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MYC translocation and/or BCL 2 protein expression are associated with poor prognosis in diffuse large B-cell lymphoma

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Key words

BCL2, BCL6, diffuse large B-cell lymphoma, MYC, prognostic factor

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Genomic alterations and protein expression levels have been established as prognostic factors for survival in patients with diffuse large B-cell lymphoma (DLBCL). In particular, double-hit DLBCL (DHL), which exhibits translocations in *MYC* and *BCL2* and/or *BCL6*, is known to be associated with a poor prognosis. However, the clinical significance of gene alterations and protein expression levels for MYC, B-cell lymphoma (BCL)2, and BCL6 are unclear. In this study, we analyzed 61 adult patients diagnosed with DLBCL without DHL, who were treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone, or similar regimens. There were no differences in the distribution of MYC expression rates among the different *MYC* gene statuses. In log–rank tests, *MYC* translocation was a prognostic factor for overall survival (OS; $P = 0.011$), whereas *BCL2* and *BCL6* translocation were not prognostic indicators ($P = 0.999$ and $P = 0.925$, respectively). Although the expression levels of MYC and BCL6 were not significantly associated with OS, the expression of BCL2 was a prognostic factor for OS ($P = 0.027$). Furthermore, copy number gains in the *MYC*, *BCL2*, and *BCL6* genes did not affect OS. *MYC* translocation (hazard ratio, 4.769; range, 1.518–14.98; $P = 0.007$) and BCL2 protein expression (hazard ratio, 3.072; range, 1.002–9.413; $P = 0.049$) were independent prognostic factors for survival in multivariate analyses. In conclusion, *MYC* translocation and BCL2 expression may need to be investigated at the initial diagnosis to predict prognosis in patients with DLBCL.

Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous, common type of aggressive B-cell lymphoma accounting for approximately one-third of all non-Hodgkin's lymphomas.[1,2] Rituximab combined with cyclophosphamide, vincristine, adriamycin, and prednisolone (R-CHOP) therapy has dramatically improved survival rates in patients with DLBCL,[3–6] however, approximately 30–40% of patients with DLBCL die from cancer-related complications.[7] In order to identify patients with DLBCL having poor prognoses, numerous studies have been undertaken to determine related prognostic factors. Clinically, the International Prognostic Index (IPI) is the best indicator for risk stratification.[8] Alternatively, according to biological and pathological features, gene expression profiling, immunohistochemistry (IHC) algorithms for detecting overexpression of specific proteins, and FISH for discovering chromosomal translocations have been developed for predicting prognosis.[9–14]

MYC, *BCL2*, and *BCL6* gene translocation and/or protein expression have been also intensively studied, with several reports showing the utility of these factors as prognostic markers.[15,16] Alterations in oncogenes such as *MYC* and anti-apoptotic genes such as *BCL2* are involved in the pathogenesis of DLBCL.[17] Deregulation of *MYC* and *BCL2* is thought to be caused by chromosomal translocation, gene copy number gains, and other mechanisms, such as transcriptional upregulation downstream of nuclear factor-κB signaling.[18] Translocation of *MYC*, *BCL2*, and *BCL6* genes, detected by FISH, has been reported to occur in approximately 10%, 14%, and 20% of patients with DLBCL, respectively.[16,19] Although the clinical impact of *BCL2* and *BCL6* gene translocations on prognosis is unclear, *MYC* translocation has been reported to predict prognosis.[20–24] However, the effects of *MYC* translocation alone on prognosis are still unclear owing to contrasting findings by different research groups. Notably, almost all studies have concluded that double-hit lymphoma (DHL), which contains translocations of *MYC* and *BCL2* and/or *BCL6*, is highly aggressive, with a poor prognosis compared with non-DHL DLBCL.[20–22,25,26]

Specific protein expression detected by IHC has been reported to predict prognosis in patients with DLBCL. However, data regarding MYC and B-cell lymphoma (BCL)2 protein expression, and the effects of these proteins on the survival of patients with DLBCL, are controversial.[16,19,27,28] Indeed, although some studies have shown that MYC expression by IHC can be used to predict prognosis in patients with DLBCL,[16,29,30] other studies have reported that there is no correlation between MYC expression by IHC and prognosis. Additionally, some studies have found that the addition of

rituximab to standard chemotherapy overcomes the adverse prognostic influence of BCL2 expression,[31,32] whereas others have shown that BCL2 expression remains a marker of poor prognostic in patients undergoing R-CHOP treatment.[33] Furthermore, several reports have indicated that double protein expression of MYC and BCL2 detected by IHC could be a prognostic indicator in patients with DLBCL. However, the combination of protein expression proportions with clinical applicability is still unclear, and no studies have shown the clinical utility of a combination of genomic translocation and protein expression patterns.

