

Case Study

# Nodal Marginal Zone Large B-Cell Lymphoma with Burkitt Translocation and Complex Chromosomal Changes Associated with Overexpression of BCL2, MYC, and BCL6

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We report the first case of a nodal marginal zone large B-cell lymphoma and the first with *MYC* rearrangement. This high proliferation rate lymphoma (40% of cells) occurred in the bilateral cervical, axillary, and para-aortic lymph nodes of an 82 year old woman. It involved extensively her bone marrow, and was lethal. Malignant B-cells were CD10 negative, harbored Burkitt translocation, and multiple chromosomal changes including trisomies of chromosomes 3 and 18, and three copies of 8q with an intact q24 cytoband (in addition to *MYC* rearrangement), associated with overexpression of BCL6, BCL2, and MYC respectively. We suggest that in aggressive nodular marginal zone lymphomas (clinical picture or high proliferation rate of lymphoma cells), fluorescence *in situ* hybridization analysis for *MYC* rearrangement, with break-apart probe, and for MYC/IGH translocation, in addition to chromosome analysis, should be performed. *MYC* rearrangement associated with a more rapid progression of the neoplasia, might warrant a more aggressive treatment. [*J Clin Exp Hematop* 55(3) : 175-180, 2015]

**Keywords:** marginal zone large B-cell lymphoma, MYC rearrangement, overexpression of bcl2, bcl6, MYC

## INTRODUCTION

Nodal marginal zone lymphoma (NMZL) is a B-cell lymphoma in which malignant cells are derived from post-germinal center B-cells located in the lymph node's marginal zone. It histologically resembles extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) and splenic marginal zone lymphoma, but by definition exclusively involves lymph nodes. NMZL is clinically indolent, and some of the synonyms are monocytoid B-cell lymphoma (Kiel), parafollicular B-cell lymphoma (Lukes-Collins), and small lymphocytic lymphoma with plasmacytoid differentiation. Diagnosis is established by a com-

ination of clinical and histological criteria since there are no specific immunophenotypic or molecular markers for NMZL. Because of overlapping morphologic and immunophenotypic features, it is sometimes difficult to distinguish NMZL from lymphoplasmacytic lymphoma and atypical cases of small lymphocytic lymphoma/B-cell chronic lymphocytic leukemia. Detection of point mutation *MYD88 L265P*<sup>1</sup> in the short arm of chromosome 3 at 3p22.2 suggests lymphoplasmacytic lymphoma and helps in differentiation [found in 96% of lymphoplasmacytic lymphomas, 4% in marginal zone lymphomas, and 2% in B-cell chronic lymphocytic leukemia/small lymphocytic lymphomas<sup>2</sup>]. NMZL is rather rare and comprises 1% to 1.8% of non-Hodgkin lymphomas.<sup>3,4,5</sup> As a distinct type of lymphoma it was discovered in 1986 and named monocytoid B-cell lymphoma<sup>6</sup> since malignant cells were morphologically similar to normal monocytoid B-cells located in the marginal zone. However, in the majority of cases NMZBCLs are composed of a mixture of cells as follows: monocytoid cells, centrocyte-like cells, small round lymphocytes, large cells resembling centroblasts or immunoblasts, and plasmacytoid differentiation may accompany all of these variants.<sup>7</sup> Any of these cell types might be predominant. NMZL with a pure population of monocytoid B-cells comprises only 10% of NMZL.<sup>3</sup> In all cases of nodal marginal B-cell lymphoma large cells resembling either large non-cleaved lymphocytes (centroblasts) or immunoblasts are

