

# Patients with localized primary non-tonsillar oral diffuse large B-cell lymphoma exhibit favorable prognosis despite a non-germinal center B-cell-like phenotype

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Diffuse large B-cell lymphomas are detected frequently in the oral cavity. Although tonsillar lymphomas have been rather well characterized, lymphomas originating from non-tonsillar regions, such as the gingiva, palate, and tongue, have not been well studied. We examined the pathology of clinical samples obtained from 21 patients with localized primary non-tonsillar oral diffuse large B-cell lymphoma. Immunohistological examination of CD10, Bcl-6, and MUM1 determined that 17 of 21 (81%) samples exhibited non-germinal center B-cell type, an increased proportion of non-germinal center B-cell type compared with previous reports in samples of tonsillar origin ( $P < 0.05$ ). The four remaining samples exhibited germinal center B-cell type, although one sample expressed MUM1. Follow-up clinical survival data were obtained from the 17 patients over a range from 4 to 173 months (mean 52 months). All patients were treated with chemotherapies, irradiation, or surgical resection. Sixteen patients achieved complete remission and two patients relapsed, but no patient has died of disease. Extranodal diffuse large B-cell lymphomas of non-germinal center B-cell type are generally characterized by poor prognosis, regardless of localized disease. Interestingly, our results indicate that, unlike similar lymphomas of tonsillar origin, localized primary non-tonsillar oral diffuse large B-cell lymphomas exhibit favorable prognosis, suggesting that these lymphomas may be clinicopathologically distinct. (*Cancer Sci* 2009; 100: 42–46)

Primary oral cavity lymphoma accounts for less than 5% of all oral malignancies,<sup>(1)</sup> and only approximately 1% of all lymphomas and 2–12% of extranodal lymphomas.<sup>(1–3)</sup> A cohort of 83 patients with oral cavity lymphoma represents 1.4 and 2.5% of all lymphoma and extranodal lymphoma cases, respectively, from our surgical pathology files. Diffuse large B-cell lymphoma (DLBCL) is the most common kind of oral cavity lymphoma.<sup>(4)</sup> Although heterogeneous by nature, DLBCL can be distinguished by genetic profiling as two distinct subtypes: germinal center B-cell-like (GCB) phenotype and non-germinal center B-cell-like (non-GCB) phenotype.<sup>(5–7)</sup> Hans *et al.* reported that these two types of DLBCL can also be distinguished easily by immunohistological staining for CD10, BCL-6, and MUM1 proteins.<sup>(6)</sup> Notably, patients belonging to the former group present better prognosis than the latter group.

In recent studies, extranodal DLBCL were subclassified into two major prognostic categories: GCB type and non-GCB type (Table 1).<sup>(8–16)</sup> Whereas tonsillar DLBCL has been characterized extensively, to date there has been no clinicopathological characterization of non-tonsillar oral DLBCL, to the best of our knowledge. We sought to examine and characterize GCB versus

non-GCB types of localized primary non-tonsillar oral DLBCL, with particular focus on patient prognosis.

## Materials and Methods

**Patients and materials.** We selected 21 Japanese patients with localized primary non-tonsillar oral DLBCL diagnosed between 1990 and 2007. We selected localized-stage lymphomas only, because primary sites of advanced-stage lymphomas were difficult to determine. All cases were retrieved from the surgical pathology files of the Department of Pathology, Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, Okayama, Japan.

**Histological examination, immunohistochemistry, and *in situ* hybridization.** Surgically resected or biopsied specimens of oral DLBCL were fixed in 10% formaldehyde and embedded in paraffin. Serial sections (4  $\mu$ m) were cut from each paraffin-embedded tissue block, and several sections were stained with hematoxylin.

Immunohistochemistry was carried out on paraffin sections using an automated Benchmark XT slide stainer (Ventana Medical Systems). The primary antibodies used were: CD20 (L26, 1:200; Novocastra), CD3 epsilon (PS-1, 1:50; Novocastra), Bcl-2 (3.1, 1:200; Novocastra), CD5 (4C7, 1:100; Novocastra), CD10 (56C6, 1:50; Novocastra), CD138 (MI15, 1:100; Dako), MUM1 (MUM1p, 1:50; Dako), Bcl-6 (polyclonal, 1:50; Dako), and Ki-67 (MIB-1, 1:5000; Novocastra).