Therefore, in this study, we analyzed genomic alterations and protein expression levels of MYC, BCL2, and BCL6 using FISH and IHC in Japanese patients with DLBCL. We surveyed the clinical relationships among genomic alterations and protein expression patterns for MYC, BCL2, and BCL6 in patients with DLBCL. We also investigated whether dual protein expression of MYC and BCL2 and/or BCL6 could be a prognostic factor and analyzed the proportions of MYC and BCL2 and/or BCL6 expression by IHC that could be predictive for survival.

Materials and Methods

Patients and samples. We enrolled 64 adult patients newly diagnosed with DLBCL, not otherwise specified between October 2003 and October 2012 at Niigata University Hospital (Niigata, Japan). Three patients with DHL were excluded, and 61 patients were analyzed. Diagnostic specimens were reviewed by two expert hematopathologists (H.M. and K.O.) according to the 2008 WHO classification. All patients were treated with R-CHOP or R-CHOP-like regimens as an initial standard therapy. Formalin-fixed, paraffin-embedded samples were obtained, and FISH analysis for *MYC*, *BCL2*, and *BCL6* was carried out at initial diagnosis in all patients. Initial treatment responses were evaluated by computed tomography (CT) scanning and/or PET-CT scanning at the end of the initial treatment. This study was carried out in accordance with recommendation of the Declaration of Helsinki and approved by the ethics review committee of Kurume University (Kurume, Japan).

Immunochemical staining. Tissue samples were processed as formalin-fixed, paraffin-embedded tissues according to standard institutional procedures. We created tissue microarrays from samples from 61 patients and undertook evaluations of IHC with antibodies using these microarrays. Antibodies (clones) used for IHC included anti-CD20 (L-26; DakoCytomation, Glostrup, Denmark), anti-BCL2 (clone124; DakoCytomation), anti-BCL6 (P1F6; Leica Microsystems, Wetzlar, Germany), anti-Multiple myeloma oncogene -1 (MUM -1) (MUM1p; DakoCytomation), anti-CD10 (56C6; Leica Microsystems), and anti-c-MYC (Y69) antibodies (Abcam, Cambridge, UK). Immunohistochemistry results were reviewed by two expert hematopathologists (H.M. and K.O.). Cut-off points for MYC, BCL2, and BCL6 protein expression were defined as DLBCL with 30% or more, 1% or more, and 30% or more positive cells, respectively, as recommended in previous studies.[12,15,34]

Fluorescence *in situ* hybridization analysis. The FISH analysis was carried out using specimens collected at the time of the initial diagnosis to detect chromosomal translocations and copy number gains. We used Vysis LSI MYC (Cat. No. 05J91-001), Vysis LSI BCL2 (Cat. No. 07J75-001), and Vysis LSI BCL6 (Cat. No. 05J68-001) dual-color break-apart rearrangement probes (Vysis/Abbott Molecular Diagnostics, Wiesbaden-Delkenheim, Germany). We used an Axio Imager M2 (Zeiss, Oberkochen, Germany) for microscopic evaluations. Cut-off levels for the break-apart probes were established by evaluating the split-signal distributions in samples of reactive lymphoid tissues, calculating the mean number of split signals. Cut-off levels were the same as those in previous studies.[19,35] If three or more gene copies were detected in tumor cells, the tumor was categorized as having copy number gains, as described in previous studies.[35,36]

Statistical evaluation. Clinicopathological characteristics of the patients were compared by χ^2-tests, Fisher's exact tests, and Mann–Whitney *U*-tests. Overall survival (OS) was defined as the time from diagnosis to the death or the last follow-up. Progression-free survival (PFS) was defined as the time from the first day of treatment to the day at which the disease progressed or the day of death from any cause. Kaplan–Meier estimates were used to predict the OS and PFS, as compared using log–rank tests. The effects of the study variables were assessed by multivariate analysis according to a Cox regression model for OS and PFS. All calculated *P*-values were two-sided, and results with *P*-values of less than 0.05 were considered statistically significant. All statistical analyses were carried out with EZR software.[37]

Results

Patient characteristics. The median age was 62 years (range, 17–85 years), and the median follow-up period was 40 months (range, 2–127 months). The clinical features of this study cohort are shown in Table 1. In this cohort, 26 (42.3%) patients were men, and 35 (57.7%) were women. Thirty-nine patients (63.9%) showed high lactate dehydrogenase levels, and 30 patients (49.2%) had IPI scores of 3–5. Moreover, 34 patients (55.7%) had late-stage (III–IV) cancer according to the Ann Arbor classification. Fifty cases (82.0%) achieved complete response.

Hans classification, FISH, and IHC status for each MYC status. Among the 61 patients, 45 cases of DLBCL (73.8%) were of germinal center B-cell type, and 16 (26.2%) were of non- germinal center B-cell type. Six patients (8.8%) exhibited *MYC* translocations, seven patients (11.5%) had *BCL2* translocations, and eight patients (13.1%) harbored *BCL6* translocations.

