

LETTER TO THE EDITOR

Impact of the double expression of MYC and BCL2 on outcomes of localized primary gastric diffuse large B-cell lymphoma patients in the rituximab era

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Diffuse large B-cell lymphoma (DLBCL) is a common subtype of primary gastric lymphoma, accounting for 50–60% of cases.¹ Prior to the rituximab era, a Japanese phase II trial evaluated three courses of cyclophosphamide, doxorubicin, vincristine and prednisolone (CHOP), followed by 40.5 Gy of involved field radiotherapy (IFRT) for localized (stage I, II₁ based on the Lugano Staging System for Gastrointestinal Lymphoma²) primary gastric DLBCL (PG-DLBCL) and the study yielded good therapeutic results.³ Rituximab plus CHOP (R-CHOP) therapy has been shown to improve the prognosis of DLBCL patients over that of patients treated with CHOP.^{4,5} The efficacy of three cycles of R-CHOP followed by IFRT for localized DLBCL was evaluated in a phase II trial by the Southwest Oncology Group and also showed favorable results.⁶ On the basis of these findings, a rituximab-containing regimen, particularly three cycles of R-CHOP followed by IFRT, is regarded as one of the standard therapies for localized DLBCL including PG-DLBCL. Recent studies demonstrated that the double rearrangement (double hit) of the *MYC* and *BCL2* genes and the double expression of the *MYC* and *BCL2* proteins were associated with a poor prognosis for patients with nodal DLBCL.^{7–10} However, the clinical impact of the double expression of *MYC* and *BCL2* on PG-DLBCL remains unknown. We retrospectively analyzed patients with localized PG-DLBCL who were initially treated with a rituximab-containing regimen, with a focus on the status of *MYC* and *BCL2*.

We retrospectively analyzed 52 consecutive patients newly diagnosed with localized PG-DLBCL at our institution between 2003 and 2013. They were initially treated or planned to be treated with a rituximab-containing regimen. All patients underwent standard staging procedures, including upper gastrointestinal endoscopy, bone marrow aspiration or biopsy, computed tomography, and/or fluorine-18-2-fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography and were assigned a clinical stage according to Lugano Staging System for Gastrointestinal Lymphomas.² This retrospective study was approved by the Institutional Review Board of the National Cancer Center in Japan. It was conducted in accordance with the international ethical recommendations stated in the Declaration of Helsinki and Japanese Good Clinical Practice Guidelines. Histopathological diagnoses were made and reviewed by two experienced hematopathologists (AMM and HT) according to the criteria of the WHO classification.¹¹ An immunohistochemical analysis of formalin-fixed, paraffin-embedded tissues was performed using a panel of monoclonal antibodies. *MYC* immunoreactivity was considered positive when the *MYC* protein was expressed in more than 40% of tumor cells. *BCL2* was considered positive when the *BCL2* protein was expressed in more than 70% of the tumor cells.⁷ Lymphoma cells were assigned a germinal center B-cell-like (GCB) or non-GCB phenotype using the Hans algorithm for cell-of-origin subtyping.¹² A fluorescence *in situ* hybridization analysis was performed on formalin-fixed, paraffin-

embedded tissue sections. The following probes were used: an LSI *MYC* dual-color break apart rearrangement probe and LSI *IGH/BCL2* dual-color dual-fusion translocation probe (Vysis, Downers Grove, IL, USA). Overall survival (OS) was defined as the interval between the date of diagnosis and date of death or last follow-up. Progression-free survival was defined as the interval between the date of diagnosis and date of death, disease progression or last follow-up. Survival analyses were performed using Kaplan–Meier's method and compared using the log-rank test. All *P*-values were based on two-sided tests and *P*-values < 0.05 were considered significant. Statistical analyses were performed using EZR version 1.27.¹³

The characteristics of all 52 patients are shown in Table 1. Twenty-four patients (46%) were male and 28 (54%) were female with a median age of 62 years (range: 29–85). Performance statuses were 0–1 in 47 patients (90%). Thirty patients (58%) presented with stage I disease, 15 (29%) with stage II₁, 2 (4%) with stage II₂ and 5 (9%) with stage IIE. Most patients (47 patients: 90%) had a low or low-intermediate risk according to the International Prognostic Index.¹⁴ Serum lactate dehydrogenase levels were normal in 45 patients (87%). Forty-eight patients (92%) lacked B symptoms.

