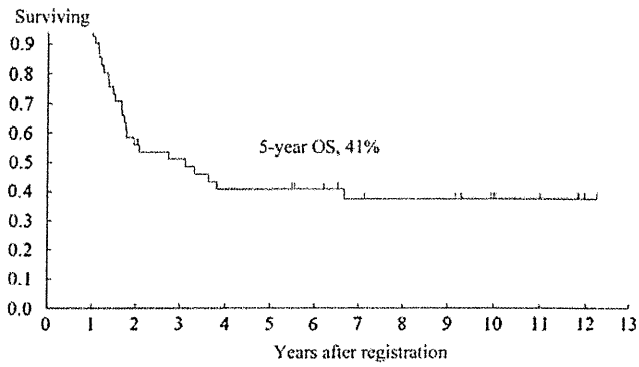

Figure 6. OS of adolescent and young adult ALL patients aged 25 years or younger. Estimated probability at 5 years was 41% (95% CI, 26–55%).

remaining three in the consolidation A, CNS prophylaxis and SCT phases, respectively. The causes of death were infection in four patients, TRD in one and hemorrhage in one. The numbers of patients who developed non-hematologic toxicities of Grade 3 or greater are shown in Table 5. The major non-hematologic toxicities of Grade 3 or greater in induction therapy were ALT (GPT) elevation (28%), infection (14%), pulmonary event (6%) and peripheral neuropathy (2%). Among the 96 patients treated with consolidation A, 7 (7%) developed ALT (GPT) elevation and 6 (6%) infection of Grade 3 or greater. Pancreatitis (Grade 4), and hyperglycemia (Grade 4) and diarrhea (Grade 3) occurred in one patient each after administration of L-asp in consolidation B, which lead to the exclusion of L-asp from consolidation B. Thereafter, no serious adverse events associated with L-asp were observed.

DISCUSSION

We conducted a multicenter Phase II study of adult ALL and LBL, JCOG9402, to evaluate an intensified post-remission chemotherapy. The primary endpoint was a 5-year PFS of 28%, which almost achieved an estimated one of 30%. It is, however, unsatisfactory in terms of similar results of 5-year OS (29%) and the %CR to our previous Phase II

Table 5. Non-hematologic toxicities of Grade 3 or greater

Regimen	Induction (*n*, %)	Consolidation A (*n*, %)	Consolidation B (*n*, %)
Total number of treated patients	115	98	83
Total number of evaluated courses	113	96	81
Toxicity			
GPT elevation	31 (28%)	7 (7%)	12 (15%)
GOT elevation	12 (11%)	2 (2%)	3 (4%)
Fever (non-infectious)	5 (4%)	3 (3%)	3 (4%)
Infection	16 (14%)	6 (6%)	5 (6%)
Pulmonary	7 (6%)	0 (0%)	0 (0%)
Diarrhea	5 (4%)	0 (0%)	3 (4%)
Constipation	3 (3%)	0 (0%)	1 (1%)
Cardiac dysfunction	3 (3%)	0 (0%)	0 (0%)
Arrhythmia	2 (2%)	0 (0%)	0 (0%)
Peripheral neuropathy	2 (2%)	0 (0%)	0 (0%)

GPT, glutamic pyruvic transaminase; GOT, glutamic oxaloacetic transaminase.

study, JCOG9004 (13), despite of an intensified chemotherapy. In JCOG9402, 81% of all eligible patients achieved CR. When compared with the %CR (83%) in JCOG9004, the %CR was almost the same. These results are equivalent to those from other multicenter trials (6,9–11). In JCOG9402, Ara-C was incorporated into consolidation A to intensify the consolidation phase with the support of G-CSF. Between consolidation and maintenance therapy, both CNS prophylaxis with interim maintenance therapy and intensification therapy were also incorporated. In maintenance therapy, long-term administration of MTX and 6-MP was combined with the intermittent use of VDS, CPA, PSL and DXR. In JCOG9402, five patients (5%) died during the protocol chemotherapy, whereas in the previous JCOG9004, using a similar strategy to JCOG9402 in view of the intensified induction and post-remission chemotherapy with the support of G-CSF, 17 patients (12%) died during the protocol chemotherapy. These results suggest that the intensified induction and post-remission chemotherapy in JCOG9402, especially including the revised consolidation B regimen excluding L-asp, could be performed more safely than those in JCOG9004. Compared with the important role of L-asp in the treatment of pediatric ALL, that of adult ALL might be limited partly because L-asp is thought to be more toxic especially in elderly patients than in pediatric patients (30). Regarding the long-term outcome, however, the median survival time in JCOG9402 (21 months) did not improve compared with JCOG9004 (26 months) (13), and the estimated probability of survival at 5 years (29%) also did not improve. Thus, it is unlikely that the strategies of JCOG9402, especially further intensification of post-remission chemotherapy, improved the overall therapeutic results, when compared with JCOG9004 (13).

In the univariate analysis of prognostic factors affecting OS, older age and chromosomal abnormality of Ph chromosome or t(4;11) were unfavorable factors. In recent years, regimens for pediatric ALL have been applied for AYA ALL (31). As far as AYA in our study, the 5-year OS (41%) was unsatisfactory compared with the previous AYA trials with pediatric regimens (31). The proportion of patients with Ph chromosome anomaly (24/108, 22%) was not different from other reports, and they showed an unfavorable prognosis, similar to other studies (3,6,10,11,16,17,32). Since it was not planned to analyze the fusion gene of *BCR-ABL* in the present study, there remains the possibility that a fraction of patients who were judged not to have Ph chromosome by conventional cytogenetics may have the fusion gene of *BCR-ABL*, as Jinnai precisely discussed in the JALSG-ALL97 study (17). The 5-year OS of Ph chromosome-negative ALL patients (37%) appears to be unsatisfactory, comparing with recent survival improvement of Ph chromosome-positive ALL (Ph+ ALL) patients with imatinib (32). It is, however, not conclusive because our study period is different from those of other studies, in which more allogeneic SCTs are conducted and imatinib is available (32). CRP did not have an impact in the present study, in contrast to the authors' previous trial, JCOG8702 (12). Female sex was not a significant prognostic factor different from JCOG9004 (13). Similar to JCOG9004, leukocytes at initial presentation were not significant. Other prognostic factors identified in this study showed consistent results with previous reports, although there were some discrepancies. In an exploratory multivariate analysis (data not shown), chromosomal abnormality was the only significant unfavorable prognostic factor. Although age of >40 years and male sex were statistically marginal, both are considered to have a clinically meaningful impact on poor outcome (hazard ratio, 1.604 in >40 years and 1.582 in male). Patients with LBL did not show a more favorable prognosis than those with ALL, as shown in Table 4; however, this is not conclusive because of the small number of LBL patients ($n = 12$) enrolled in the present study.

Because there is considerable heterogeneity in the stem cell source, conditioning regimen etc., in the 31 patients who underwent SCT in first CR, it is impossible to draw meaningful conclusions; however, the results of OS suggest that SCT, particularly allogeneic SCT, in first CR is worthy of further investigation as well as JCOG9004 (13). In the recent meta-analysis, allogeneic SCT proved to be superior to autologous SCT or chemotherapy for patients with ALL in first CR (33).

The therapeutic outcome of JCOG9402 is still unsatisfactory. One of the plausible reasons for the unfavorable therapeutic outcome is age. In the present study, the median age of the 108 eligible patients was 33.5 years, and that of the 96 eligible patients with ALL was 38.5 years. When this was

compared with the median age of 25–40 years in the reported results (3,6,9–12,16,32), the median age in the present study, especially that of ALL, is rather older. Considering that age is a strong prognostic factor in most studies of adult ALL and LBL (34,35), the therapeutic results in this study could be worse. In addition, the median age of eligible patients in the present JCOG9402 study (33.5 years) was lower than in JCOG9004 (41 years). Therefore, it seems that intensification of induction and post-remission chemotherapy by available agents with the support of G-CSF for adult ALL and LBL had reached its limit (36). To further improve the therapeutic results, application of a dose-intensive pediatric regimen (7,37–40), incorporation of effective new agents such as nelarabine for T-ALL and T-LBL (41), imatinib and new tyrosine kinase inhibitors for Ph+ ALL (42–47) and other new targeted agents including monoclonal antibodies (48) may be needed, in addition to the risk-adapted SCT (48), considering the status of minimal residual disease (MRD) (48,49).

In conclusion, JCOG9402 did not show improvement in long-term follow-up results in adult patients with ALL and LBL when compared with the investigators' latest historical control, JCOG9004, although intensified post-remission chemotherapy, especially its revised version excluding L-asp from the consolidation B regimen, was performed safely in most patients with an achievement of the primary endpoint of estimated 5-year PFS. To further improve the therapeutic outcomes of adult patients with ALL and LBL, new strategies including that for non-AYA ALL with Ph-negative chromosome using pediatric regimens, MRD-guided strategy and developing of new agents such as liposomal-doxorubicin (50), peg-asparaginase (51) and clofarabine (52) must be considered.

Acknowledgements

We are greatly appreciative of all members of JCOG-LSG for their excellent cooperation in this long-term follow-up study. We thank the treating physicians, nurses, laboratory technicians and patients. We wish to thank Ms Yuko Watanabe (JCOG Data Center) for data management.

Funding

This study was supported by Grants-in-Aid for Cancer Research from the Ministry of Health, Labor and Welfare of Japan (5S-1, 8S-1, 11S-1, 11S-4, 14S-1, 14S-4, 17S-1, 17S-5, 20S-1, 20S-6, 1-1, 4-5, 7-29, 9-10) and by the National Cancer Center Research and Development Fund (23-A-16, 23-A-17) (1993–present).

Conflict of interest statement

None declared.

References

1. Swerdlow SH, Campo E, Harris NL, et al., editors. *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon: IARC Press 2008.
2. Slater DE, Mertelsmann R, Koziner B, et al. Lymphoblastic lymphoma in adults. *J Clin Oncol* 1986;4:57–67.
3. Larson RA, Dodge RK, Burns CP, et al. A five-drug remission induction regimen with intensive consolidation for adults with acute lymphoblastic leukemia: Cancer and Leukemia Group B study 8811. *Blood* 1995;85:2025–37.
4. Hoelzer D, Gokbuget N, Digel W, et al. Outcome of adult patients with T-lymphoblastic lymphoma treated according to protocols for acute lymphoblastic leukemia. *Blood* 2002;99:4379–85.
5. Thomas DA, O'Brien S, Cortes J, et al. Outcome with hyper-CVAD regimens in lymphoblastic lymphoma. *Blood* 2004;104:1624–30.
6. Kantarjian H, Thomas D, O'Brien S, et al. Long-term follow-up results of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (Hyper-CVAD), a dose-intensive regimen, in adult acute lymphocytic leukemia. *Cancer* 2004;101:2788–801.
7. Huguet F, Leguay T, Raffoux E, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. *J Clin Oncol* 2009;27:911–8.
8. Rivera GK, Pinkel D, Simone JV, Hancock ML, Crist WM. Treatment of acute lymphoblastic leukemia—30 years' experience at St. Jude Children's Research Hospital. *N Engl J Med* 1993;329:1289–95.
9. Hoelzer D, Thiel E, Loftier H, et al. Prognostic factors in a multicenter study for treatment of acute lymphoblastic leukemia in adults. *Blood* 1988;71:123–31.
10. Larson RA, Dodge RK, Linker CA, et al. A randomized controlled trial of filgrastim during remission induction and consolidation chemotherapy for adults with acute lymphoblastic leukemia: CALGB study 9111. *Blood* 1998;92:1556–64.
11. Thomas X, Boiron JM, Huguet F, et al. Outcome of treatment in adults with acute lymphoblastic leukemia: analysis of the LALA-94 trial. *J Clin Oncol* 2004;22:4075–86.
12. Kobayashi T, Tobinai K, Shimoyama M, et al. Long-term follow-up results of adult patients with acute lymphocytic leukemia or lymphoblastic lymphoma treated with short-term, alternating non-cross-resistant chemotherapy: Japan Clinical Oncology Group study 8702. *Jpn J Clin Oncol* 1999;29:340–8.
13. Tobinai K, Takeyama K, Arima F, et al. Phase II study of chemotherapy and stem cell transplantation for adult acute lymphoblastic leukemia or lymphoblastic lymphoma: Japan Clinical Oncology Group study 9004. *Cancer Sci* 2007;98:1350–7.
14. Tanimoto M, Miyawaki S, Ino T, et al. Response-oriented individualized induction therapy followed by intensive consolidation and maintenance for adult patients with acute lymphoblastic leukemia: the ALL-87 study of the Japan Adult Leukemia Study Group (JALSG). *Int J Hematol* 1998;68:421–9.
15. Ueda T, Miyawaki S, Asou N, et al. Response-oriented individualized induction therapy with six drugs followed by four courses of intensive consolidation, 1 year maintenance and intensification therapy: the ALL90 study of the Japan Adult Leukemia Study Group. *Int J Hematol* 1998;68:279–89.
16. Takeuchi J, Kyo T, Naito K, et al. Induction therapy by frequent administration of doxorubicin with four other drugs, followed by intensive consolidation and maintenance therapy for adult acute lymphoblastic leukemia: the JALSG ALL93 study. *Leukemia* 2002;16:1259–66.
17. Jinnai I, Sakura T, Tsuzuki M, et al. Intensified consolidation therapy with dose-escalated doxorubicin did not improve the prognosis of adults with acute lymphoblastic leukemia: the JALSG-ALL97 study. *Int J Hematol* 2010;92:490–502.
18. Fiere D, Lepage E, Sebban C, et al. Adult acute lymphoblastic leukemia: a multicentric randomized trial testing bone marrow transplantation as postremission therapy: the French Group on therapy for adult acute lymphoblastic leukemia. *J Clin Oncol* 1993;11:1990–2001.
19. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of acute leukemias. *Br J Haematol* 1976;33:451–8.
20. Bennett JM, Catovsky D, Daniel MT, et al. Proposal for the recognition of minimally differentiated acute myeloid leukaemia (AML-M0). *Br J Haematol* 1991;78:325–9.