We subsequently compared the cells of origin, FISH results, and IHC results among the three groups (*MYC* translocation group, *MYC* copy number gains group, and normal *MYC* group). Hans classification, FISH, and IHC status in these three groups are shown in Table 2. There were no significant differences between the *MYC* translocation group ($n = 6$) and the normal *MYC* group ($n = 42$), or between the *MYC* copy number gains group ($n = 13$) and the normal *MYC* group ($n = 42$).

Association between genomic alterations and protein expression for MYC, BCL2, and BCL6. Next, we analyzed the associations between gene alterations detected by FISH and protein expression measured by IHC. We statistically compared the differences in the distributions of positive MYC staining rates by IHC between the *MYC* translocation group and normal *MYC* group and between the *MYC* copy number gains group and normal *MYC* group. There were no significant differences between the two comparisons (Fig. 1a,b). Similar results were observed for *BCL2* and *BCL6* (Fig. 1c–f). Examples of pathological images of DLBCL cases in this study are shown in Fig. S1.

Table 1. Characteristics of patients with diffuse large B-cell lymphoma (*n* = 61)

Patient characteristic	*n*	%
Sex		
Male	26	42.3
Female	35	57.3
Age, median (range), years	62 (17–85)	
≥61 years	39	63.9
<61 years	22	36.1
Stage		
I/II	27	44.3
III/IV	34	55.7
Serum LDH		
Normal	22	36.1
Elevated	39	63.9
ECOG performance status		
0–2	44	72.1
3–5	17	27.9
Extranodal sites		
≥2	9	14.8
<2	52	85.2
IPI score		
0	6	9.8
1	15	24.6
2	10	16.4
3	20	32.8
4	8	13.1
5	2	3.3
IPI 0–2	31	50.8
IPI 3–5	30	49.2
Initial therapy response		
Complete response	50	82.0
Partial response	5	8.2
Stable disease	2	3.3
Progressive disease	4	6.5

ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; LDH, lactate dehydrogenase.

Survival analysis. In the analysis by log–rank tests, high IPI score (IPI 3–5) was a significant prognostic factor for poor PFS ($P = 0.006$) and OS ($P = 0.0045$; Fig. 2a,b). In contrast, cell of origin by Hans classifier was not a prognostic factor for PFS ($P = 0.207$) or OS ($P = 0.093$; Fig. 2c,d).

MYC translocation was a prognostic factor for PFS ($P = 0.015$) and OS ($P = 0.006$; Fig. 3a,b), whereas copy number gain in the *MYC* gene had no effect on survival (Fig. 3c,d). Although copy number gain in the *BCL2* gene was not a prognostic factor for OS, it was a prognostic factor for PFS (log–rank test). Translocation and copy number gain in the *BCL6* gene were not prognostic factors for PFS or OS (Figs S2,S3).

We subsequently investigated whether protein expression patterns of *MYC*, *BCL2*, and *BCL6* by IHC affected survival. There was no significant association between MYC expression and survival in patients with DLBCL (Fig. 4a,b). However, patients with DLBCL with 1% or more BCL2 expression showed poorer prognoses than those without BCL2 expression (Fig. 4c,d). There was no significant association between BCL6 expression and survival in patients with DLBCL (Fig. 4e,f).

Univariate and multivariate analysis. Univariate and multivariate analyses showed that *MYC* translocation (hazard ratio [HR], 4.227 [range, 1.385–12.90] for univariate analysis; HR, 4.769 [range, 1.518–14.98] for multivariate analysis) and 1% or more BCL2 expression (HR, 3.481 [range, 1.158–10.46] for univariate analysis; HR, 3.072 [range, 1.002–9.413] for multivariate analysis) were independent prognostic factors for OS. Furthermore, *MYC* translocation (HR, 3.353 [range, 1.089–10.32] for univariate analysis; HR, 5.645 [range, 1.725–18.47] for multivariate analysis) and 1% or more *BCL2* expression (HR, 3.838 [range, 1.433–10.28] for univariate analysis; HR, 3.776 [range, 1.389–10.27] for multivariate analysis) were independent prognostic factors for PFS (Table 3). Although IPI 3–5 was a prognostic factor for PFS in univariate and multivariate analyses, it was not a prognostic factor for OS in univariate or multivariate analyses.