The details of treatments were as follows: 43 patients (83%) received R-CHOP followed by IFRT, 7 (13%) received R-CHOP alone, 1 (2%) underwent total gastrectomy followed by rituximab because the patient denied chemotherapy and IFRT, and 1 (2%) received CHOP plus IFRT. The median number of CHOP cycles was three (range: 2–8). Most patients (43 patients: 83%) were treated with R-CHOP plus IFRT. The median dose of IFRT was 40 Gy (range: 30–40 Gy).

The Ki-67 index was ≥ 90% in 25 out of 46 evaluable patients (54%). The cell-of-origin subtype was assigned in 48 of the 52 patients (30 patients (63%) GCB and 18 patients (37%) non-GCB). The double expression of *MYC* and *BCL2* was assessed in 47 out of 52 patients (90%), and confirmed in 7 (15%). *MYC* break apart was detected in 1 out of 24 patients (4%) evaluated by fluorescence

Table 1. Patient characteristics

N = 52	n	(%)
Median age (range)	62 years	(29–85)
Male/female	24/28	(46/54)
PS 0–1	47	(90)
<i>Stage</i> ^a		
Stage I	30	(58)
Stage II ₁	15	(29)
Stage II ₂	2	(4)
Stage IIE	5	(9)
Normal serum LDH	45	(87)
IPI low, low-intermediate	47	(90)
B symptoms absent	48	(92)

Abbreviations: IPI, International Prognostic Index; LDH, lactate dehydrogenase; PS, performance status. ^aLugano Staging System for gastrointestinal lymphomas.

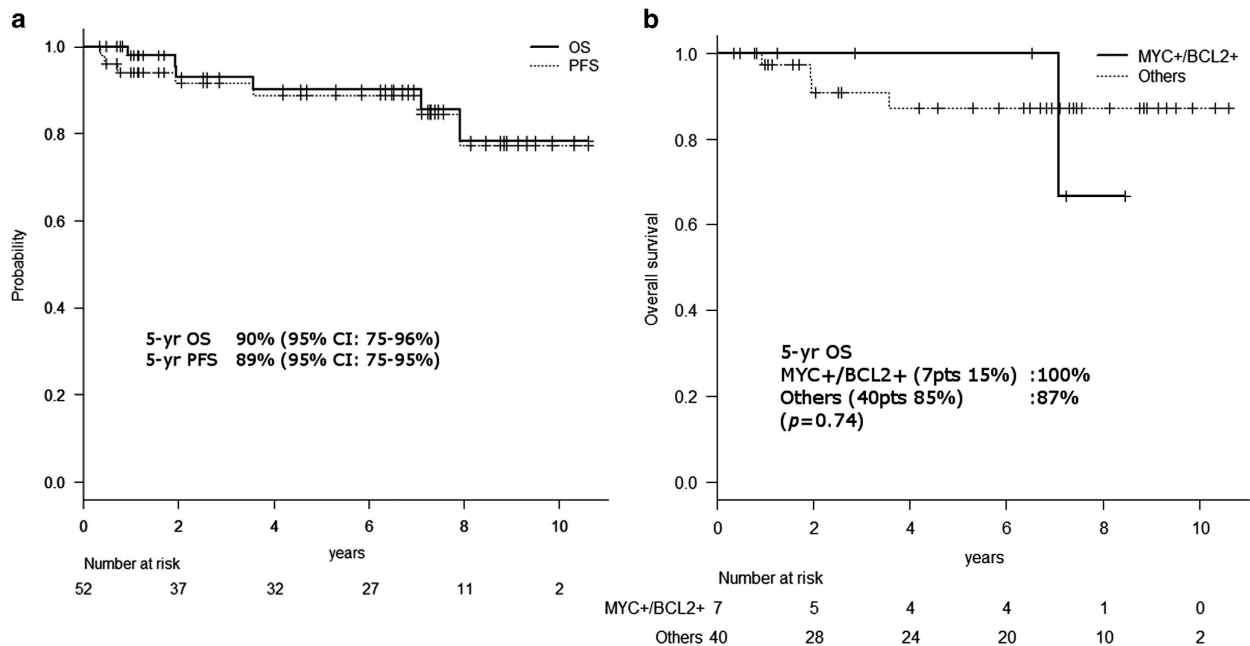


Figure 1. (a) Overall and progression-free survival (PFS) rates of all 52 patients. The estimated 5-year OS and PFS rates of all 52 patients were 90% (95% CI, 75–96%) and 89% (95% CI, 75–95%), respectively. (b) Overall survival rates according to the MYC/BCL2 status. Kaplan–Meier curves of OS rates according to the MYC/BCL2 status. No significant difference was observed between the two groups.