21. The Non-Hodgkin's Lymphoma Pathologic Classification Project: National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphoma: summary and description of a Working Formulation for clinical usage. *Cancer* 1982;49:2112–35.
22. Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *Am J Clin Oncol* 1982;5:649–55.
23. Shimoyama M, Fukuda H, Saijo N, Yanaguchi N. Japan Clinical Oncology Group (JCOG). *Jpn J Clin Oncol* 1998;28:158–62.
24. Tutschka PJ, Copelan EA, Klein JP. Bone marrow transplantation for leukemia following a new busulfan and cyclophosphamide regimen. *Blood* 1987;70:1382–8.
25. Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med* 1986;314:729–35.
26. Morishima Y, Morishita Y, Tanimoto M, et al. Low incidence of acute graft-versus-host disease by the administration of methotrexate and cyclosporine in Japanese leukemia patients after bone marrow transplantation from human leukocyte antigen compatible siblings; possible role of genetic homogeneity. *Blood* 1989;74:2252–6.
27. *WHO Handbook for Reporting Results of Cancer Treatment.* WHO offset publication no. 48. Geneva: World Health Organization 1979.
28. Tobinai K, Kohno A, Yasuhiro S, et al. Toxicity grading criteria of the Japan Clinical Oncology Group. *Jpn J Clin Oncol* 1993;23:250–7.
29. Makuch RW, Simon RM. Sample size considerations for non-randomized comparative studies. *J Chr Dis* 1980;33:175–81.
30. Douer D. Is asparaginase a critical component in the treatment of acute lymphoblastic leukemia? *Best Pract Res Clin Haematol* 2008;21:647–58.
31. Wendy S. Adolescents and young adults with acute lymphoblastic leukemia. *Hematology Am Soc Hematol Educ Program* 2010;2010:489–93.
32. Kantarjian H, O'Brien S, Smith TL, et al. Results of treatment with Hyper-CVAD, a dose-intensive regimen, in adult acute lymphocytic leukemia. *J Clin Oncol* 2000;18:547–61.
33. Ram R, Gafter-Gvili A, Vidal L, et al. Management of adult patients with acute lymphoblastic leukemia in first complete remission: systematic review and meta-analysis. *Cancer* 2010;116:3447–57.
34. Marks DI. Treating the 'older' adult with acute lymphoblastic leukemia. *Hematology Am Soc Hematol Educ Program* 2010;2010:13–20.
35. Larson RA. Acute lymphoblastic leukemia: older patients and newer drugs. *Hematology Am Soc Hematol Educ Program* 2005;131–6.
36. Bassan R. Randomized clinical trials in adult acute lymphoblastic leukemia: which is the question? *Haematologica* 1998;83:193–6.
37. DeAngelo DJ. The treatment of adolescents and young adults with acute lymphoblastic leukemia. *Hematology Am Soc Hematol Educ Program* 2005;123–30.
38. Stock W, La M, Sanford B, et al. What determines the outcomes for adolescents and young adults with acute lymphoblastic leukemia treated on cooperative group protocols? A comparison of Children's Cancer Group and Cancer and Leukemia Group B studies. *Blood* 2008;112:1646–54.
39. Litzow MR. Evolving paradigms in the therapy of Philadelphia chromosome-negative acute lymphoblastic leukemia in adults. *Hematology Am Soc Hematol Educ Program* 2009;362–70.
40. Nachman JB, La MK, Hunger SP, et al. Young adults with acute lymphoblastic leukemia have an excellent outcome with chemotherapy alone and benefit from intensive postinduction treatment: a report from the Children's Oncology Group. *J Clin Oncol* 2009;27:5189–94.
41. Berg SL, Blaney SM, Devidas M, et al. Phase II study of nelarabine (compound 506U78) in children and young adults with refractory T-cell malignancies: a report from the Children's Oncology Group. *J Clin Oncol* 2005;23:3376–82.
42. Yanada M, Takeuchi J, Sugiura I, et al. High complete remission rate and promising outcome by combination of imatinib and chemotherapy for newly diagnosed BCR-ABL-positive acute lymphoblastic leukemia: a phase II study by the Japan Adult Leukemia Study Group. *J Clin Oncol* 2006;24:460–6.
43. Wassmann B, Pfeifer H, Goekbuget N, et al. Alternating versus concurrent schedules of imatinib and chemotherapy as front-line therapy for Philadelphia positive acute lymphoblastic leukemia (Ph+ ALL). *Blood* 2006;108:1469–77.
44. Bassan R, Rossi G, Pogliani EM, et al. Chemotherapy-phased imatinib pulses improve long-term outcome of adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: Northern Italy Leukemia Group protocol 09/00. *J Clin Oncol* 2010;28:3644–52.
45. Talpaz M, Shah NP, Kantarjian H, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. *N Engl J Med* 2006;354:2531–41.
46. Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib-resistant CML and Philadelphia chromosome-positive ALL. *N Engl J Med* 2006;354:2542–51.
47. Ottmann OG, Pfeifer H. Management of Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL). *Hematology Am Soc Hematol Educ Program* 2009;371–81.
48. Bassan R, Hoelzer D. Modern therapy of acute lymphoblastic leukemia. *J Clin Oncol* 2011;29:532–43.
49. Bassan R, Spinelli O, Oldani E, et al. Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). *Blood* 2009;113:4153–62.
50. Hunault BM, Leguay T, Thomas X, et al. A randomized study of pegylated liposomal doxorubicin versus continuous-infusion doxorubicin in elderly patients with acute lymphoblastic leukemia: the GRAALL-SA1 study. *Haematologica* 2011;96:245–52.
51. Wetzler M, Sanford BL, Kurtzberg J, et al. Effective asparagine depletion with pegylated asparaginase results in improved outcomes in adult acute lymphoblastic leukemia: Cancer and Leukemia Group B Study 9511. *Blood* 2007;109:4164–7.
52. Hijiya N, Thomson B, Isakoff MS, et al. Phase 2 trial of clofarabine in combination with etoposide and cyclophosphamide in pediatric patients with refractory or relapsed acute lymphoblastic leukemia. *Blood* 2011;118:6043–9.

Appendix

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Cancer Science
The official journal of the Japanese Cancer Association

Bcl-2, Bcl-6, and the International Prognostic Index are prognostic indicators in patients with diffuse large B-cell lymphoma treated with rituximab-containing chemotherapy

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(Received June 10, 2012/Revised July 5, 2012/Accepted July 9, 2012/Accepted manuscript online July 11, 2012/Article first published online August 13, 2012)

This study aimed to clarify the clinicopathological prognostic parameters of *de novo* diffuse large B-cell lymphoma (DLBCL) in the rituximab era. We examined the correlation of 22 clinicopathological parameters with progression-free survival (PFS), overall survival (OS), and primary refractory disease in 285 DLBCL patients treated with rituximab-containing chemotherapy. Complete response rate was 87%, overall response rate was 91%, 5-year PFS rate was 72%, and 5-year OS rate was 91%. By log–rank test, higher International Prognostic Index (IPI) ($P < 0.0001$), Bcl-2 positivity ($P = 0.0013$), Bcl-6 negativity ($P = 0.0112$), and no irradiation ($P = 0.0371$) were significantly correlated with shorter PFS; higher IPI ($P = 0.0107$), starry sky pattern ($P = 0.0466$), and no irradiation ($P = 0.0264$) correlated with shorter OS. In multivariate analyses, higher IPI ($P = 0.0006$), Bcl-2 positivity ($P = 0.0015$), and Bcl-6 negativity ($P = 0.04$) were significantly correlated with shorter PFS; higher IPI ($P = 0.0045$) correlated with shorter OS. Bcl-2 ($P = 0.0029$), Bcl-6 ($P = 0.002$), and IPI ($P < 0.0001$) were significantly correlated with primary refractory disease. In conclusion, Bcl-2 positivity, Bcl-6 negativity, and higher IPI were indicators of shorter PFS and OS plus primary refractory disease in patients with DLBCL in the rituximab era. (*Cancer Sci* 2012; 103: 1898–1904)

Diffuse large B-cell lymphoma (DLBCL) represents the largest and most widely heterogeneous category of aggressive non-Hodgkin lymphomas.[1] Several histopathological prognostic parameters of DLBCL have been reported. In the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues published in 2008, several DLBCL variants, subgroups, and subtypes were proposed.[1] Morphological variants include centroblastic, immunoblastic, and anaplastic variants. Subtypes of DLBCL include T cell/histiocyte-rich large B-cell lymphoma and Epstein–Barr virus (EBV)-positive DLBCL of the elderly. Among these morphological variants and subtypes, immunoblastic variant DLBCL,[2,3] T cell/histiocyte-rich large B-cell lymphoma,[4] and EBV-positive DLBCL of the elderly[5] have been reported to have poor prognoses. Immunohistochemical expression of Bcl-2[3,6–9] and CD5[10] has been reported to be associated with an unfavorable prognosis; expression of Bcl-6[11] and CD10[3] are associated with a favorable prognosis. High Ki-67 index[12] has also been reported to be a poor prognostic parameter. However, these results were obtained mainly in the pre-rituximab era. Therefore, these prognostic parameters should be re-evaluated in the rituximab era. As anti-CD30 mAb therapy was found to be effective for classical Hodgkin's lymphoma and anaplastic large cell lymphoma,[13] the prognostic implication of CD30 expression should be examined in DLBCLs. *cMYC* rearrangement was reported to be a poor prognostic parameter of DLBCL in the rituximab era,[14] and *cMYC* rearrangement and immunohistochemical cMyc expression were reported to be correlated.[15] Therefore, immunohistochemical cMyc expression should also be evaluated.

Since 2000, DLBCL has been subdivided into germinal center B-cell (GCB) phenotype and non-GCB phenotype (including the activated B-cell phenotype and type 3 phenotype) using the cDNA microarray technique.[16,17] For use in clinical practice, Hans *et al.*[18] showed that a panel of immunohistochemical markers comprising CD10, Bcl-6, and MUM1 could be used on paraffin-embedded tissues to classify DLBCL into tumors with a GCB or non-GCB phenotype. The GCB phenotype showed a better outcome in the pre-rituximab era;[5,16,17] however, it was reported that the addition of rituximab to the cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) regimen eliminated the prognostic value of the GCB phenotype.[19] Therefore, the use of the GCB phenotype as a prognostic marker is controversial in the rituximab era.