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present in varying proportions from rare to relatively numerous.<sup>7</sup> When large cells are numerous enough to form sheets and erase the marginal zone pattern, nodal marginal B-cell lymphoma has transformed to diffuse large B-cell lymphoma. Nodal marginal large B-cell lymphoma with a pure marginal zone pattern has not been previously reported. NMZL does not possess characteristic or consistent chromosomal/cytogenetic abnormalities. The most common are numerical abnormalities as follows: trisomy 3, trisomy 12, and trisomy 18.<sup>8,9</sup> There may be simultaneous partial duplications of chromosome 3 in several regions (for example 3q11-q29), as identified by comparative genomic hybridization.<sup>10,11</sup> Genetic and genomic changes in NMZL are different than those in extranodal marginal zone lymphomas<sup>11,12</sup> or lymphoplasmacytic lymphoma which harbors previously mentioned characteristic mutation.<sup>1,2</sup> The exception is both somatic mutations and genomic deletions of the NF- $\kappa$ B-negative regulator TNFAIP3 (A20) providing evidence for its constitutive activation in all types of marginal zone lymphoma.<sup>13</sup> There is also a hypothesis that because marginal zone B-cell lymphomas of extranodal, nodal, and splenic type show similar morphology, immunophenotype and cytogenetics, they might basically represent different clinical manifestations of the same disease.<sup>14</sup>

## CASE REPORT

An 82 year old woman noticed a right submandibular mass. Computerized tomography demonstrated multiple enlarged bilateral cervical, bilateral axillary and para-aortic lymph nodes. Neck lymph node biopsy and bone marrow biopsy were performed. After the diagnosis had been established she received one cycle of CVP/R (cyclophosphamide, vincristine, prednisolone, and rituximab). Doxorubicin hydrochloride was omitted due to left ventricle ejection fraction of 45%. She died two months later.

## MATERIALS AND METHODS

The patient's history was obtained from the Electronic Medical Record of Charleston Area Medical Center, Charleston WV, USA. Hematoxylin and eosin and immunoperoxidase stained sections of the neck lymph node biopsy and bone marrow biopsy were obtained according to the standard protocols. The five-color flow cytometric immunophenotyping of the lymph node biopsy and of the bone marrow aspirate was performed on Beckman Coulter Cytomics FC 500, Miami FL, USA. Chromosome and fluorescence *in situ* hybridization (FISH) analyses were performed according to standard protocol at the Integrated Oncology, a business unit of Esoterix Genetic Laboratories, LLC New York, NY, USA.

## RESULTS

Hematoxylin and eosin and immunoperoxidase stained sections of the neck lymph node (Figs. 1-3) demonstrated nodal marginal zone large B-cell lymphoma (NMZLBCL) with a high proliferation rate (40%). All lymphoma cells were large, with watery (open) chromatin and a conspicuous single nucleolus or 2 conspicuous nucleoli. Malignant cells to some extent resembled centroblasts. Malignant large B-cells effaced normal marginal zone cells and populated areas between the remnants of secondary follicles consisting only of germinal centers and mantle zones thus creating marginal zone large B-cell lymphoma. (Figs. 1-3). Focal colonization of germinal centers was noted.

Flow cytometric analysis of the same lymph node biopsy (Fig. 4) has revealed that the population of large lymphocytes is monoclonal, immunoglobulin  $\kappa$  light chain restricted ( $\kappa$ : $\lambda$  = 87) and that malignant cells express CD19, CD20, CD23 and CD38 without expression of CD5 or CD10. Small lymphocytes are polyclonal ( $\kappa$ : $\lambda$  = 3).