For each section, 10 high-power fields were recorded, quantitated, and averaged for the estimated percentage of positively immunostained cells. The following categories were defined: negative (<30% positively stained tumor cells) or positive (>30% positively stained tumor cells).<sup>(6)</sup> Ki-67 immunoreactivity was evaluated semiquantitatively using the average estimated percentage of positive cells in 10 recorded high-power fields.

*In situ* hybridization (ISH) with Epstein–Barr virus (EBV)-encoded small RNA (EBER) oligonucleotides was carried out to test for the presence of EBV small RNA in formalin-fixed paraffin-embedded sections using the automated Benchmark XT slide stainer.

**Statistical analysis.** All statistical analyses were carried out with SPSS software (version 14.0; SPSS Inc., Japan). Actuarial overall survival curves were calculated using the Kaplan–Meier method,<sup>(17)</sup> and differences were examined using the log-rank test to detect

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significant prognostic factors.<sup>(18)</sup> Overall survival was defined as the time from diagnosis to death from any cause or from last follow up.

## Results

**Characterization of patients with non-tonsillar oral DLBCL.** The median age of the 21 patients with localized primary non-tonsillar oral DLBCL was 68 years (range 53–86 years) (Table 2). There were 11 men and 10 women. Eleven patients (52%) presented with disease in the gingival mucosa, six patients (28%) in the hard palate mucosa, two patients (9%) in the soft palate mucosa, one patient (4%) in the dorsum of the tongue, and one patient (4%) in the buccal mucosa. The anatomical presenting site was typically a painless oral mucosal mass, which increased gradually in size. According to the Ann Arbor classification, 17 patients were clinical stage IE, and four patients were stage IIE. Thirteen

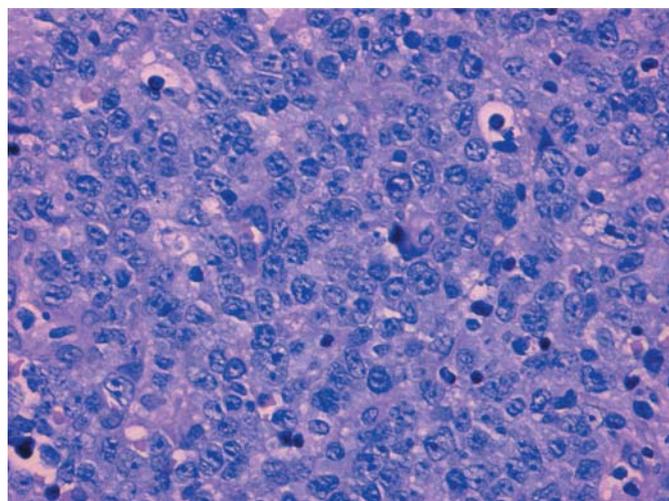
of 21 patients exhibited superficial ulceration of the tumor mass. Seven patients (33%) were at low–intermediate risk and the remaining 14 patients were at low risk according to the International Prognostic Index.<sup>(19)</sup> Histologically, all cases were classified as diffuse large B-cell lymphoma (Fig. 1). All patients were newly presenting, with no prior treatment history.

**Immunohistochemical and ISH analysis of non-tonsillar oral DLBCL.** The B-cell immunophenotype of the lymphomas was confirmed by immunoreactivity with antibodies to CD20 in 21 cases (Table 3). Although no cases were positive for CD5 and CD138, four cases (19%) were positive for CD10, 12 cases (57%) were positive for Bcl-2, and 10 cases (48%) were positive for BCL-6.

**Table 1. Immunophenotype of extranodal diffuse large B-cell lymphomas (DLBCL)**

Extranodal DLBCL	Major immunophenotype
Central nervous system <sup>(8)</sup>	Non-GCB
Breast <sup>(9)</sup>	Non-GCB
Stomach <sup>(10)</sup>	Non-GCB
Leg-type cutaneous <sup>(11)</sup>	Non-GCB
Testis <sup>(12)</sup>	Non-GCB
Intravascular type <sup>(13)</sup>	Non-GCB
Bone <sup>(14)</sup>	GCB
Intestine <sup>(10)</sup>	GCB
Tonsil <sup>(15)</sup>	GCB
Thyroid <sup>(16)</sup>	GCB
Non-leg cutaneous <sup>(11)</sup>	GCB

GCB, germinal center B-cell-like.