In the pre-rituximab era, the International Prognostic Index (IPI), which is based on clinical parameters such as age, stage, serum lactate dehydrogenase (LDH) level, performance status (PS), and extent of extranodal involvement (EN), proved to be highly valuable for the prediction of prognosis in patients with DLBCL.[20] However, the IPI seems to have lost some of its high predictive value in the rituximab era.[21]

The aim of this study was to clarify the clinicopathological prognostic parameters of *de novo* DLBCL in the rituximab era. Thirteen histopathological parameters including DLBCL morphological variant, necrosis, starry sky pattern, CD5, CD10, CD30, Bcl-2, Bcl-6, MUM1, GCB/non-GCB, cMyc, Ki-67, and EBV-encoded RNA (EBER)-1, as well as nine clinical parameters, including IPI, influencing progression-free survival (PFS) and/or overall survival (OS) were evaluated by log–rank tests and multivariate analyses. Correlation of primary refractory disease and these clinicopathological parameters was also examined.

Materials and Methods

Patient selection. The study subjects were 285 consecutive patients with de novo DLBCL, treated at the National Cancer Center Hospital (Tokyo, Japan) between 2003 and 2010. Clinical information was extracted from medical records. The Ann Arbor system was used for staging. The staging procedures

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doi: 10.1111/j.1349-7006.2012.02382.x

included bone marrow aspiration or biopsy, endoscopy of the upper gastrointestinal tract, computed tomography, and optionally, PET. After initial diagnoses, all of the patients received rituximab-containing chemotherapy that consisted of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) with or without involved-field radiotherapy (258 patients), R-CHOP-like regimen (three patients), or modified rituximab, cyclophosphamide, vincristine, doxorubicin, and methotrexate (R-CODOX-M)/ifosfamide, etoposide, and cytarabine (IVAC) (24 patients).[22] High-dose chemotherapy with autologous peripheral blood stem cell transplantation was carried out in three patients. Four groups of clinical course were defined as follows: group 1, patients achieving complete response (CR) or partial response (PR) with the initial therapy and no relapse; group 2, patients achieving CR or PR with the initial therapy and relapse after 1 year or later (late relapse); group 3, patients achieving CR or PR with the initial therapy and relapse within 1 year (early relapse); and group 4, patients showing no change or progressive disease after the initial therapy. Group 4 were defined as having primary refractory disease in this study. Median follow-up time was 41 months (range, 1–97 months). Informed consent was obtained from each patient. The study was approved by the institutional review board of the National Cancer Center.

Morphological review. Biopsy materials were fixed in 10% neutral-buffered formalin, embedded in paraffin, cut into sections 4-μm thick, and stained with H&E for histopathological evaluation. All specimens were diagnosed by two pathologists (AMM and HT) according to the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2008.[1] Diffuse large B-cell lymphoma was subclassified as centroblastic, anaplastic, immunoblastic, or T cell/histiocyte-rich variant. Diffuse large B-cell lymphoma preceded by low-grade B-cell lymphoma or DLBCL with coexisting low-grade B-cell lymphoma was excluded. Necrosis and starry sky pattern were also evaluated.

Immunohistochemistry and *in situ* hybridization. Immunohistochemistry on formalin-fixed paraffin-embedded tissues was carried out using a panel of mAbs. The number of available specimens differed for each stain. Sections 4-μm thick were cut from each paraffin block, deparaffinized, and incubated at 121°C in citrate buffer, pH 6.0, for 10 min for antigen retrieval. The antibodies used included those against the following antigens: CD3 (PS1, ×25; Novocastra, Newcastle-upon-Tyne, UK), CD5 (4C7, ×100; Novocastra), CD10 (56C6, ×100; Novocastra), CD20 (L26, ×200; Dako, Glostrup, Denmark), CD30 (Ber-H2, ×100; Dako), Bcl-2 (124, ×100; Dako), Bcl-6 (PG-B6p, ×20; Dako), cMyc (Y69, ×50; Epitomics, Burlingame, CA, USA), cyclin D1 (SP4, ×25; Nichirei, Tokyo, Japan), Ki-67 (MIB-1, ×100; Dako), and MUM1 (MUM1p, ×200; Dako). An autostainer was used with the standard polymer method (Dako Autostainer Plus). All cases were positive for CD20 and negative for CD3. Immunoreactivity for CD5, CD10, CD30, Bcl-2, Bcl-6, and MUM1 was judged positive if more than 20% of the tumor cells were stained. Labeling index was counted for Ki-67 and cMyc; 90% or more was defined as high labeling index for Ki-67, and 80% or more as high labeling index for cMyc. The area with the greatest staining was selected, 500 nuclei were counted manually, and the proportion of positive cells was calculated. To classify each case as having either a GCB phenotype or a non-GCB phenotype, a panel of three antigens (CD10, Bcl-6, and MUM1) was used according to the protocol reported by Hans *et al.*[18] If the tumor revealed CD5 positivity, cyclin D1 negativity was confirmed. *In situ* hybridization with an EBER-1 probe (Dako) was carried out to detect possible EBV infection.

Statistical analysis. All survival curves were estimated by the Kaplan–Meier method. Statistical differences between survival curves were compared using the log–rank test for clinicopathological parameters. Multivariate analysis was carried out using Cox's proportional hazard model for the significant parameters detected by the log–rank test. Correlation between primary refractory disease and clinicopathological parameters was analyzed by Spearman's rank correlation coefficient test. Differences were considered significant when the P-value was <0.05.

Results

Patient characteristics and histopathological results. Clinical information and histopathological results are summarized in Table 1. Patients comprised 148 men and 137 women, ranging in age from 17 to 88 years with a median age of 55 years. Initial site was nodal in 164 patients and extranodal in 121 patients. The %CR for initial treatment was 87%, and the overall response rate was 91%. The 5-year PFS rate was 72%, and the 5-year OS rate was 91%.

By log–rank test, higher IPI ($P < 0.0001$), advanced stage ($P = 0.0003$), a high level of LDH ($\geq$230 U/L) ($P < 0.0001$), high PS (2–4) ($P = 0.0317$), more than two incidences of EN ($P = 0.0008$), no irradiation ($P = 0.0371$), Bcl-6 negativity ($P = 0.0112$; Figs 1a,2), and Bcl-2 positivity ($P = 0.0013$; Figs 1b,3) were significant parameters of shorter PFS (Table 1). Likewise, higher IPI ($P = 0.0107$), a high level of LDH ($P = 0.0121$), no irradiation ($P = 0.0264$), and starry sky pattern ($P = 0.0466$; Fig. 1c) were significant parameters of shorter OS (Table 1). Bcl-6 negativity was marginal as a prognostic parameter of OS ($P = 0.0691$). Patients with CD30 positivity and EBER-1 positivity revealed 100% 5-year OS; however, the number of CD30-positive and EBER-1-positive cases was small.

In the multivariate analyses carried out for significant parameters detected by log–rank test, with the exception of parameters such as stage, LDH, PS, and EN already included in the IPI scoring system, higher IPI, Bcl-2 positivity, and Bcl-6 negativity were independently correlated with lower PFS rate (Table 2), and only higher IPI was independently correlated with lower OS rate (Table 3).

We carried out subgroup analyses to examine the predictive value of Bcl-2 and Bcl-6 with PFS and OS. Bcl-2 was a significant prognostic factor of PFS in the low (L)/low-intermediate (LI) IPI group ($P = 0.009$), but not in the high-intermediate (HI)/high (H) IPI group (Fig. 4a). Bcl-6 was a significant prognostic factor of PFS in the HI/H IPI group ($P = 0.0451$), but not in the L/LI IPI group (Fig. 4b). The PFS was compared among the following four groups: $Bcl2^+/Bcl6^-$; $Bcl2^-/Bcl6^+$; $Bcl2^+/Bcl6^+$; and $Bcl2^-/Bcl6^-$. The $Bcl2^-/Bcl6^+$ group had a significantly better PFS than the other three groups ($P = 0.0486$), but significant differences were not found among the other three groups (Fig. 5).

Correlation of primary refractory disease with clinicopathological parameters. Group 1 comprised 220 (77%) patients, group 2 comprised 13 (5%) patients, group 3 comprised 25 (9%) patients, and group 4 comprised 27 (9%) patients (Table 4). The PFS curves for all 285 patients stratified by these four groups are shown in Figure 6. Five-year PFS rates were 100% in group 1, 8% in group 2, and 0% in groups 3 and 4. The OS curves for all 285 patients stratified by the four groups are shown in Figure 7. Five-year OS rates were 97% in group 1, 92% in group 2, 62% in group 3, and 52% in group 4. The OS rates significantly differed between groups 1 and 2 versus group 3 ($P < 0.0001$), and between group 3 versus group 4 ($P = 0.0499$). Correlation of primary refractory disease with clinicopathological parameters is shown in Table 4. Primary refractory disease was significantly correlated with higher IPI, advanced stage, high LDH, PS (2–4), EN (≥ 2), Bcl-2 positivity, and Bcl-6 negativity.

Histopathology of rebiopsied material at the time of relapse indicated DLBCL in five patients (site: bone marrow, 1; liver,

Table 1. Prognostic significance of 22 clinicopathological parameters in 285 patients with diffuse large B-cell lymphoma

Parameters	No. of patients (%)	5-year PFS (%)	*P*-value*	5-year OS (%)	*P*-value*
Total	285	72		90	
Clinical parameters					
Age					
≤60 years	158 (55)	74	NS	94	NS
>60 years	127 (45)	70		85	
Gender					
Male	148 (52)	69	NS	88	NS
Female	137 (48)	76		93	
IPI					
L, LI	218 (76)	79	<0.0001	94	0.0107
HI, H	67 (24)	51		79	
Stage					
I, II	198 (69)	79	0.0003	93	NS
III, IV	87 (31)	58		84	
LDH					
Normal range	144 (51)	82	<0.0001	95	0.0121
High	141 (49)	62		85	
PS					
0, 1	249 (87)	75	0.0317	91	NS
2–4	36 (13)	58		84	
EN					
0–1	236 (83)	76	0.0008	92	NS
≥2	49 (17)	55		83	
Initial site					
Nodal	164 (58)	74	NS	89	NS
Extranodal	121 (42)	70		92	
Irradiation					
Not performed	151 (53)	78	0.0371	94	0.0264
Performed	134 (47)	68		85	
Histopathological parameters					
DLBCL variant					
C or A	259 (91)	73	NS	91	NS
I or T/H	26 (9)	67		91	
Necrosis					
Absent	223 (78)	72	NS	90	NS
Present	62 (22)	77		93	
Starry sky					
Absent	263 (92)	73	NS	91	0.0466
Present	22 (8)	65		84	
CD5					
Negative	245 (92)	72	NS	90	NS
Positive	22 (8)	71		85	
CD10					
Negative	192 (74)	74	NS	92	NS
Positive	67 (26)	64		90	
Bcl-6					
Negative	69 (33)	65	0.0112	85	NS
Positive	140 (67)	76		93	
MUM1					
Negative	96 (46)	70	NS	94	NS
Positive	112 (54)	75		88	
GCB/non-GCB					
GCB	100 (43)	73	NS	93	NS
Non-GCB	132 (57)	71		89	
Bcl-2					
Negative	102 (46)	86	0.0013	96	NS
Positive	122 (54)	67		92	
cMyc index					
<80%	190 (90)	73	NS	90	NS
≥80%	20 (10)	72		94	
Ki-67 index					

doi: 10.1111/j.1349-7006.2012.02382.x

Table 1. (continued)

Parameters	No. of patients (%)	5-year PFS (%)	*P*-value*	5-year OS (%)	*P*-value*
<90%	139 (70)	76	NS	94	NS
≥90%	60 (30)	67		86	
CD30					
Negative	156 (91)	75	NS	93	Not calculated
Positive	15 (9)	87		100	
EBER-1 ISH					
Negative	197 (94)	74	NS	91	Not calculated
Positive	13 (6)	66		100	

*P-value was calculated by log–rank test. A, anaplastic; C, centroblastic; EN, extranodal involvement; GCB, germinal center B-cell phenotype; H, high; HI, high intermediate; I, immunoblastic; IPI, international prognostic index; ISH, *in situ* hybridization; L, low; LDH, lactate dehydrogenase (normal range, 119–229 U/L); LI, low intermediate; NS, not significant; OS, overall survival; PFS, progression-free survival; PS, performance status; T/H, T-cell/histiocyte rich.