Table 2. Results of Hans classifier, FISH, and immunohistochemistry (IHC) analyses in patients with diffuse large B-cell lymphoma with *MYC* translocation (A), *MYC* copy number gains (B), or normal *MYC* (C)

	All patients (*n* = 61)		(A) *MYC* translocation (*n* = 6)		(B) *MYC* amplification (*n* = 13)		(C) *MYC* normal (*n* = 42)		(A)–(C)	(B)–(C)
	n	%	*n*	%	*n*	%	*n*	%	*P*-value	*P*-value
Hans classification										
GCB type	45	73.8	5	83.3	12	92.3	28	66.7	0.650	0.086
Non-GCB type	16	26.2	1	16.7	1	7.7	14	33.3		
FISH results										
MYC translocation	6	8.8	6	100	0	0.0	0	0.0	N.A.	N.A.
BCL2 translocation	7	11.5	0	0.0	0	0.0	7	100.0	0.573	0.179
BCL6 translocation	8	13.1	0	0.0	0	0.0	8	100.0	0.571	0.176
IHC results										
MYC ≥30%	32	52.3	4	66.7	8	61.5	20	47.6	0.666	0.528
BCL2 ≥1%	36	59.0	3	50.0	7	53.8	26	61.9	0.669	0.748
BCL6 ≥30%	42	68.9	2	33.3	12	92.3	28	66.7	0.200	0.087
MYC ≥30% and BCL2 ≥1%	22	36.1	2	33.3	4	30.8	16	38.1	1.000	0.749
MYC ≥30% and BCL6 ≥30%	24	39.3	1	16.7	8	61.5	15	35.7	0.648	0.119

N.A., not available; BCL, B-cell lymphoma; GCB, germinal center B-cell.

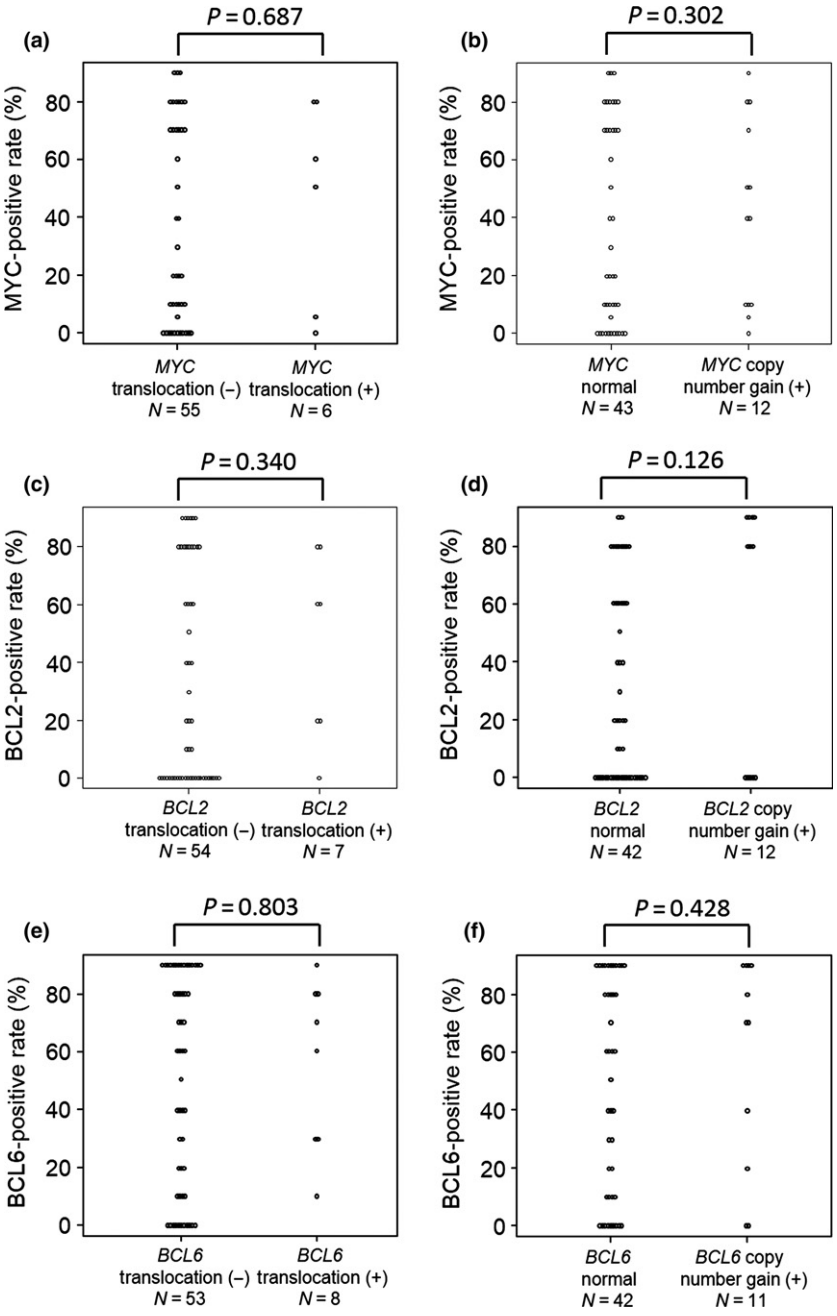

Fig. 1. Distributions of positive rates of MYC, B-cell lymphoma (BCL)2, and BCL6 immunohistochemical staining in different groups of patients with diffuse large B-cell lymphoma. (a) Correlation between MYC staining and *MYC* translocation. (b) Correlation between *MYC* copy number gains and normal *MYC*. (c,d) Correlations between BCL2 staining and *BCL2* translocation (c) and between BCL2 staining and *BCL2* copy number gains (d). (e,f) Correlations between BCL6 staining and *BCL6* translocation (e) and between BCL6 staining and *BCL6* copy number gains (f).