**Fig. 1.** Diffuse infiltration and proliferation of large lymphoma cells in oral mucosa. Hematoxylin–eosin staining. Original magnification  $\times 200$ .

**Table 2. Clinical data of 21 primary non-tonsillar oral diffuse large B-cell lymphomas**

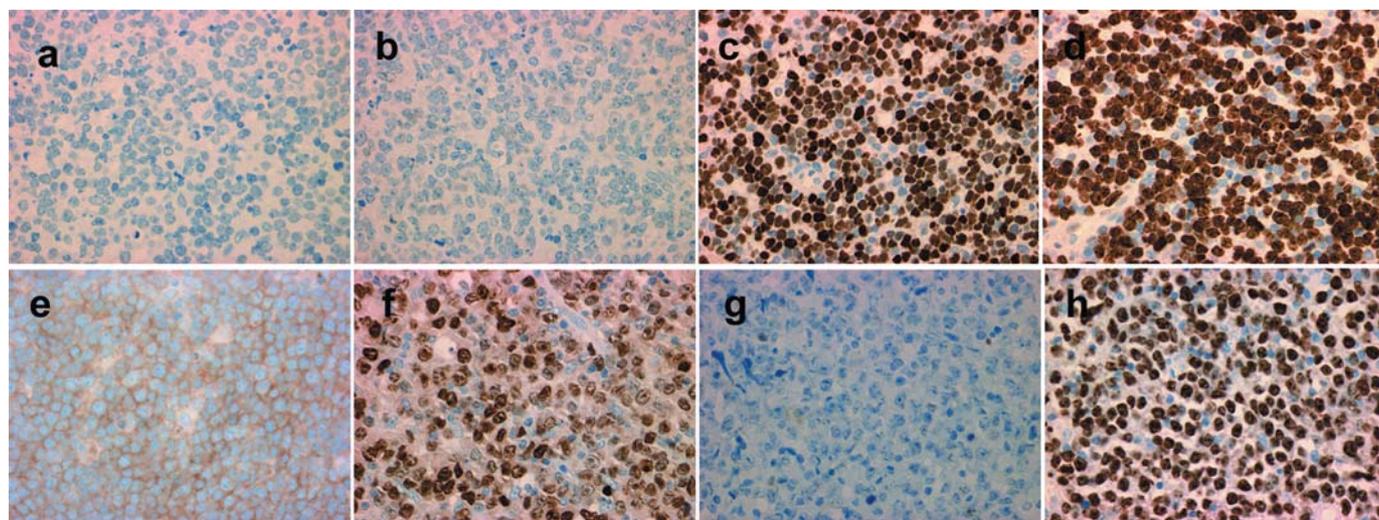
Patient no.	Age (years)	Sex	Primary site	IPI	LDH > normal values	Tumor size (maximum diameter; cm)	Ulcer	CS	Treatment	Relapse	Follow-up period (months)	Follow-up status
1	69	M	Hard palate	L	No	2.5	–	IE	CHOP + RT	+	59	Alive, FOD
2	57	F	Gingiva	L	No	2.5	+	IE	RT	–	79	Alive, FOD
3	65	F	Gingiva	LI	Yes	3.5	+	IE	R-CHOP + RT	–	13	Alive, FOD
4	68	F	Gingiva	LI	No	3.5	+	IE	CHOP + RT	–	22	Alive, FOD
5	60	M	Gingiva	L	No	2	+	IE	R-CHOP + RT	–	16	Alive, FOD
6	68	F	Gingiva	LI	Yes	5<	–	IE	NA	NA	NA	NA
7	62	M	Gingiva	LI	No	2, multiple	+	IE	R-CHOP	–	19	Alive, FOD
8	74	F	Hard palate	L	No	3	–	IE	CHOP + RT	–	129	Alive, FOD
9	85	M	Soft palate	L	No	3.5	–	IE	NA	NA	NA	NA
10	76	M	Gingiva	L	No	4	+	IE	R-THP-COP + RT	–	33	Alive, FOD
11	53	M	Hard palate	L	No	2	–	IE	CHOP	NA	NA	NA
12	72	F	Gingiva	L	No	3	–	IE	NA	NA	NA	NA
13	85	M	Hard palate	L	No	3	–	IE	R-CHOP	–	37	Alive, FOD
14	86	M	Soft palate	LI	Yes	2	+	IE	CHOP + RT	–	126	Alive, FOD
15	71	M	Buccal	L	No	4.5	+	IE	CHOP + RT	+	67	Alive, FOD
16	63	F	Hard palate	L	No	3	+	IE	CHOP + RT	NA	4	LTF
17	62	F	Gingiva	LI	Yes	1.5	+	IE	CHOP + RT	–	177	Alive, FOD
18	77	F	Gingiva	L	No	3.5	+	IIE	Partial resection + CHOP	–	31	Alive, FOD
19	57	M	Gingiva	L	No	3	–	IIE	R-CHOP	–	52	Alive, FOD
20	71	F	Tongue	L	No	2.5	+	IIE	THP-COP	–	34	Alive, FOD
21	67	M	Hard palate	LI	Yes	5<	+	IIE	CHOP + RT	–	29	Alive, FOD