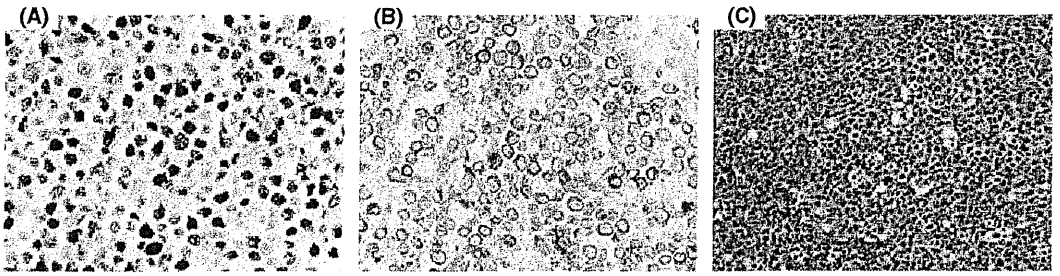

Fig. 1. Diffuse large B-cell lymphoma with a Bcl-6-positive phenotype (magnification, ×400) (A), a Bcl-2-positive phenotype (magnification, ×400) (B), and with starry sky pattern (H&E staining; magnification, ×200) (C).

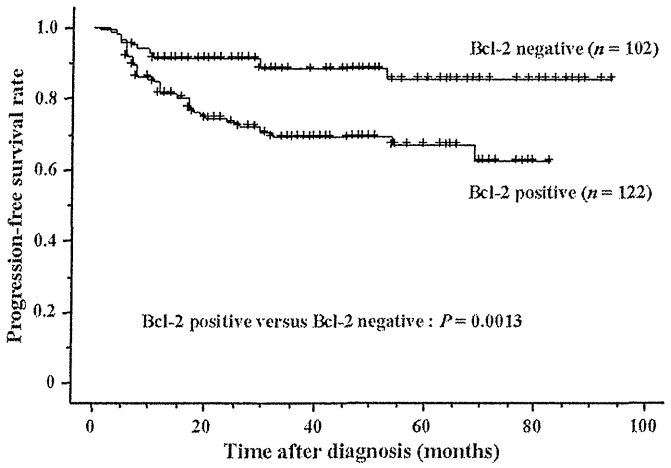

Fig. 2. Progression-free survival (PFS) curves for 224 patients with diffuse large B-cell lymphoma stratified by Bcl-2 immunoreactivity. Five-year PFS rates were 67% in the Bcl-2-positive group and 86% in the Bcl-2-negative group. The PFS rate of 122 patients with Bcl-2 positivity was significantly worse than that of 102 patients with Bcl-2 negativity ($P = 0.0013$).

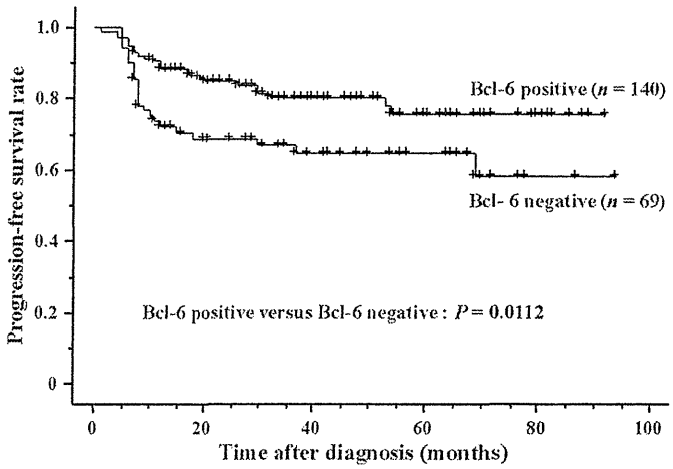

Fig. 3. Progression-free survival (PFS) curves for 209 patients with diffuse large B-cell lymphoma stratified by Bcl-6 immunoreactivity. Five-year PFS rates were 76% in the Bcl-6 positive group and 65% in the Bcl-6 negative group. The PFS rate of 69 patients with Bcl-6 negativity was significantly worse than that of 140 patients with Bcl-6 positivity ($P = 0.0112$).

1; skin, 2; and stomach, 1), follicular lymphoma grade 1 in one patient (site: jejunum), and mucosa-associated lymphoid tissue lymphoma in four patients (site: orbit, 1; parotid gland, 1; and thyroid, 2) in group 2. In group 3, histopathology indicated DLBCL in 16 patients (site: central nervous system, 1; bone marrow and skin, 1; chest wall, 1; lung, 1; lymph node, 5; mediastinum, 2; skin, 2; testis and central nervous system, 2; and urinary bladder, 1), and DLBCL and follicular lymphoma grade 3B in one patient (site: lymph node).

Table 2. Results of Cox's multivariate proportional hazards analysis for progression-free survival in 285 patients with diffuse large B-cell lymphoma

Parameters	Hazard ratio	95% confidence interval	*P*-value
IPI	1.610	1.226–2.116	0.0006
Bcl-2	1.833	1.260–2.668	0.0015
Bcl-6	0.516	0.275–0.970	0.0400

IPI, International Prognostic Index.

Table 3. Results of Cox's multivariate proportional hazards analysis for overall survival in 285 patients with diffuse large B-cell lymphoma

Parameter	Hazard ratio	95% confidence interval	*P*-value
IPI	1.792	1.199–2.680	0.0045

IPI, International Prognostic Index.

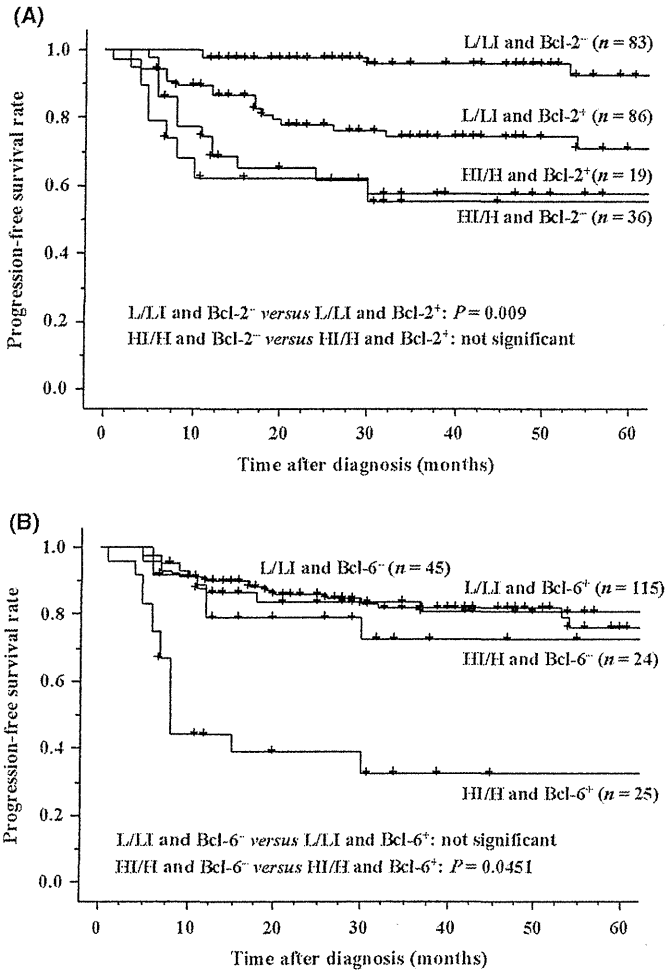

Fig. 4. (A) Progression-free survival (PFS) curves for 224 patients with diffuse large B-cell lymphoma stratified by the International Prognostic Index (IPI) and Bcl-2 immunoreactivity. Five-year PFS rates were 92% in the low (L)/low-intermediate (LI) IPI and Bcl-2⁻ group, 72% in the L/LI and Bcl-2⁺ group, 58% in the high-intermediate (HI)/high (H) and Bcl-2⁺ group, and 56% in the HI/H and Bcl-2⁻ group. The PFS of the L/LI and Bcl-2⁻ group was significantly better than that of the other three groups ($P = 0.009$). (B) The PFS curves for 209 patients stratified by IPI and Bcl-6 immunoreactivity. Five-year PFS rates were 75% in the L/LI and Bcl-6⁻ group, 80% in the L/LI and Bcl-6⁺ group, 72% in the HI/H and Bcl-6⁻ group, and 33% in the HI/H and Bcl-6⁺ group. The PFS of the HI/H and Bcl-6⁺ group was significantly worse than that of the other three groups ($P = 0.0451$).

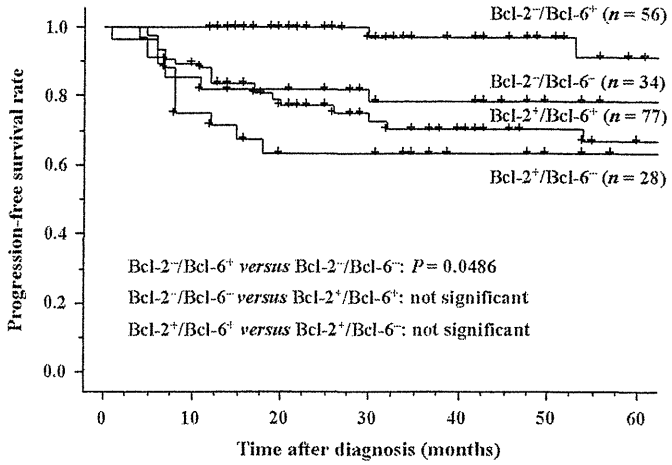

Fig. 5. Progression-free survival (PFS) curves for 195 patients stratified by Bcl-2 and Bcl-6 immunoreactivity. Five-year PFS rates were 92% in the Bcl-2⁻/Bcl-6⁺ group, 79% in the Bcl-2⁻/Bcl-6⁻ group, 68% in the Bcl-2⁺/Bcl-6⁺ group, and 63% in the Bcl-2⁺/Bcl-6⁻ group. The PFS of the Bcl-2⁻/Bcl-6⁺ group was significantly better than that of the other three groups ($P = 0.0486$).

Table 4. Correlation between groups 1–4 and clinicopathological parameters in 285 patients with diffuse large B-cell lymphoma

Parameters	Group	1	2	3	4	*P*-value
Clinical parameters	IPI (L, LI/HI, H)	183/37	9/4	15/10	11/16	<0.0001
	Stage (I, II/III, IV)	164/56	8/5	12/13	14/13	<0.0001
	LDH (normal/high)	126/93	8/5	4/21	5/22	<0.0001
	PS (0–1/2–4)	195/24	12/1	21/4	20/7	<0.0001
	EN (0–1/≥2)	191/29	9/4	18/7	18/9	0.0011
Histopathological parameters	Bcl-6 (negative/positive)	4/116	3/6	9/9	12/9	0.0029
	Bcl-2 (negative/positive)	91/88	2/7	3/12	6/15	0.0020

P-value was calculated by Spearman's co-efficiency test. EN, extranodal involvement; Group 1, patients with complete response (CR) or partial response (PR) after the first therapy and no relapse; Group 2, patients with CR or PR after the first therapy and relapse after 1 year or later (late relapse); Group 3, patients with CR or PR after the first therapy and relapse within 1 year (early relapse); Group 4, patients with no change or progressive disease after the first therapy; H, high; HI, high intermediate; IPI, International Prognostic Index; L, low; LDH, lactate dehydrogenase (normal range, 119–229 U/L); LI, low intermediate; NS, not significant; PS, performance status.