From the above results, *MYC* translocation detected by FISH and BCL2 expression measured by IHC were prognostic factors for poor OS and PFS. Patients who had *MYC* translocation and/or *BCL2* expression showed markedly poorer clinical outcomes than other patients (OS, $P = 0.003$; PFS, $P = 0.009$; Fig. 5).

Discussion

In this study, we found that *MYC* translocation detected by FISH and BCL2 expression detected by IHC were important factors for predicting the prognosis of patients with DLBCL. Additionally, copy number gains in *MYC*, *BCL2*, and *BCL6* were not prognostic factors for OS.

Our results showed that IPI, but not cells of origin by Hans classifier, was a prognostic factor for OS and PFS. Interestingly, before the use of rituximab (an anti-CD20 mAb), IPI and cells of origin were representative prognostic indicators in patients with DLBCL.[8,12] However, rituximab has been reported to overcome the impairments caused by cell of origin,[38] and IPI is currently the primary clinical tool used to predict outcomes in patients with DLBCL, even in the post-rituximab era.[39] Therefore, based on these previous works, our study cohort was assumed to be adequate for analysis.

Our study showed that *MYC* translocation was an independent prognostic factor for PFS and OS, although *BCL2* and *BCL6* translocations were not prognostic factors. Individually, *BCL2* and *BCL6* translocations are not thought to be prognostic factors in patients undergoing rituximab-based therapy.[16,19] However, *MYC* translocation has been reported to be a prognostic factor in patients with DLBCL treated with R-CHOP therapy,[20–24] although these results are controversial.[19] According to a

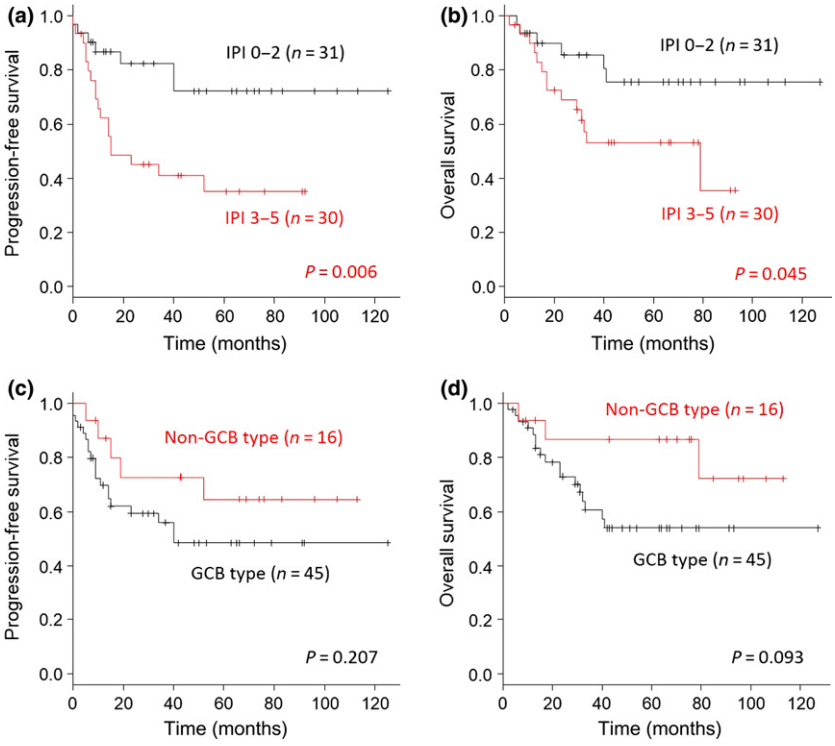

Fig. 2. Progression-free survival (PFS) and overall survival (OS) according to the International Prognostic Index (IPI) and cells of origin in patients with diffuse large B-cell lymphoma. (a,b) Analysis of IPI score as a prognostic factor for PFS (a) and OS (b). (c,d) Analysis of cells of origin (Hans classifier) as a prognostic factor for PFS (c) and OS (b). GCB, germinal center B-cell.