in situ hybridization. *IGH/BCL2* fusion was confirmed in 1 out of 27 evaluable patients (4%). *MYC* break apart and *IGH/BCL2* fusion were both assessed in 22 patients including 5 with the double expression of *MYC* and *BCL2*. However, no patient had the double hit of *MYC* break apart and *IGH/BCL2* fusion.

The median follow-up duration was 76 months (range: 4–127 months). Fifty patients (96%) achieved complete response, and the remaining two patients without the double expression of *MYC* and *BCL2* had primary refractory disease. The estimated 5-year OS and progression-free survival rates of all 52 patients were 90% (95% confidence interval (CI), 75–96%) and 89% (95% CI, 75–95%), respectively (Figure 1a). The estimated 5-year OS rates of patients with and without the double expression of *MYC* and *BCL2* were 100% and 87% (95% CI, 69–95%), respectively (Figure 1b). No significant difference was observed between the two cohorts ($P = 0.74$). The estimated 5-year OS rates of the GCB phenotype and non-GCB phenotype were 86% (95% CI, 63–96%) and 93% (95% CI, 59–99%), respectively, and this difference was not significant ($P = 0.99$). Six out of 52 patients died. The causes of death were as follows; two were due to the progression of DLBCL without the double expression of *MYC* and *BCL2*, and the remaining four died of other cancers without DLBCL.

The results of the present study revealed the good prognosis of patients with localized PG-DLBCL treated with rituximab-containing chemotherapy with or without IFRT, and demonstrated that double expression of *MYC* and *BCL2* did not influence patient outcomes. Although CHOP followed by IFRT is regarded as one of the standard therapies for localized PG-DLBCL, information in the rituximab era has been limited. Our study confirmed the efficacy and feasibility of R-CHOP plus IFRT, and this strategy is considered to be a reasonable standard therapy for localized PG-DLBCL in the rituximab era.

The prognosis of nodal DLBCL patients with the double expression of *MYC* and *BCL2* treated with R-CHOP therapy was significantly worse in previous studies,^{7–10} and it was also shown that the prognosis of patients with the double expression and double hit was not significantly different.⁷ There is currently no standard therapy for DLBCL patients with the double hit or double

expression. Several studies have suggested that more intensive chemotherapies than R-CHOP or stem cell transplantation may overcome the poor prognosis of double hit lymphoma.¹⁵ However, these hypotheses have mainly been discussed for nodal DLBCL. The results of the present study suggest that R-CHOP followed by IFRT is sufficient for most localized PG-DLBCL patients with the double expression of *MYC* and *BCL2*. On the other hand, as no double hit patients were included, it was not possible to evaluate the impact of the translocation in our study.

There were several limitations to this study. This was a retrospective analysis conducted at a single institution. The number of patients and events was too small to draw any definitive conclusions. Although most patients received R-CHOP plus IFRT, 17% were treated with other regimens. Specimen availability differed in each patient; therefore, the profiles of all patients were not assessed for the double hit or double expression of *MYC* and *BCL2*.

In conclusion, the results of the present study showed good prognoses, and suggest that the double expression of *MYC* and *BCL2* did not influence the outcomes of localized PG-DLBCL patients treated with rituximab-containing chemotherapy with or without RT. Further investigations are needed in order to confirm our results.

CONFLICT OF INTEREST

KY has received funding from Otsuka Pharmaceutical Co., Ltd, ARIAD Pharmaceuticals, Inc., Boehringer Ingelheim GmbH. TK, Zenyaku Kogyo has received funding from Chugai Pharmaceutical. The remaining authors declare no conflict of interest.

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