Discussion

In this study, clinicopathological prognostic parameters of patients with de novo DLBCL in the rituximab era were examined. Histopathological parameters studied included DLBCL morphological variant, necrosis, starry sky pattern, CD5, CD10, CD30, Bcl-2, Bcl-6, MUM1, GCB/non-GCB, cMyc, Ki-67, and EBER-1. The results revealed that higher IPI, Bcl-2 positivity, and Bcl-6 negativity were significantly correlated with shorter PFS and primary refractory disease in the rituximab era.

Concerning the outcome of patients with DLBCL, Récher *et al.*[23] reported that the 3-year PFS rate was 73% and the 3-year OS rate was 84%. Sehn *et al.*[21] reported that the 4-year PFS rate was 70% and the 4-year OS rate was 70%. Patients in the present study might have had better outcomes than the patients in these reports: 5-year PFS rate was 72% and 5-year OS rate was 91%, which might be partly associated with the

doi: 10.1111/j.1349-7006.2012.02382.x

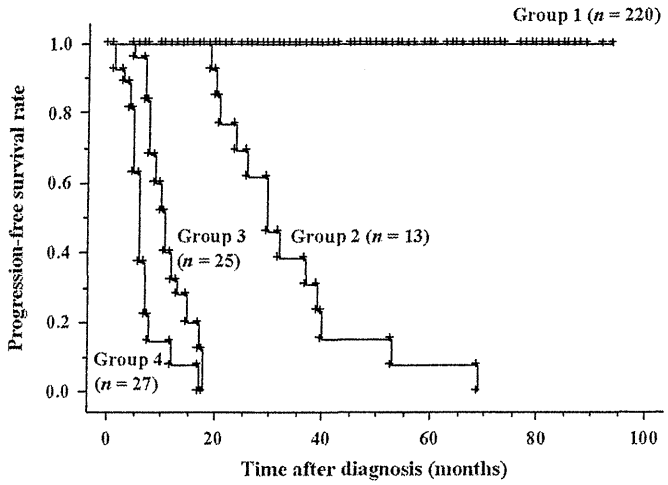

Fig. 6. Progression-free survival (PFS) curves for 285 patients with diffuse large B-cell lymphoma stratified into four groups. Five-year PFS rates were 100%, 8%, 0%, and 0% in groups 1 (220 patients), 2 (13 patients), 3 (25 patients), and 4 (27 patients), respectively.

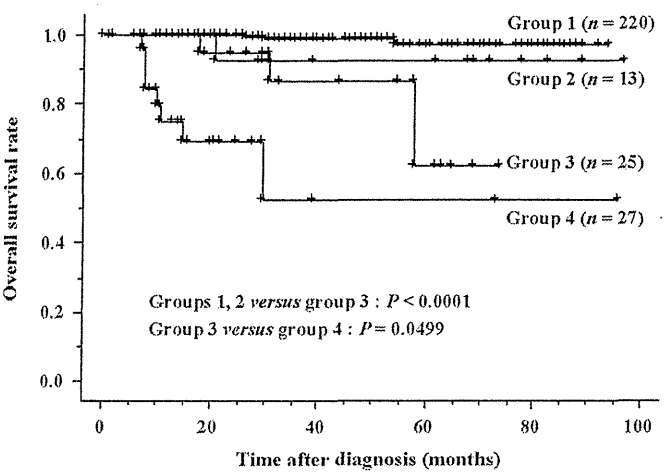

Fig. 7. Overall survival (OS) curves for 285 patients with diffuse large B-cell lymphoma stratified into four groups. The OS rates differed significantly ($P < 0.0001$) between groups 1 (220 patients) and 2 (13 patients) versus group 3 (25 patients); OS rates differed significantly ($P = 0.0499$) between group 3 versus group 4 (27 patients). Five-year OS rates were 97%, 92%, 62%, and 52% in groups 1, 2, 3, and 4, respectively.

low median age (55 years) and high incidence of low/low intermediate IPI and stage I/II disease in the current study.

Bcl-2 protein, an antiapoptotic molecule, is expressed on resting B and T cells, but not on normal germinal center cells.[24] Bcl-2 is expressed in 22–80% of DLBCLs,[25] and Bcl-2 positivity has been reported to be associated with an unfavorable prognosis.[3,6–9] Bcl-6 protein is expressed in B and $CD4^+$ T cells within the germinal center,[26] and is expressed in 47–84% of DLBCLs.[1] The Bcl-6 rearrangement observed in 30–40% of DLBCLs leads to deregulation of Bcl-6 gene expression.[27,28] Bcl-6 rearrangement was reported to correlate with a favorable outcome.[29] However, the level of Bcl-6 protein expression is not correlated with the presence or absence of Bcl-6 gene rearrangement and mutation.[30,31] Bcl-6 protein expression was reported to be associated with favorable prognosis.[11] The addition of rituximab to treatment regimens has considerably improved the survival of patients with DLBCL[32] and was reported to have eliminated the negative impact of Bcl-2 expression and the positive impact of Bcl-6 expression on clinical outcome.[33–35] However, in the present study, Bcl-2 positivity and Bcl-6 negativity were found to be parameters predicting a significantly shorter PFS (particularly Bcl-2 in the L/LI IPI group, and Bcl-6 in the HI/H IPI group) and primary refractory disease even in the rituximab era.

CD10 shows restricted expression in the germinal center B cells of reactive lymphoid tissue and is expressed in 30–60% of DLBCLs.[1] MUM1 is a lymphoid-specific member of the interferon regulatory factor family of transcription factors.[36] Normally expressed in plasma cells and a minor subset of germinal center B cells, MUM1 has been reported to be expressed in 35–65% of DLBCLs.[1] CD10 has been reported to be a favorable prognostic parameter.[3] CD10, Bcl-6, and MUM1 are included in the panel of markers used to assess GCB or non-GCB phenotype.[18] Some previous studies examining the difference in prognosis between patients with GCB phenotype and those with non-GCB phenotype DLBCL revealed that the former group had a more favorable prognosis.[16,17] However, Colomo *et al.*[7] found no prognostic difference between these groups; thus, this has recently become a controversial issue. In the present study, GCB versus non-GCB was not a significant prognostic factor of DLBCL and neither were CD10 or MUM1.

Ki-67 index, cMyc index, starry sky pattern, and necrosis are considered to be correlated immunohistochemical and histopathological findings, and all of them are associated with proliferation activity of tumors. High Ki-67 index and high cMyc index reflecting *cMYC* rearrangement were reported to be poor prognostic parameters.[12,14] The predictive value of Ki-67 index was reported in the pre-rituximab era.[12] In the present study, only starry sky pattern was a marginally significant predictor of OS by log–rank test; however, this result was not maintained in multivariate analysis. Our results suggested that the predictive values of these factors are limited in the rituximab era.

Our results suggested that CD5 was not a significant poor prognostic factor in the rituximab era. Yamaguchi *et al.*[10] reported that CD5 was a significant poor prognostic factor of OS in the pre-rituximab era, but not in the rituximab era.[37] In addition, expression of CD30, and EBER-1 and morphological DLBCL variant were not significant prognostic parameters in the rituximab era.

In the pre-rituximab era, IPI proved to be highly valuable in predicting the prognosis of DLBCLs;[20] however, IPI seems to have lost some of its predictive value in the rituximab era.[21] In the present study, conventional IPI was a significant prognostic parameter for predicting PFS, OS, and primary refractory disease. Several parameters comprising IPI, such as stage, LDH, PS, and EN, were also significant prognostic parameters predicting PFS, OS, or primary refractory disease.

In group 2, histopathology of rebiopsied material at the time of relapse revealed DLBCL in five patients and low-grade B-cell lymphoma in five patients. The latter could have represented transformed low-grade B-cell lymphoma from initial presentation. Therefore, it was speculated that approximately 50% of late relapsed DLBCLs had transformed from low-grade B-cell lymphomas.

In conclusion, our study shows that Bcl-2 positivity, Bcl-6 negativity, and higher IPI are significant indicators of shorter PFS, that IPI is a significant indicator of shorter OS, and that Bcl-2 positivity, Bcl-6 negativity, and higher IPI are indicators of primary refractory disease. Our results clarify the significant clinicopathological prognostic parameters of DLBCL in the rituximab era.

Acknowledgments

The authors would like to thank Ms C. Kina and Ms S. Miura for technical assistance with immunohistochemistry. The study was supported

in part by the National Cancer Center Research and Development Fund, and a Grant-in-Aid for the Third-Term Comprehensive 10-Year Strategy for Cancer Control from the Ministry of Health, Labor and Welfare, Japan.

Disclosure Statement

Kensei Tobinai received research grants from Zenyaku Kogyo and Chugai Pharmaceutical.