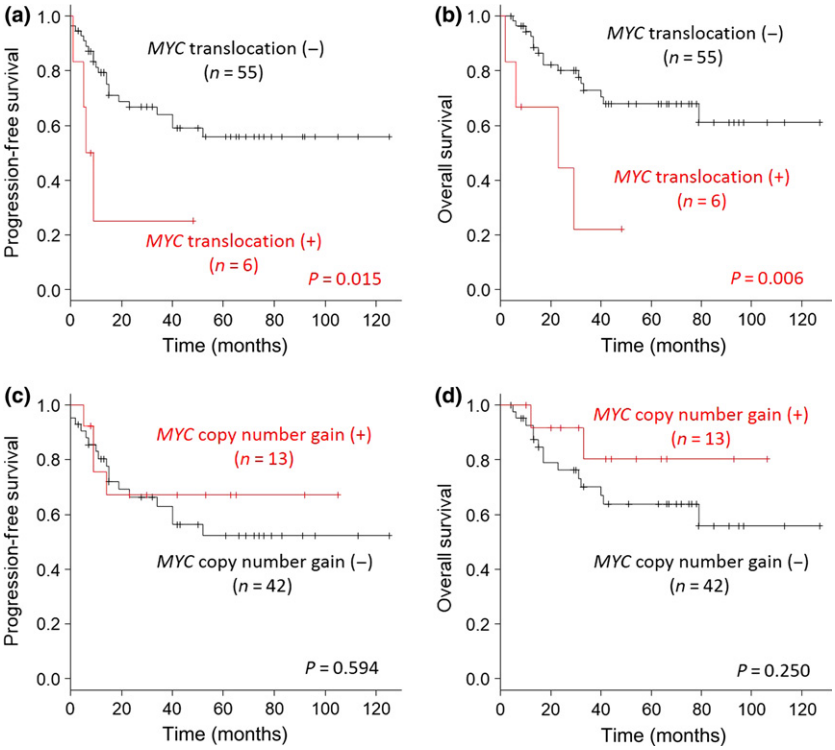

Fig. 3. Progression-free survival (PFS) and overall survival (OS) according to *MYC* translocation and copy number gains in patients with diffuse large B-cell lymphoma. (a,b) Analysis of *MYC* translocation as a prognostic factor for PFS (a) and OS (b). (c,d) Analysis of *MYC* copy number gains as a prognostic factor for PFS (c) and OS (d).

previous report examining *MYC* single translocation cases in DLBCL,[40] fusion of the *MYC* gene and immunoglobulin (IG) gene (IG heavy chain gene [*IGH*] or light chain genes κ [*IGK*] or λ [*IGL*]) was reported to be associated with a poorer prognosis than that of cases without fusion of the *MYC* gene and immunoglobulin gene (non-IG). In this study, we confirmed the detection *MYC/IGH* fusion by FISH in three of six cases (50%). Although there was no significant difference in prognosis between *MYC/IGH* fusion-positive cases ($n = 3$) and non-*MYC/IGH* fusion cases ($n = 3$), there may be some cases with *MYC* and *IGK* or *IGL* in non-*MYC/IGH* cases. Fusion of the *MYC* gene and *IGK* or *IGL* should also be investigated among cases without *MYC/IGH* fusion. Future studies are needed to identify and characterize *MYC* translocation partners.