CHOP, cyclophosphamide, adriamycin, vincristine, prednisolone; CS, clinical stage; F, female; FOD, free of disease; IPI, International Prognostic Index; L, low; LDH, lactate dehydrogenase; LI, low–intermediate; LTF, lost to follow up; M, male; NA, not available; R-, with rituximab; RT, radiation; THP-COP, cyclophosphamide, THP-doxorubicin (pirarubicin), vincristine, prednisolone.

**Table 3. Immunohistochemical findings of primary non-tonsillar oral diffuse large B-cell lymphoma**

Patient no.	EBER	CD3	CD5	CD10	CD20	CD138	BCL-6	MUM1	Bcl-2	Ki-67 labeling (%)	Immunophenotype
1	-	-	-	-	+	-	-	+	+	50	Non-GCB
2	-	-	-	-	+	-	-	+	-	85	Non-GCB
3	+	-	-	-	+	-	-	+	+	75	Non-GCB
4	-	-	-	-	+	-	-	+	-	60	Non-GCB
5	-	-	-	-	+	-	+	+	+	90	Non-GCB
6	-	-	-	+	+	-	+	-	+	80	GCB
7	+	-	-	-	+	-	+	+	+	70	Non-GCB
8	-	-	-	-	+	-	-	+	+	60	Non-GCB
9	-	-	-	-	+	-	-	+	+	80	Non-GCB
10	-	-	-	-	+	-	+	+	+	80	Non-GCB
11	-	-	-	-	+	-	-	+	-	90	Non-GCB
12	-	-	-	+	+	-	+	+	-	50	GCB
13	-	-	-	+	+	-	+	-	+	55	GCB
14	-	-	-	-	+	-	-	+	-	90	Non-GCB
15	-	-	-	-	+	-	-	+	+	90	Non-GCB
16	-	-	-	+	+	-	+	-	-	50	GCB
17	-	-	-	-	+	-	+	+	-	65	Non-GCB
18	-	-	-	-	+	-	+	+	-	80	Non-GCB
19	-	-	-	-	+	-	-	+	-	90	Non-GCB
20	-	-	-	-	+	-	+	+	+	40	Non-GCB
21	-	-	-	-	+	-	-	+	+	60	Non-GCB

EBER, Epstein-Barr virus-encoded small RNA; GCB, germinal center B-cell.



**Fig. 2.** The immunohistochemical panel for case 2 (upper row), belonging to the non-germinal center B-cell-like group, with (a) CD10-negative, (b) Bcl-6-negative, and (c) MUM1-positive staining, and (d) high proliferative activity as labeled by Ki-67. The immunohistochemical panel for case 6 (lower row), belonging to the germinal center B-cell-like group, with (e) CD10-positive, (f) Bcl-6-positive, and (g) MUM1-negative staining, and (h) high proliferative activity as labeled by Ki-67.

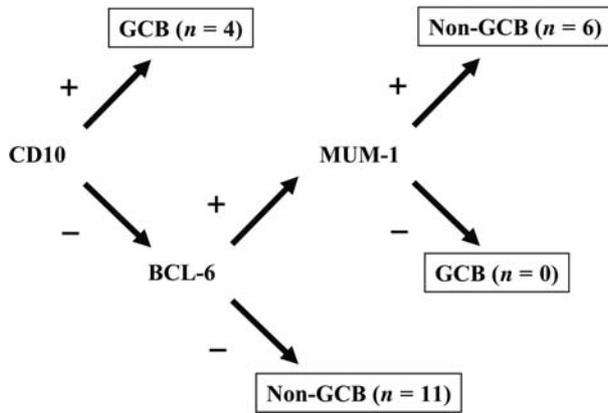
Ki-67  $\geq$  80% was observed in 10 cases (48%), and 18 cases (86%) displayed MUM1-positive staining. As shown in Figures 2 and 3, the four CD10-positive cases (19%) were classified as GCB type, and one of the four was positive for MUM1. Of the other 17 (81%), which were classified as non-GCB type, 11 were CD10-negative and BCL-6-negative, and six were CD10-negative, BCL-6-positive, and MUM1-positive. Of the 21 cases examined, EBER was detected in two cases (9%) by ISH.