References

1 Swerdlow SH, Campo E, Harris NL *et al. World Health Organization Classification of Tumors of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press, 2008.
2 Endlhard M, Brittinger G, Huhn D *et al.* Subclassification of diffuse large B-cell lymphomas according to the Kiel classification: distinction of centroblastic and immunoblastic lymphomas is a significant prognostic risk factor. *Blood* 1997; **89**: 2291–7.
3 De Paepe P, Achten R, Verhoef G *et al.* Large cleaved and immunoblastic lymphoma may represent two distinct clinicopathologic entities within the group of diffuse large B-cell lymphomas. *J Clin Oncol* 2005; **23**: 7060–8.
4 Achten R, Verhoef G, Vanuytsel L, Wolf-Peeters C. T-cell/histiocyte-rich large B-cell lymphoma: a distinct clinicopathologic entity. *J Clin Oncol* 2002; **20**: 1269–77.
5 Park S, Lee J, Ko YH *et al.* The impact of Epstein–Barr virus status on clinical outcome in diffuse large B-cell lymphoma. *Blood* 2007; **110**: 972–8.
6 Berglund M, Thunberg U, Amini RM *et al.* Evaluation of immunophenotype in diffuse large B-cell lymphoma and its impact on prognosis. *Mod Pathol* 2005; **18**: 1113–20.
7 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.
8 Gascoyne RD, Adomat SA, Krajewski S *et al.* Prognostic significance of Bcl-2 protein expression and Bcl-2 gene rearrangement in diffuse aggressive non-Hodgkin's lymphoma. *Blood* 1997; **90**: 244–51.
9 Hermine O, Haioun C, Lepage E *et al.* Prognostic significance of bcl-2 protein expression in aggressive non-Hodgkin's lymphoma. Groups d'Etude des Lymphomes de l'Adulte (GELA). *Blood* 1996; **87**: 265–72.
10 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.
11 Lossos IS, Jones CD, Warnke R *et al.* Expression of a single gene, *BCL-6*, strongly predicts survival in patients with diffuse large B-cell lymphoma. *Blood* 2001; **98**: 945–51.
12 Miller TP, Grogan TM, Dahlberg S *et al.* Prognostic significance of the Ki-67-associated proliferative antigen in aggressive non-Hodgkin's lymphomas: a prospective Southwest Oncology Group trial. *Blood* 1994; **83**: 1460–6.
13 Younes A, Barlett NL, Leonard JP *et al.* Brentuximab vedotin (SGN-35) for relapsed CD30-positive lymphomas. *N Engl J Med* 2010; **363**: 1812–21.
14 Hummel M, Bentink S, Berger H *et al.* A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. *N Engl J Med* 2006; **354**: 2419–30.
15 Ruzinova MB, Caron T, Rodig SJ. Altered subcellular localization of c-Myc protein identifies aggressive B-cell lymphomas harboring a *c-MYC* translocation. *Am J Surg Pathol* 2010; **34**: 882–91.
16 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.
17 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 Engl J Med* 2002; **346**: 1937–47.
18 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.
19 Nyman H, Adde M, Karjalainen-Lindsberg ML *et al.* Prognostic impact of immunohistochemically defined germinal center phenotype in diffuse large B-cell lymphoma patients treated with immunochemotherapy. *Blood* 2007; **109**: 4930–5.
20 Armitage JO, Weisenburger DD. New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. *J Clin Oncol* 1998; **16**: 2780–95.
21 Sehn LH, Berry B, Chhanabhai M *et al.* The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. *Blood* 2007; **109**: 1857–61.
22 Maruyama D, Watanabe T, Maeshima AM *et al.* Modified cyclophosphamide, vincristine, doxorubicin, and methotrexate (CODOX-M)/ifosfamide, etoposide, and cytarabine (IVAC) therapy with or without rituximab in Japanese adult patients with Burkitt lymphoma (BL) and B cell lymphoma, unclassifiable, with features intermediate between diffuse large B cell lymphoma and BL. *Int J Hematol* 2010; **92**: 732–43.
23 Récher C, Coiffier B, Haioun C *et al.* Intensified chemotherapy with ACVBP plus rituximab versus standard CHOP plus rituximab for the treatment of diffuse large B-cell lymphoma (LNH03-2B): an open-label randomised phase 3 trial. *Lancet* 2011; **378**: 1858–67.
24 Nunez G, London L, Hockenbery D *et al.* Deregulated Bcl-2 gene expression selectively prolongs survival of growth factor-deprived hemopoietic cell lines. *J Immunol* 1990; **144**: 3602–10.
25 Anagnostopoulos I, Dallenbach F, Stein H. Diffuse large B-cell lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*, 2nd edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2001; 855–913.
26 Falini B, Fizzotti M, Pileri S *et al.* Bcl-6 protein expression in normal and neoplastic lymphoid tissues. *Ann Oncol* 1997; **8**: 101–4.
27 Chaganti SR, Chen W, Parsa N *et al.* Involvement of BCL6 in chromosomal aberrations affecting band 3q27 in B-cell non-Hodgkin lymphoma. *Genes Chromosom Cancer* 1998; **23**: 323–7.
28 Ye BH, Chaganti S, Chang CC *et al.* Chromosomal translocations cause deregulated BCL6 expression by promoter substitution in B cell lymphoma. *EMBO J* 1995; **14**: 6209–17.
29 Offit K, Lo Coco F, Louie DC *et al.* Rearrangement of the Bcl-6 gene as a prognostic marker in diffuse large B-cell lymphoma. *N Engl J Med* 1994; **331**: 74–80.
30 Pittaluga S, Ayoubi TA, Wlodarska I *et al.* Bcl-6 expression in reactive lymphoid tissue and in B-cell non-Hodgkin's lymphomas. *J Pathol* 1996; **179**: 145–50.
31 Skinnider BF, Horsman DE, Dupuis B *et al.* Bcl-6 and Bcl-2 protein expression in diffuse large B-cell lymphoma and follicular lymphoma: correlation with 3q27 and 18q21 chromosomal abnormalities. *Hum Pathol* 1999; **30**: 803–8.
32 Coiffer B. Rituximab therapy in malignant lymphoma. *Oncogene* 2007; **26**: 3603–13.
33 Mounier N, Briere J, Gisselbrecht C *et al.* Rituximab plus CHOP (R-CHOP) overcomes bcl-2-associated resistance to chemotherapy in elderly patients with diffuse large B-cell lymphoma (DLBCL). *Blood* 2003; **101**: 4279–84.
34 Mounier N, Briere J, Gisselbrecht C, Reyes F, Gaulard P, Coiffier B. Estimating the impact of rituximab on bcl-2-associated resistance to CHOP in elderly patients with diffuse large B-cell lymphoma. *Haematologica* 2006; **91**: 715–6.
35 Winter JN, Weller EA, Horning SJ *et al.* Prognostic significance of Bcl-6 protein expression in DLBCL treated with CHOP or R-CHOP: a prospective correlative study. *Blood* 2006; **107**: 4207–13.
36 Mamane Y, Heylbroeck C, Genin P *et al.* Interferon regulatory factors: the next generation. *Gene* 1999; **237**: 1–14.
37 Miyazaki K, Yamaguchi M, Suzuki R *et al.* CD5-positive diffuse large B-cell lymphoma: a retrospective study in 337 patients treated by chemotherapy with or without rituximab. *Ann Oncol* 2011; **22**: 1601–7.

doi: 10.1111/j.1349-7006.2012.02382.x

Ann Hematol (2012) 91:383–390
DOI 10.1007/s00277-011-1306-0

ORIGINAL ARTICLE

Retrospective analysis of primary gastric diffuse large B cell lymphoma in the rituximab era: a multicenter study of 95 patients in Japan

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Received: 18 February 2011 / Accepted: 26 July 2011 / Published online: 6 August 2011

Abstract Primary gastric diffuse large B cell lymphoma (PG-DLBCL) is common subtype of extranodal non-Hodgkin lymphoma. The optimal treatment strategy for PG-DLBCL in the rituximab era still remains unknown. To evaluate clinical outcomes of PG-DLBCL in the rituximab era, we conducted a retrospective, multicenter analysis of 95 patients with PG-DLBCL. In 58 patients with localized disease, 3-year progression-free survival (PFS) and overall survival (OS) were 91% and 91% for patients with six cycles of rituximab plus CHOP (R-CHOP) and 92% and 95% for patients with three to four cycles of R-CHOP plus radiotherapy (Log-rank test, P=0.595 and P=0.278, respectively). In 37 patients with advanced disease, 3-year PFS and 3-year OS were 43% and 64% for patients with R-CHOP chemotherapy with or without radiotherapy. On multivariate analysis, advanced stage and elevated serum LDH levels were independent predictors of survival in patients with PG-DLBCL. One patient with localized disease relapsed in lymph node, and eight patients with advanced disease relapsed in lymph node (n=3), stomach (n=2), central nervous system (CNS; n=2), and duodenum (n=1). Intriguingly, CNS relapse developed within 6 months after initial series of treatment (4.9 and 5.8 months, respectively), and stomach relapse developed in later phase (27.2 and 32.9 months, respectively). Clinical outcomes of PG-DLBCL were extremely favorable for localized-stage patients in the rituximab era, although these might be poor for advanced-stage patients even in the rituximab era. Further prospective analyses are warranted.

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Keywords Primary gastric lymphoma · Diffuse large B cell lymphoma · Rituximab · Radiotherapy · Relapse

Introduction

Primary gastric diffuse large B cell lymphoma (PG-DLBCL) is the most common histologic type of extranodal non-Hodgkin lymphoma [1]. Regarding initial treatment for this condition, various modalities have long been used, including surgery, chemotherapy, and radiotherapy, either alone or in combination [2]. In a randomized controlled trial in patients with localized-stage PG-DLBCL, chemotherapy alone had a 90% cure rate, and 10-year overall survival was equivalent to that of surgery plus chemotherapy [3] while, in a subsequent prospective study in patients with localized-stage PG-DLBCL, chemotherapy followed by radiotherapy was shown to be highly effective [4]. These results lead to the replacement of surgical resection with more stomach-preserving therapy and chemotherapy followed by radiotherapy is commonly used treatment in localized disease. Nevertheless, it remains unclear whether optimal treatment is provided by chemotherapy alone or chemotherapy followed by radiotherapy [5].

With regard to advanced-stage PG-DLBCL, a prospective study by the *Groupe d'Etude des Lymphomes de l'Adult* (GELA) showed that gastrointestinal lymphomas behaved similarly to nodal lymphomas in patients treated with chemotherapy alone [6]. Since the appearance of this study, patients with advanced-stage PG-DLBCL have been mainly treated with chemotherapy alone because of the effectiveness and feasibility [1, 7].

The advent of rituximab, a chimeric anti-CD20 monoclonal antibody, has changed clinical treatment for DLBCL. A number of randomized clinical trials, conducted mainly for advanced-stage DLBCL, have shown that the addition of rituximab to cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) chemotherapy provides superior survival to CHOP chemotherapy alone [8, 9], and this combination has achieved consensus as the standard treatment especially in patients with advanced-stage DLBCL.

In PG-DLBCL, prospective analyses have been reported mainly in patients with localized disease treated with rituximab plus CHOP (R-CHOP) chemotherapy [10, 11]. However, the role of R-CHOP chemotherapy followed by radiotherapy in localized disease has not yet been evaluated. On the other hand, in advanced disease, there has been no detailed data in patients treated with R-CHOP chemotherapy even retrospective series. Here, we retrospectively analyzed a cohort of 95 patients with localized- and advanced-stage PG-DLBCL receiving R-CHOP chemotherapy with or without radiotherapy.

Methods

Patients

We conducted a retrospective analysis of 95 patients who were newly diagnosed with PG-DLBCL from January 1995 to January 2009 at Nagoya University Hospital and seven associated hospitals. PG-DLBCL was diagnosed if lesions were predominantly in the stomach when the expansion of disease is checked in full body at initial diagnosis [12]. Clinical stage was evaluated according to the Lugano staging system for gastrointestinal non-Hodgkin's lymphoma [13], in which stages I and II1 are categorized as localized disease, and II2, IIE, and IV as advanced disease [13]. All patients received staging investigations, including physical examination, laboratory data analysis, computed tomography (CT) of the chest and abdomen, gallium scintigraphy, or fluorine-18-fluorodeoxyglucose positron emission tomography, bone marrow aspiration/biopsy, and gastrofiberscopy (GF) with biopsy. Evaluation of central nervous system (CNS) involvement was by either or both computed tomography/magnetic resonance imaging and lumber puncture with cerebrospinal fluid analysis where indicated. The following clinical and laboratory data were available at the time of diagnosis: age; sex; performance status (PS); presence of B symptoms, bulky mass, bone marrow involvement, and CNS involvement; serum lactate dehydrogenase (LDH) level; clinical stage; and number of extranodal sites. For this study, International Prognostic Index (IPI) scores were determined, and the patients were categorized into low- (score 0–2) or high-risk groups (score 3–5) [14]. This study was approved by the institutional review board at each participating hospital and complied with all provisions of the Declaration of Helsinki.

Pathological studies

Histological sections were reviewed, and diagnosis was confirmed as DLBCL according to the fourth edition of the World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues [15]. The review was performed by two pathologists (S.N. and T.T.) at the Department of Pathology and Clinical Laboratories, Nagoya University Hospital. Immunohistochemical staining and scoring for CD10, BCL-6, and MUM-1/IRF4 were performed on formalin-fixed paraffin-embedded tissues from patients diagnosed with PG-DLBCL and scored as positive if 30% or more of tumor cells were labeled [16]. The patients were then assigned as germinal center B cell-like (GCB) phenotype or non-GCB phenotype using the algorithm of Hans et al. [16].

Treatment

Analysis was restricted to patients who received CHOP chemotherapy (CHOP or CHOP-like regimen) plus rituximab (R-CHOP) or R-CHOP chemotherapy followed by radiotherapy as initial therapy. Rituximab dosage for all patients was 375 mg/m^2. Therapeutic strategies were determined by the attending physician in each hospital. Regarding localized-stage PG-DLBCL, selection of R-CHOP chemotherapy alone, or R-CHOP chemotherapy followed by radiotherapy was not decided in advance of diagnosis.

Response to treatment

Complete response (CR) was defined as the disappearance of all clinical evidence of disease, negative gastric biopsy, and recovery of all laboratory and radiological abnormalities related to the disease. Partial response (PR) was indicated by a decrease of more than 50% in the sum of the products of the maximum perpendicular diameters of each measurable lesion. Progressive disease (PD) was indicated by at least a 25% increase in the size of any preexisting lesions or by the appearance of any new lesions during or after therapy. Stable disease was neither PR nor PD. Relapse disease (RD) was the appearance of any new lesion in patients who had achieved CR. Overall survival (OS) was defined as the time from initial diagnosis to the date of death from any cause or of last follow-up. PFS was defined as the duration from initial diagnosis to the date of progression, relapse, death from any cause, or last follow-up, whichever occurred first.

Gastrointestinal-specific toxicities

Gastrointestinal-specific toxicities such as gastric hemorrhage, gastric perforation, and gastric obstruction during initial treatment were evaluated. Gastric hemorrhage was defined as symptoms of melena or hematemesis and the presence of hemorrhage confirmed by GF; gastric obstruction as symptoms of vomiting, eating difficulty, and the presence of stenosis confirmed by GF; and gastric perforation as the presence of free air around the stomach in the abdominal cavity on CT.