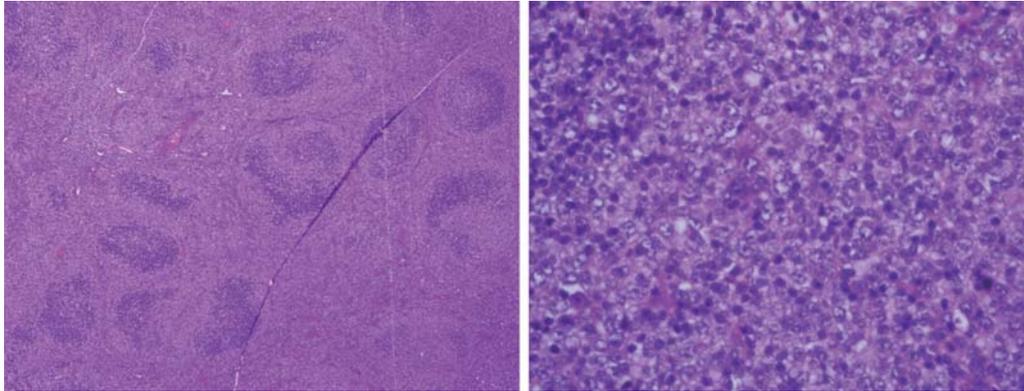
FISH study of the neck lymph node biopsy showed rearrangement involving one copy of *MYC* with three intact copies of *MYC* in 40.5% of cells (Fig. 5), and positive *MYC/IGH* fusion signal with extra copies of intact *MYC* in 41% of cells (Fig. 6). In addition three copies of *BCL6* were observed in 42.5% of cells, three copies of *IGH* (reflecting split of one of the *IGH* signals due to translocation with *MYC*) and *BCL2* were observed in 32.5% of cells, and three copies of *MALT1* were observed in 31.0% of cells suggesting trisomy 3 and trisomy 18 (Fig. 7).

Chromosomal analysis revealed complex changes (Fig. 8). There is a translocation between the long arms of chromosome 2 and 13, an additional copy of chromosomes 3 and 18, an unbalanced whole arm translocation between two copies of 8 (isochromosome 8q) with a translocation between one of the long arms of the 8 and a 14,t(8;14)(q24.1;q32), and a derivative 14 resulting from a translocation between the long arm of 8 and the homolog of 14.

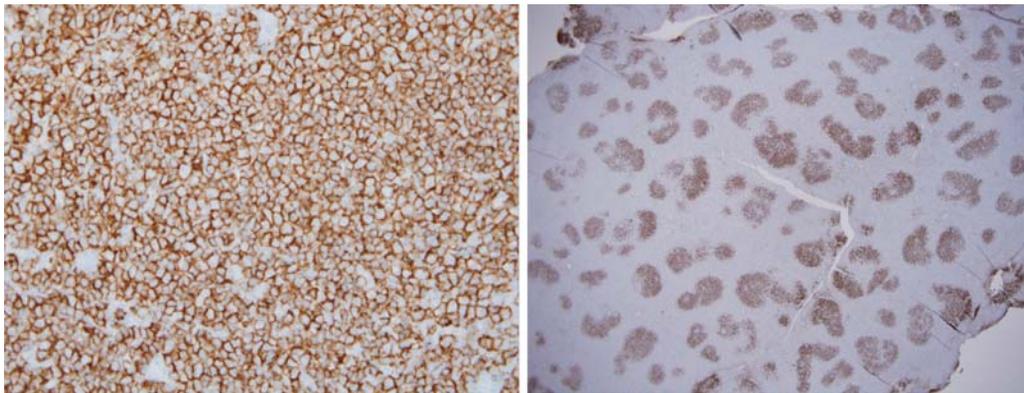
FISH analysis of the bone marrow sample showed rearrangement of one copy of *MYC* and three intact *MYC* signals in 14.4% of cells, three copies of *BCL6* in 14.6% of cells, four copies of *IGH* with three copies of *BCL2* in 10.8% of cells.