**Clinical data and statistics analysis in patients with non-tonsillar oral DLBCL.** Follow-up clinical data were available for 17 patients. The duration of follow up ranged from 4 to 173 months (mean 52 months). Ten patients were treated initially with chemotherapy plus irradiation, five were treated with chemotherapy alone, one was treated with irradiation alone, and one was treated with

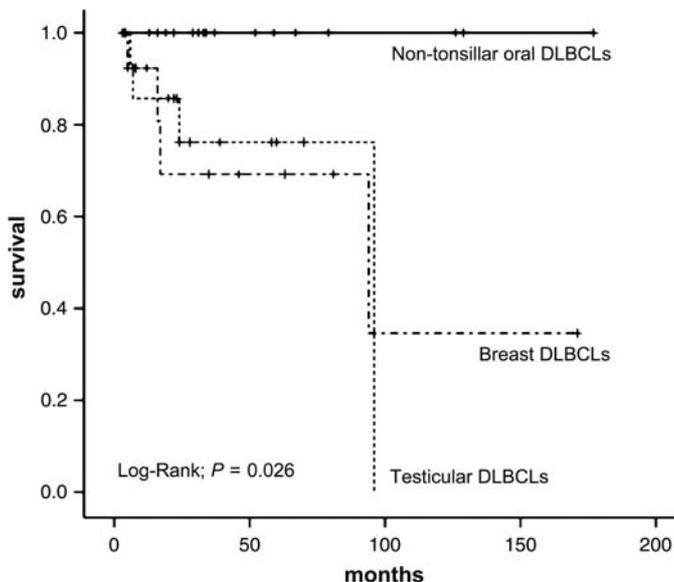
chemotherapy plus surgery. Sixteen patients achieved complete remission. Although two patients relapsed, complete remission was achieved with alternative chemotherapy. Initial treatments for the two relapsed patients were cyclophosphamide, adriamycin, vincristine, and prednisolone plus radiation. To date, 16 patients are disease free and no patients died of disease (Table 2). These results differ from those of previous reports on localized non-GCB type DLBCL (Fig. 4).<sup>(9,12)</sup>

## Discussion

Diffuse large B-cell lymphoma is the most frequent and aggressive lymphoma, and represents a heterogeneous group that includes *de novo* large-cell lymphomas, as well as transformed



**Fig. 3.** Distribution of germinal center B-cell-like (GCB) and non-GCB type non-tonsillar oral diffuse large B-cell lymphomas according to Hans *et al.*<sup>(6)</sup>



**Fig. 4.** Comparison of localized non-tonsillar oral diffuse large B-cell lymphomas (DLBCL) with localized testicular and breast DLBCL in overall survival.<sup>(9,12)</sup>

lymphomas from follicular or mucosa-associated lymphoid tissue lymphomas.<sup>(20)</sup>

Recent studies have clarified that DLBCL can be further sub-classified into two major prognostic categories: GCB type and non-GCB type.<sup>(5,6)</sup> Non-GCB type DLBCL had a significantly poorer prognosis than GCB type DLBCL.

We carried out immunohistochemical analysis of 21 patients with localized primary non-tonsillar oral DLBCL. Our results show that the majority (81%) of localized primary non-tonsillar oral DLBCL can be identified as non-GCB type, whereas Ree *et al.* reported that 51% of Asian tonsillar DLBCL are GCB type.<sup>(15)</sup> Comparing our data, there was a statistically significant difference ( $P < 0.05$ ,  $\chi^2$ -test) in the ratio of GCB type to non-GCB type between the subjects of the two studies. Moreover, one patient in our study was positive for CD10 and MUM-1, which was categorized as GCB type according to Hans *et al.*<sup>(6)</sup> However, Chang *et al.* reported that such double-positive expression

is characterized by poor prognosis similar to that of non-GCB type.<sup>(21)</sup> This outlying double expression pattern suggests that our patient cohort might in fact have more non-GCB type. Our data indicated that localized primary non-tonsillar oral DLBCL exhibit a rather uniform immunophenotype. Interestingly, patients with localized primary non-tonsillar oral DLBCL presented with a good clinical course, despite having non-GCB type.