Statistical analysis

Patient characteristics between treatment groups were compared with Fisher's exact test and median age with the Mann–Whitney *U* test. OS and PFS were assessed by the Kaplan–Meier method and compared between groups by the log-rank test. The impact of independent prognostic factors on OS was evaluated by univariate and multivariate analyses using a Cox proportional hazards model. Variable factors were as follows: sex; age; performance status; presence of B symptoms, bulky mass, and bone marrow involvement; expression of the GCB phenotype; number of extranodal sites; serum LDH level; addition of rituximab; and addition of radiotherapy. All *P* values were based on two-sided tests and *P* values less than 0.05 were considered significant. All statistical analyses were performed using the Statistical Software Package for the Social Sciences (SPSS version 11.0 for Windows; SPSS Inc., Chicago, IL).

Results

Patient characteristics

Patient characteristics are shown in Table 1. Of the 95 patients analyzed in this study, 50 were male and 45 were female with a median age of 68 years (range, 32–86 years). The proportion of GCB phenotype was lower compared with that of non-GCB type (42% and 58%, respectively). Seven variables showed a significant difference between localized- and advanced-stage groups, namely PS, number of extranodal sites, serum LDH level, IPI risk group, bulky mass, and radiotherapy. Frequent extranodal involvements other than the stomach were liver in four patients, spleen duodenum, and bone marrow in three patients and bone in two patients. *Helicobacter pylori* infection was found in 27 of 49 patients (55%) who could be examined for *H. pylori* status in PG-DLBCL. Eleven of 27 patients (41%) with *H. pylori*-positive PG-DLBCL received eradication therapy before or after initial chemotherapy. In 95 patients diagnosed with PG-DLBCL, eight patients (8%) had DLBCL with marginal-zone lymphoma of mucosa-associated lymphoid tissue (MALT) component. *H. pylori* status was recognized in four of six patients (67%) with DLBCL in the presence of MALT component and not examined in two patients. In eight patients of DLBCL with MALT component, all patients were classified into non-GCB phenotype on the immunohistochemical staining.

Treatment

Of the 58 patients with localized disease, 35 patients (60%) received a median of three courses (range, three to four) of R-CHOP chemotherapy followed by radiotherapy, while the remaining 23 (40%) received a median of six courses (range, two to eight) of R-CHOP chemotherapy without radiotherapy. Of the 37 patients with advanced disease, 35 patients (95%) received R-CHOP chemotherapy alone and CHOP (n=35) or CHOP-like regimen (n=2) combined with rituximab. Two patients (5%) received three cycles of R-CHOP chemotherapy combined with radiotherapy.

Table 1 Patient characteristics

Variable	Total (N=95) N (%)	Localized stage (n=58) n (%)	Advanced stage (n=37) n (%)	P value*
Age				
Median age	68	68	67	0.722
Range	32–86	32–84	35–86	
Sex				
Male	50 (52)	30 (51)	20 (54)	0.824
Female	45 (48)	28 (49)	17 (46)	
Performance status				
0–1	89 (94)	57 (98)	31 (84)	0.013
2–4	6 (6)	1 (2)	6 (16)	
Lugano stage				
I	33 (35)	33 (57)	–	
II1	25 (26)	25 (43)	–	
II2	10 (11)	–	10 (27)	
IIE	4 (4)	–	4 (11)	
IV	23 (24)	–	23 (62)	
Extranodal sites				
Fewer than 2 (stomach only)	81 (85)	58 (100)	23 (62)	<0.0001
2 or more	14 (15)	0	14 (38)	
Serum LDH level				
Elevated	29 (31)	9 (15)	20 (54)	0.0002
IPI score				
<3	75 (79)	57 (98)	18 (49)	<0.0001
≥3	20 (21)	1 (2)	19 (51)	
B symptom present	19 (20)	10 (17)	9 (24)	0.438
Bulky mass present	9 (9)	1 (2)	8 (22)	0.002
Bone marrow involvement	3 (3)	0	3 (8)	0.056
Treatment				
Six cycles of R-CHOP	58 (61)	23 (39)	35 (95)	<0.0001
Three to four cycles of R-CHOP +Radiotherapy	37 (39)	35 (61)	2 (5)	
ASCT				
Yes	1 (1)	0	1 (3)	0.389
No	94 (99)	58	36 (97)	
Hans' algorism				
GCB phenotype	40 (42)	22 (37)	18 (49)	0.302
Non-GCB phenotype	45 (58)	36 (63)	19 (51)	

Abbreviations: *LDH* lactate dehydrogenase, *ASCT* autologous stem cell transplantation, *GCB* germinal center B cell-like

*P values are for the comparison of localized- and advanced-stage group

Efficacy

Localized-stage patient

Of the 58 patients with localized disease, 51 patients (88%) and seven patients (12%) achieved CR and PR. No patient developed PD. With a median follow-up for surviving patients of 34.5 months (range, 4.9–89.3 months), 3-year PFS and OS were 93%. With regard to radiotherapy, CR rate in the localized disease was 83% and 91% in six cycles of R-CHOP and in three to four cycles of R-CHOP plus radiotherapy, respectively. 3-Year PFS and OS were 91% and 91% in patients with six cycles of R-CHOP and 92% and 95% in those with three to four cycles of R-CHOP plus radiotherapy (Log-rank test, P=0.595 and P=0.278, respectively; Fig. 1a, b). Twenty-two patients (38%) were classified as the GCB phenotype and 36 (62%) as the non-GCB phenotype. No significant difference in 3-year OS was seen between the GCB and non-GCB phenotypes (92% vs 96%; P=0.886).

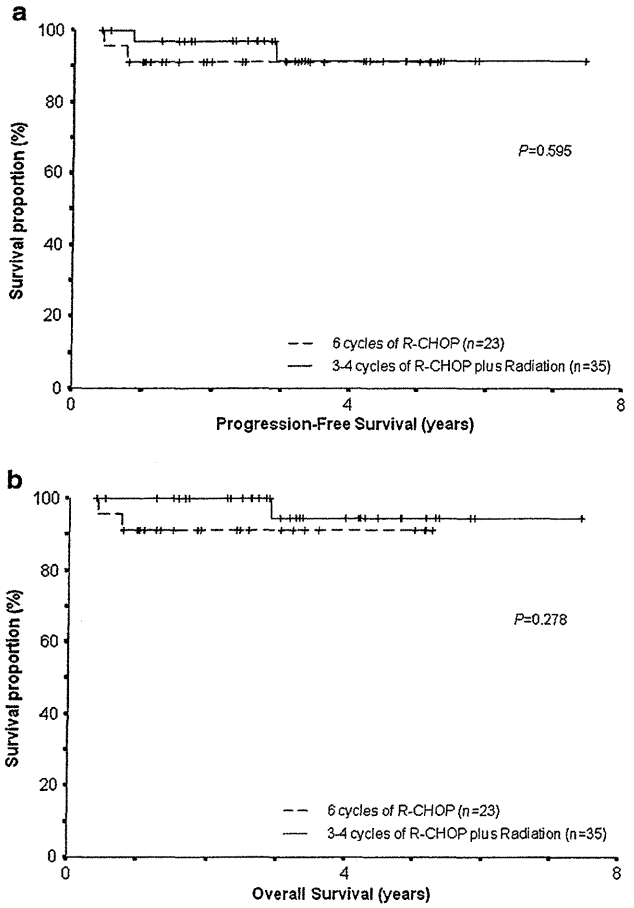

Fig. 1 **a** Progression-free and **b** overall survival of 58 patients receiving six cycles of R-CHOP (n=23) and three to four cycles of R-CHOP plus radiotherapy (n=35) in localized disease

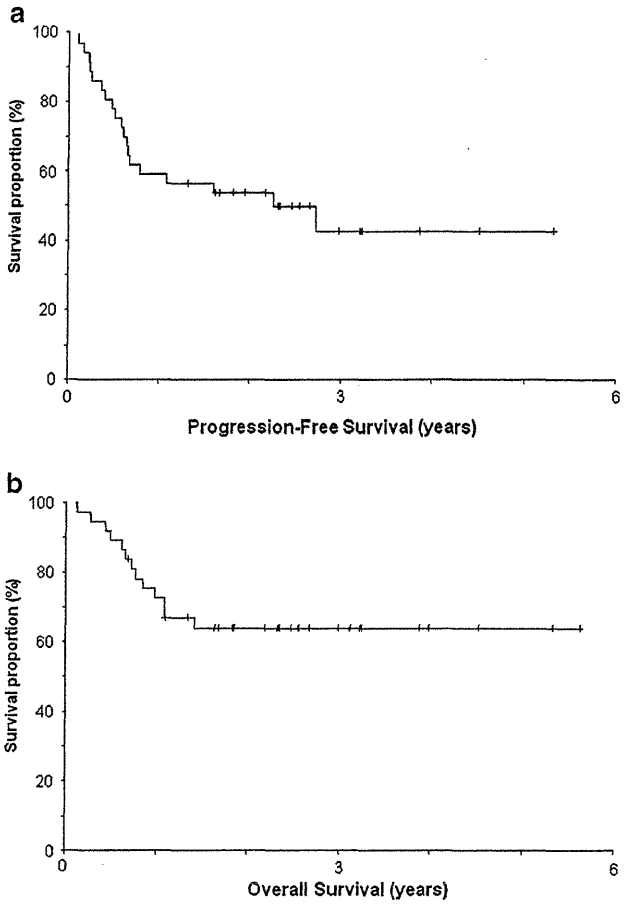

Fig. 2 **a** Progression-free and **b** overall survival of 37 patients receiving R-CHOP chemotherapy with or without radiotherapy in advanced disease

Advanced-stage patient

Of the 37 patients with advanced disease, 29 (78%) and two (5%) achieved CR and PR. Four patients (11%) developed PD. With a median follow-up for the surviving patients of 30.2 months (range, 8.2–67.5 months), 3-year PFS and OS were 43% and 64%, respectively (Fig. 2a, b). Eighteen patients (49%) were classified as the GCB phenotype and 19 (51%) as the non-GCB phenotype. No significant difference in 3-year OS was seen between the GCB and non-GCB phenotypes (58% vs 71%; P=0.303).

Toxicity

Surgical events such as gastric hemorrhage, gastric perforation, and gastric obstruction are shown in Table 2. Gastric perforation was not identified in any patient. Gastric hemorrhage occurred in one patient (1%) in the localized stage and two (5%) in the advanced stage, and gastric obstruction in two patients (3%) in the localized stage and four (5%) in the advanced stage. The frequency of gastric hemorrhage and gastric obstruction between the localized and advanced stage did not significantly differ (P=0.558 and P=0.999, respectively).

Relapsed disease

Localized-stage patient

Among the 51 patients achieving CR after initial treatment, only one patient (2%) developed RD in lymph node with 10.4 months of interval between initial diagnosis and relapse (Table 3).

Advanced-stage patient

Among 29 patients achieving CR, eight patients (28%) developed RD. Sites of relapse were lymph node (n=3), stomach (n=2), CNS (n=2), and duodenum (n=1). Median interval between initial diagnosis and relapse was 7.8 months (range, 4.9–32.9 months). In patients with RD in the CNS or stomach, median interval between initial

Table 2 The frequency of gastric perforation, hemorrhage, and obstruction

Variable	Localized stage (n=58)			Advanced stage (n=37)			
	Six cycles of R-CHOP (n=23)	Three to four cycles of R-CHOP+radiation (n=35)	Total	Six cycles of R-CHOP (n=35)	Three to four cycles of R-CHOP+radiation (n=2)	Total, N (%)	P value
Hemorrhage	0	1	1 (1)	2	0	2 (5)	0.558
Perforation	0	0	0	0	0	0	
Obstruction	1	1	2 (3)	2	0	2 (5)	0.999

diagnosis and relapse was 5.4 and 30.0 months, respectively (Table 3). Of the two patients relapsed in stomach, one was *H. pylori*-positive DLBCL with MALT component and achieved CR with six cycles of R-CHOP chemotherapy. Eradication therapy was not performed before or after chemotherapy. MALT lymphoma occurred in the same lesion of the stomach 27 months later. After eradication therapy, the relapsed lesion disappeared. The other who was *H. pylori*-negative DLBCL relapsed with DLBCL in different lesion of the stomach 32 months later.