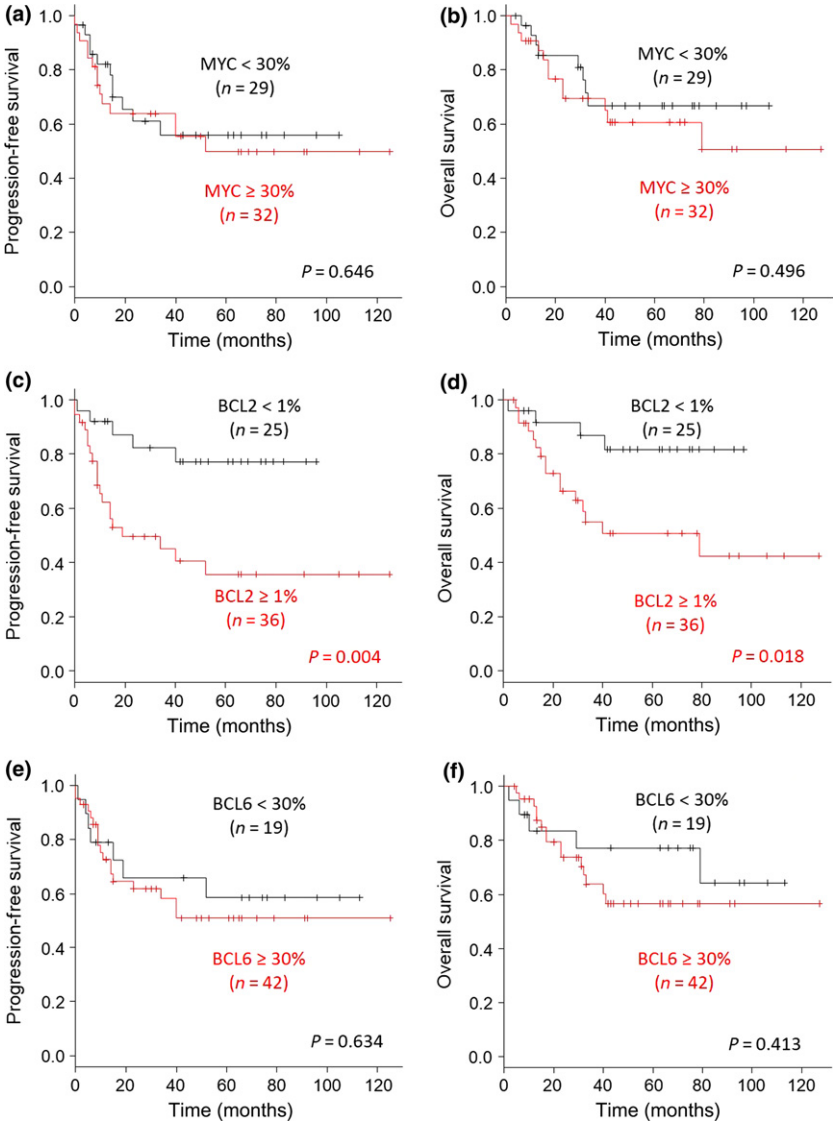

Fig. 4. Progression-free survival (PFS) and overall survival (OS) according to MYC, B-cell lymphoma (BCL)2, and BCL6 protein expression in patients with diffuse large B-cell lymphoma. (a,b) Analysis of clinical differences in PFS (a) and OS (b) between the ≥30% MYC expression group and the <30% expression group. (c,d) Analysis of ≥1% BCL2 expression as a prognostic factor for PFS (c) and OS (d). (e,f) Analysis of <30% BCL6 expression as a prognostic factor for PFS (e) and OS (f).

The clinical significance of MYC, BCL2, and BCL6 expression as evaluated by IHC has recently been extensively studied. However, the specific effects of MYC, BCL2, and BCL6 expression in patients with DLBCL treated with R-CHOP therapy remain unclear. These conflicting results may be explained by the differences in cut-off values for each study. For example, 40% or more MYC protein expression detected by IHC has been reported to be a prognostic factor in several studies;[15,16,19,35] however, our results showed that overexpression of MYC at any cut-off level had no effect on survival in patients with DLBCL (Fig. S4). Similarly, BCL6 protein expression at any cut-off level had no effect on survival (data not shown). In contrast, 1% or more BCL2 protein expression as detected by IHC was a prognostic factor for survival in this study. The reasons for setting the cut-off value of BCL2 expression to ≥1% were: to extract as many patients with poor prognosis as possible, to be able to judge clearly and easily whether there was expression of BCL2 for applications by pathologists in daily clinical practice, and to suggest the possibility that a BCL2 inhibitor could be used for as many patients with BCL2-positive DLBCL as possible. Recently, BCL2 inhibitor has been suggested to be effective for DLBCL.[41] Based on these considerations, the cut-off value of BCL2 was set to ≥1% in this study. A previous report also showed that 1% or more BCL2 protein expression was a prognostic factor in patients with DLBCL.[19] Moreover, BCL2 protein expression at any cut-off value was found to affect OS (Fig. S5). Several studies have shown that R-CHOP therapy overcomes the clinical effects of BCL2 expression.[15,31,32] In contrast, some researchers concluded that BCL2 expression remains an adverse prognostic marker in patients treated with R-CHOP.[16,19,33] Based on our current results, we suggest that BCL2 staining may be important for predicting prognosis in patients with DLBCL.

Coexpression of MYC and BCL2 protein in DLBCL, termed double-expresser lymphoma, has been suggested to be a prognostic factor. However, the cut-off values for MYC and BCL2 expression have not been consistent among published reports.[16,19,27,28,42,43] In addition, because these studies have included DHL, which has been shown to be associated with a poor prognosis, the results may reflect the heterogeneous nature of DLBCL.[44] Although we investigated whether coexpression of MYC and BCL2 at any cut-off level, only coexpression of MYC ≥ 30% and BCL2 ≥ 30% was a

Table 3. Univariate and multivariate analysis for predicting prognosis of diffuse large B-cell lymphoma

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Overall survival				
IPI 3–5	2.576 (0.985–6.735)	0.054	2.453 (0.926–6.498)	0.071
MYC translocation	4.227 (1.385–12.90)	0.011	4.769 (1.518–14.98)	0.007
BCL2 translocation	1.001 (0.231–4.333)	0.999		
BCL6 translocation	1.061 (0.310–3.627)	0.925		
MYC copy number gain	0.906 (0.329–2.497)	0.849		
BCL2 copy number gain	1.620 (0.582–4.506)	0.356		
BCL6 copy number gain	0.890 (0.297–2.667)	0.835		
MYC expression ≥30%	1.361 (0.556–3.334)	0.499		
BCL2 expression ≥1%	3.481 (1.158–10.46)	0.027	3.072 (1.002–9.413)	0.049
BCL6 expression ≥30%	1.526 (0.548–4.249)	0.418		
Progression–free survival				
IPI 3–5	3.169 (1.321–7.600)	0.009	3.248 (1.331–7.924)	0.009
MYC translocation	3.353 (1.089–10.32)	0.035	5.645 (1.725–18.47)	0.004
BCL2 translocation	1.885 (0.636–5.583)	0.253		
BCL6 translocation	0.740 (0.221–2.475)	0.625		
MYC copy number gain	1.129 (0.470–2.709)	0.786		
BCL2 copy number gain	2.163 (0.896–5.218)	0.086		
BCL6 copy number gain	0.681 (0.234–1.987)	0.482		
MYC expression ≥30%	1.182 (0.536–2.605)	0.679		
BCL2 expression ≥1%	3.838 (1.433–10.28)	0.007	3.776 (1.389–10.27)	0.009
BCL6 expression ≥30%	1.235 (0.514–2.965)	0.637		