Rituximab, a chimeric mouse–human monoclonal antibody against CD20, is used for monotherapy, as well as combination chemotherapy, against CD20-positive B-cell lymphoma.<sup>(22)</sup> Recent application of rituximab improved DLBCL patient prognosis, but only six patients in our cohort were treated with rituximab.

The oral cavity is very sensitive and it is easy to notice abnormalities, which might lead to earlier diagnosis of disease. This could be associated with rather favorable patient prognoses. However, unexpectedly almost all patients had tumors that were  $\geq 2$  cm in diameter.

According to previous reports, DLBCL of the central nervous system,<sup>(8)</sup> breast,<sup>(9)</sup> stomach,<sup>(10)</sup> leg type,<sup>(11)</sup> testis,<sup>(12)</sup> and intravascular type<sup>(13)</sup> predominantly exhibit non-GCB type, consistent with our study of localized primary non-tonsillar oral DLBCL cases. However, patients with central nervous system, breast, and testicular DLBCL exhibit poor prognosis, regardless of localized disease.<sup>(8,9,12,23)</sup> The patients with leg-type cutaneous DLBCL presented multiple skin lesions on one or both legs, and had disease-related 5-year survival of 45 and 36%, respectively, which is significantly poorer than non-leg-type cutaneous DLBCL.<sup>(11,24)</sup> In contrast, when comparing localized primary non-tonsillar oral DLBCL with localized non-GCB type DLBCL, there was a statistically significant difference in overall survival (Fig. 4). Localized-stage gastric DLBCL have good prognosis,<sup>(25,26)</sup> despite their non-GCB type. Connor and Ashton-Key have reported that gastric DLBCL might reflect a relationship with mucosa-associated lymphoid tissue (MALT) lymphoma.<sup>(10)</sup>

Our data show that localized primary non-tonsillar oral DLBCL have biologically similar behavior to DLBCL of gastric origin. The reasons for this remain unclear, but MALT lymphomas might show a histological transformation to high-grade lymphoma of this region (e.g. the stomach), although the incidences of MALT lymphoma in these organs are not similar.<sup>(27,28)</sup>

High-grade transformation might be recognized as such if a residual area of MALT lymphoma can be identified. In the absence of such areas, the tumors are morphologically indistinguishable from DLBCL.<sup>(29)</sup> We checked the biopsied specimens thoroughly, and there were no low-grade MALT lymphomatous components. As the biopsied specimens from the oral cavity were generally small in size, associated low-grade components could not be entirely excluded. Interestingly, Kojima *et al.* reported that the high-grade type of nodal marginal zone B-cell lymphoma was categorized as an indolent tumor.<sup>(30)</sup> They suggested that non-GCB type DLBCL arising from MALT-type nodal marginal zone B-cell lymphoma may be separated from conventional non-GCB type DLBCL.

The proliferating fraction of the lymphomas that we examined was generally high, with an average of 71%. Leval and Harris reported that a proliferating fraction of  $\geq 80\%$  is an adverse prognostic factor.<sup>(31)</sup> Although 48% of our cases showed a proliferating fraction of  $\geq 80\%$ , no significant correlation was detected in the patients' clinical courses and in the Ki-67 labeling index.

Previous reports have shown that lymphomas associated with overexpression of Bcl-2 behave aggressively and reduce overall survival.<sup>(32–36)</sup> In our series, however, although Bcl-2 expression was detected in 57% of the cases examined, there was no difference between the Bcl-2-positive and Bcl-2-negative groups.

In conclusion, we have demonstrated that localized primary non-tonsillar oral DLBCL exhibited uniform clinicopathological

characteristics, especially those with favorable prognoses in spite of the non-GCB phenotype. A review of previous reports indicated that in most extranodal sites, each organ has a clear deviation of GCB versus non-GCB in DLBCL, and the patients' prognoses depend on these lymphoma phenotypes, except for non-tonsillar oral lymphoma.

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