Prognostic factors

All patients with localized and advanced disease were analyzed together. In univariate analysis, seven factors were associated with shorter survival, namely poor performance status, involvement of two or more extranodal sites, advanced stage, elevated serum LDH level, presence of bulky mass, presence of B symptoms, and presence of bone marrow involvement. The other three factors, namely sex, age, and expression of the GCB phenotype were not predictive of survival on univariate analysis. In addition, the GCB phenotype was not predictive of survival in both patients with localized and advanced group. Multivariate analysis identified advanced stage (hazard ratio (HR), 4.807; 95% confidence interval (CI), 1.075-21.739; P=0.039) and elevated serum LDH level as independent predictors of survival (HR, 4.901; 95% CI, 1.035–23.255; P=0.045; Table 4).

Discussion

We found that the clinical outcomes in patients with localized-stage PG-DLBCL were extremely favorable in the both groups treated with three cycles of R-CHOP plus radiotherapy and six cycles of R-CHOP, and those tended to be similar. Furthermore, the clinical outcome in patients with advanced-stage PG-DLBCL treated with R-CHOP chemotherapy might be poor. Although retrospective, these findings might be informative in patients with PG-DLBCL in the rituximab era.

In this study, patients with localized-stage PG-DLBCL treated with six cycles of R-CHOP had a CR rate of 83% and 3-year OS of 91%. There have been two reported studies that have prospectively evaluated PG-DLBCL mainly in localized-stage using R-CHOP chemotherapy alone as follows: Wohrer et al. reported a CR rate of 87% (13 of 15 patients) in patients treated with six cycles of R-CHOP [10]. Aviles et al. showed 5-year OS of 95% in 42 patients treated with six cycles of R-CHOP [11]. Although current study was retrospective, our

Table 3 Site of relapse in patients with a CR after initial therapy

Case no.	Age/sex	Stage	Lugano	LDH	IPI score	Extranodal involvement (excluding stomach)	Therapy	Course	Site of relapse	Time to relapse (months)
1	52/F	Localized	I	294	1		R-CHOP+Rad	3	Cervical LN	11.1
2	57/M	Advanced	II2	461	1		R-CHOP	8	CNS	5.8
3	53/M	Advanced	IIE	220	0	Duodenum	R-CHOP	8	Duodenum	8.0
4	71/M	Advanced	IV	398	3		R-CHOP	6	CNS	4.9
5	57/M	Advanced	IV	237	2	Spleen, liver	R-CHOP	7	Mediastinal LN	6.2
6	35/M	Advanced	IV	209	1		R-CHOP	6	Stomach	27.2
7	73/F	Advanced	IV	188	2		R-CHOP	8	Stomach	32.9
8	67/M	Advanced	IV	390	3		R-CHOP	8	Paraorta LN	7.6
9	69/F	Advanced	IV	434	4	Pancreas	R-CHOP	8	Paraorta LN	20.7

CNS central nervous system

Table 4 Univariate and multivariate analysis for OS in patients with PG-DLBCL

Variable	Subgroup	Univariate analysis Hazard ratio [95% CI]	*P* value	Multivariate analysis Hazard ratio [95% CI]	*P* value
Sex	Female vs. male	1.129 [0.420–3.039]	0.885	3.636 [0.952–13.888]	0.058
Age	<60 vs. ≥60	2.096 [0.596–7.352]	0.248	3.194 [0.605–16.949]	0.171
Performance status	0–1 vs. 2–4	5.917 [1.893–18.518]	0.002	2.028 [0.458–8.928]	0.351
Extranodal site	One vs. two or more	3.846 [1.386–10.638]	0.009	1.381 [0.104–7.209]	0.660
Lugano stage	Localized vs. advanced	8.064 [2.298–28.571]	0.001	4.807 [1.075–21.739]	0.039
Serum LDH level	Normal vs. high	6.535 [2.267–18.867]	0.0005	4.901 [1.035–23.255]	0.045
Bulky mass	No vs. yes	3.533 [1.137–10.989]	0.029	1.054 [0.252–4.418]	0.942
B symptom	No vs. yes	3.300 [1.125–8.849]	0.018	2.906 [0.822–10.309]	0.097
Bone marrow involvement	No vs. yes	6.250 [1.385–27.777]	0.017	1.738 [0.224–13.484]	0.596
GCB phenotype	GCB vs. non-GCB	1.293 [0.470–3.558]	0.618	1.769 [0.469–6.666]	0.398

CI confidence interval

result was comparable with previous prospective data in localized-stage PG-DLBCL.

Our analysis of all patients treated with rituximab-containing regimen showed that three to four cycles of R-CHOP plus radiotherapy tended to be similar to six cycles of R-CHOP in terms of PFS and OS. These results suggested that the optimal treatment strategy for localized-stage PG-DLBCL in the rituximab era, in other words, the relative merit of three cycles of R-CHOP followed by involved field radiation versus six cycles of R-CHOP thus remains uncertain. Our results support the use of six cycles of R-CHOP without involved field radiation as an important treatment option for localized-stage PG-DLBCL in the rituximab era.

With regard to advanced-stage PG-DLBCL, our study showed that 3-year OS was 64% with half proportion of high-risk group (IPI score ≥3). However, compared with previous study in patients with DLBCL treated with R-CHOP chemotherapy, 3-year OS was similar to patients with DLBCL in high-risk group [14]. In fact, 7 of 12 patients who developed PD or RD died within 1 year after PD or RD despite the use of salvage therapies, and five of eight patients who developed RD did not achieve CR despite salvage therapies. Considering this poor survival for advanced disease, another therapeutic strategy should be developed. In our case, one patient who received autologous stem cell transplantaion (ASCT) in the initial treatment survived without relapse at the end of the study. ASCT in the initial treatment might be worthy of evaluation as a treatment option for advanced patients especially with elevated LDH level as a poor prognostic factor.

We found two notable remarks in the site of relapse. First, relapse in the stomach was frequent, and *H. pylori* eradication therapy should be performed even if CR was obtained, especially in patients with DLBCL with MALT component. Second, CNS relapse was frequent when time to relapse was short (median, 5.4 months). Given previous findings that early relapse in the CNS within 6 months of initial therapy might have been due to subclinical CNS involvement at the time of diagnosis, however, this finding requires careful interpretation [17]. Of the two patients experiencing CNS relapse in the present study, neither of patients had undergone CNS evaluation at initial diagnosis, and the possibility of subclinical CNS involvement at the time of initial diagnosis could not be excluded.

Massive hemorrhage, gastric obstruction, or gastric perforations in patients with PG-DLBCL are surgical events related to chemotherapy and radiotherapy. In previous studies, the rate of these complications with chemotherapy with or without rituximab was 12% to 25% [18, 19]. In our study, however, the rate of surgical events was 7% with no gastric perforation, suggesting that the frequency of surgical complications was not high in the rituximab era.

Several limitations of our study warrant mention. First, this retrospective study might have been influenced by unrecognized bias. Second, the number of treatment courses was not standardized and thus treatment intensity varied. This variation in our present study, which was also present in previous clinical trials for localized DLBCL [9, 20], might have lead to the underestimation of effects.

In conclusion, we found the clinical outcome in patients with localized-stage PG-DLBCL treated with three cycles of R-CHOP plus radiotherapy tended to be similar to six cycles of R-CHOP with an extremely favorable effect. Furthermore, the clinical outcome in patients with advanced-stage PG-DLBCL might be poor even in the rituximab era. Further prospective analyses are warranted.

Acknowledgments We are indebted to Dr. Tatsuya Ito, Department of Hematology, Anjo Kosei Hospital, and Dr. Hisamitsu Suzuki, Department of Hematology, Okazaki Municipal Hospital, for their contribution to the collection of patient data.

Funding This work was supported in part by a Grant-in-aid for Cancer Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and a Grant-in-aid for delineation of molecular biological profile of the refractory lymphoid malignancy and the development of its tumor type-specific management from the Ministry of Health, Labor, and Welfare, Japan.

Conflict of interest disclosure The authors declare no competing financial interests.

References

1. Koch P, del Valle F, Berdel WE, et al. German Multicenter Study Group. Primary gastrointestinal non-Hodgkin's lymphoma: II. Combined surgical and conservative or conservative management only in localized gastric lymphoma—results of the prospective German Multicenter Study GIT NHL 01/92. J Clin Oncol. 2001;19:3874–83.
2. Brands F, Monig SP, Raab M (1997) Treatment and prognosis of gastric lymphoma. Eur J Surg 163:803–813, Review
3. Aviles A, Nambo MJ, Neri N et al (2004) The role of surgery in primary gastric lymphoma: results of a controlled clinical trial. Ann Surg 240:44–50
4. Ishikura S, Tobinai K, Ohtsu A et al (2005) Japanese multicenter phase II study of CHOP followed by radiotherapy in stage I-II, diffuse large B-cell lymphoma of the stomach. Cancer Sci 96:349–352
5. Martinelli G, Gigli F, Calabrese L et al (2009) Early stage gastric diffuse large B-cell lymphomas: results of a randomised trial comparing chemotherapy alone versus chemotherapy+involved field radiotherapy. (IELSG 4). Leuk Lymphoma 50:925–931
6. Salles G, Herbrecht R, Tilly H et al (1991) Aggressive primary gastrointestinal lymphomas: review of 91 patients treated with the LNH-84 regimen. A study of the Groupe d'Etude des Lymphomes Agressifs. Am J Med 90:77–84
7. Coiffier B, Salles G (1997) Does surgery belong to medical history for gastric lymphomas? Ann Oncol 8:419–421
8. Coiffier B, Lepage E, Briere J et al (2002) CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 346:235–242
9. Pfreundschuh M, Trümper L, Osterborg A, MabThera International Trial Group et al (2006) CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. Lancet Oncol 7:379–391
10. Wöhrer S, Püspök A, Drach J et al (2004) Rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) for treatment of early-stage gastric diffuse large B-cell lymphoma. Ann Oncol 15:1086–1090
11. Avilés A, Castañeda C, Cleto S et al (2009) Rituximab and chemotherapy in primary gastric lymphoma. Cancer Biother Radiopharm 24:25–28
12. Lewin KJ, Ranchod M, Dorfman RF (1978) Lymphomas of the gastrointestinal tract: a study of 117 cases presenting with gastrointestinal disease. Cancer 42:693–707
13. Rohatiner A, d'Amore F, Coiffier B et al (1994) Report on a workshop convened to discuss the pathological and staging classifications of gastrointestinal tract lymphoma. Ann Oncol 5:397–400
14. Sehn LH, Berry B, Chhanabhai M et al (2007) The revised International Prognostic Index (R-IPI) is a better predictor of outcome than the standard IPI for patients with diffuse large B-cell lymphoma treated with R-CHOP. Blood 109:1857–1861
15. Swerdlow SH, Campo E, Harris NL et al (2008) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. International Agency for Research on Cancer, Lyon
16. Hans CP, Weisenburger DD, Greiner TC et al (2004) Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 103:275–282
17. Bernstein SH, Unger JM, Leblanc M, Friedberg J, Miller TP, Fisher RI (2009) Natural history of CNS relapse in patients with aggressive non-Hodgkin's lymphoma: a 20-year follow-up analysis of SWOG 8516–the Southwest Oncology Group. J Clin Oncol 27:114–119
18. Huang J, Jiang W, Xu R et al (2010) Primary gastric non-Hodgkin's lymphoma in Chinese patients: clinical characteristics and prognostic factors. BMC Cancer 10:358
19. Spectre G, Libster D, Grisariu S et al (2006) Bleeding, obstruction, and perforation in a series of patients with aggressive gastric lymphoma treated with primary chemotherapy. Ann Surg Oncol 13:1372–1378
20. Persky DO, Unger JM, Spier CM et al (2008) Phase II study of rituximab plus three cycles of CHOP and involved-field radiotherapy for patients with limited-stage aggressive B-cell lymphoma: Southwest Oncology Group Study 0014. J Clin Oncol 26:2258–2263

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木下朝博

臨床血液　第53巻第2号 別刷
(2012年2月)