## DISCUSSION

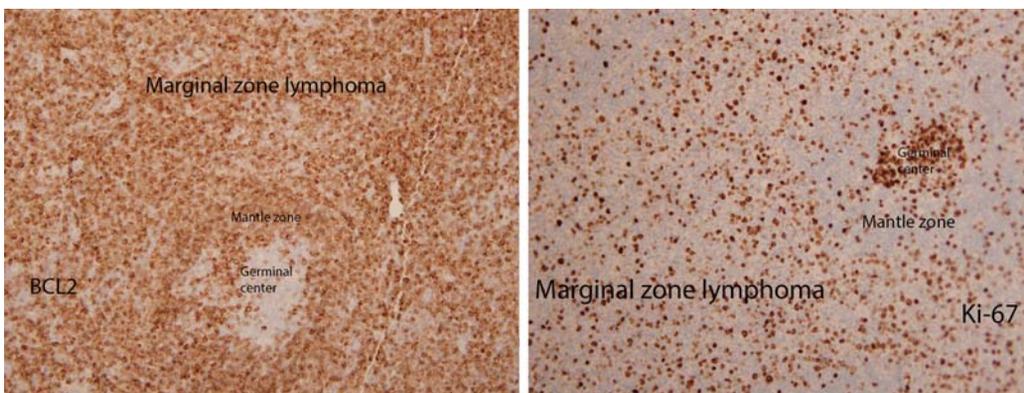
Our patient's primary NMZLBCL with a high proliferation rate displays multiple chromosomal changes including characteristic Burkitt lymphoma translocation t(8;14)(q24.1;q32). It also displays trisomy 3, trisomy 18, and three copies of 8q with intact q24 cytoband, the locus for the *MYC* gene, in addition to the 8q with rearranged *MYC* (fourth copy of *MYC* gene). It is known that trisomy 3 and trisomy 18 are not innocent bystanders but by overexpressing *BCL6* and *BCL2*



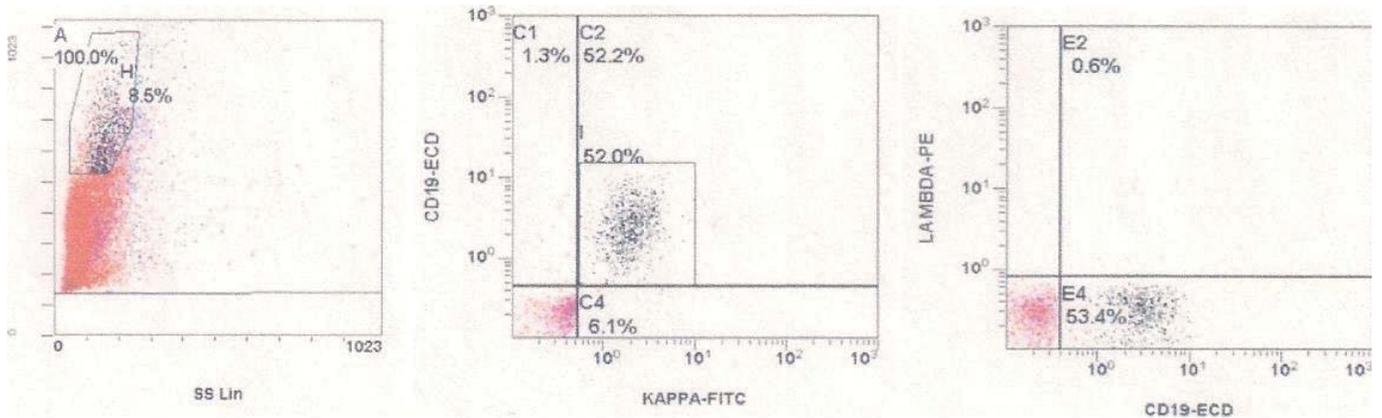
**Fig. 1.** Marginal zone lymphoma with malignant cells between the remnants of benign secondary follicles expanding marginal zones (*left hand side*). Large cells of marginal zone lymphoma with watery chromatin and prominent nucleolus/nucleoli are visible on the *right hand side* of the figure. Mantle zone cells are in the left upper corner of the right picture. Lymph node, H&E stain, original magnification  $\times 40$  (*left*) and  $\times 600$  (*right*).