BCL, B-cell lymphoma; CI, confidence interval; HR, hazard ratio; IPI, International Prognostic Index.

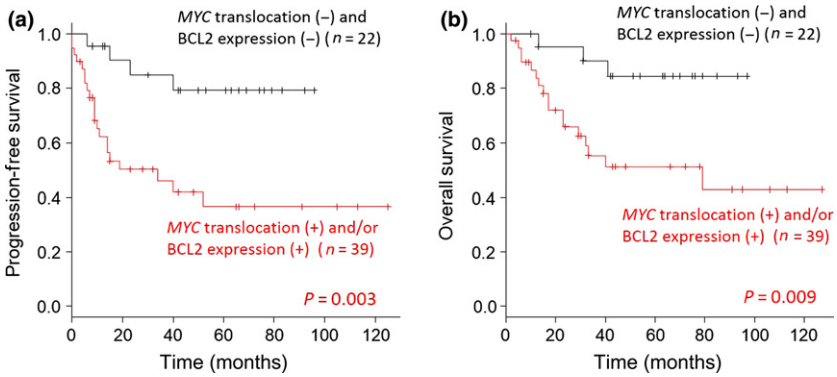

Fig. 5. Progression-free survival (a) and overall survival (OS) (b) according to *MYC* translocation detected by FISH or B-cell lymphoma (BCL)2 expression detected by immunohistochemistry in patients with diffuse large B-cell lymphoma.

prognostic factor (data not shown; OS, $P = 0.0024$; PFS, $P = 0.0017$). Namely, excluding coexpression of MYC ≥ 30% and BCL2 ≥ 30%, the coexpression of MYC and BCL2 at any cut-off level including MYC ≥ 30% and BCL2 ≥ 1%, was not a prognostic factor (Fig. S6; OS, $P = 0.140$; PFS, $P = 0.176$). Patients with coexpression of MYC ≥ 30% and BCL2 1–29% ($n = 3$) showed comparatively good prognosis in this study. However, if we compared the respective prognosis curves, the clinical significance of coexpression of MYC and BCL2 was considered to be small because, in this study, the expression of BCL2 had stronger effects than MYC expression did on prognosis. In addition, double expression of MYC and BCL6 was not a prognostic factor for survival. However, survival of lymphoma cells in the context of coexpression of BCL2 and c-MYC has been shown to depend on BCL2 function, and inhibition of BCL2 function by ABT-737 (a selective inhibitor of BCL-2, BCL–extra large [BCL-xL], and BCL-w) could can also induce cell death in a mouse model of MYC-driven lymphoma.[45] This report also suggested that BCL2 expression may be more important for prognosis than overexpression of MYC in double-expresser lymphoma.

This study had several limitations. First, we included relatively few cases. Additionally, the study was carried out at a single center with a Japanese cohort. The number of patients in this study may have been too small to reach strong conclusions. Therefore, further studies are needed to establish the validity of our results in a large cohort.

In conclusion, MYC translocation as detected by FISH and BCL2 expression as measured by IHC may be important for predicting prognosis. Patients with DLBCL harboring *MYC* translocation as detected by FISH and BCL2 expression as detected by IHC may achieve improved outcomes using a therapeutic strategy including intensive chemotherapy rather than conventional R-CHOP therapy.

Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. MYC, B-cell lymphoma (BCL)2, and BCL6 detected by immunohistochemistry in patients with diffuse large B-cell lymphoma.

Fig. S2. Progression-free survival (a,c) and overall survival (b,d) according to translocation (a,b) and copy number gain (c,d) of *BCL2* in patients with diffuse large B-cell lymphoma.

Fig. S3. Progression-free survival (a,c) and overall survival (b,d) according to translocation (a,b) and copy number gain (c,d) of *BCL6* in patients with diffuse large B-cell lymphoma.

Fig. S4. Overall survival according to MYC protein expression in patients with diffuse large B-cell lymphoma.

Fig. S5. Overall survival according to B-cell lymphoma (BCL)2 protein expression in patients with diffuse large B-cell lymphoma.

Fig. S6. Progression-free survival (a,c) and overall survival (b,d) according to levels of MYC and B-cell lymphoma (BCL)2 or BCL6 expression in patients with diffuse large B-cell lymphoma.