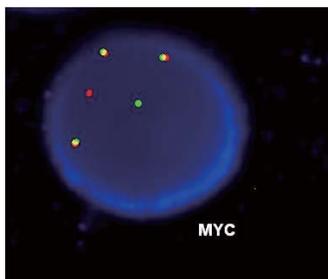
**Fig. 2.** The CD20 positive B-cells of marginal zone lymphoma (*left*). The CD23 has highlighted the dendritic cells of the follicular skeletons (*right*). Malignant cells of the marginal zone lymphoma are unstained. Lymph node, immunoperoxidase stain for CD20, magnification  $\times 400$  (*left*), and immunoperoxidase stain for CD23, magnification  $\times 20$  (*right*)



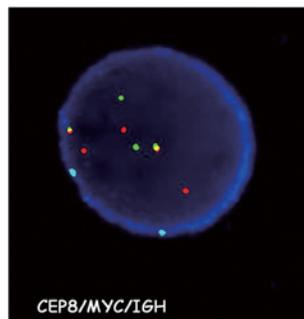
**Fig. 3.** Marginal zone lymphoma cells overexpress BCL-2 while the secondary follicle exhibits reactive (benign) donut pattern. Marginal zone lymphoma cells exhibit high proliferation rate by Ki-67 study, about 40% while the germinal center proliferation rate is 95%. Lymph node, immunoperoxidase stain for BCL2, magnification  $\times 200$  (*left*), and immunoperoxidase stain for Ki-67, magnification  $\times 200$  (*right*).



**Fig. 4.** Scatter plots: Forward/Side, CD19/ $\kappa$ , and  $\lambda$ /CD19 showing that large lymphocytes are monoclonal immunoglobulin  $\kappa$  light chain restricted ( $\kappa$ : $\lambda$  = 87:1).



**Fig. 5.** Fluorescence *in situ* hybridization study of the neck lymph node biopsy. Three copies of intact *MYC* (fusion signals) and one copy of rearranged *MYC* (one red, one green), break apart probe.



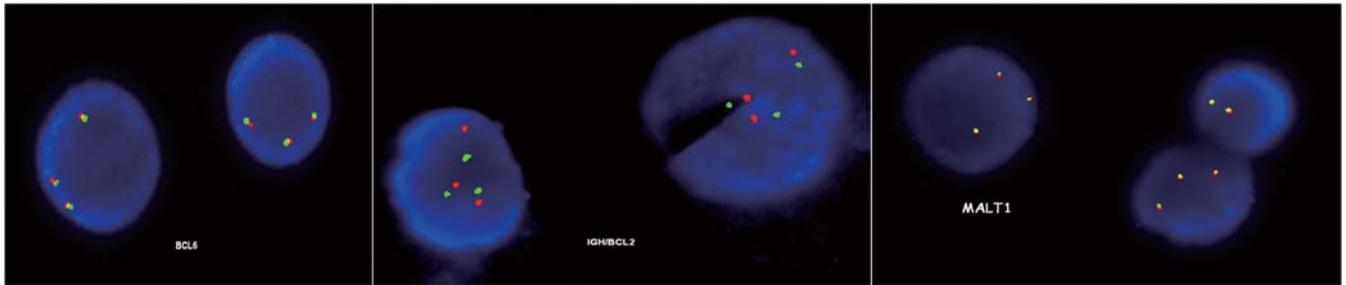
**Fig. 6.** Fluorescence *in situ* hybridization study of the neck lymph node biopsy. *MYC/IGH* fusion probe indicates *MYC/IGH* translocation (2 fusion signals, yellow) in addition to three intact *MYC* signals on 8q (red), two centromeres for chromosome 8 (aqua) reflecting isochromosome 8q (two centromeres and three q arms), and two *IGH* signals (green).

are drivers of hematological malignancies.<sup>15-18</sup> Usually indolent nodular marginal zone lymphoma does not exhibit a characteristic chromosomal translocation, however trisomy 3 and 18 are quite common. While the *BCL2* overexpression increases the cell survival due to the antiapoptotic protein, the *MYC* protooncogene keeps control (high pace) of cell proliferation. *MYC* overexpression, a driver of tumorigenesis, often requires co-expression of the antiapoptotic *BCL2* protein. The detection of the *MYC/IGH* translocation might signal progression of the lymphoma from overexpression of *BCL2*, *BCL6* and *MYC*. However, the small B-cells in our patient's lymphoma, by flow cytometric study, are polyclonal

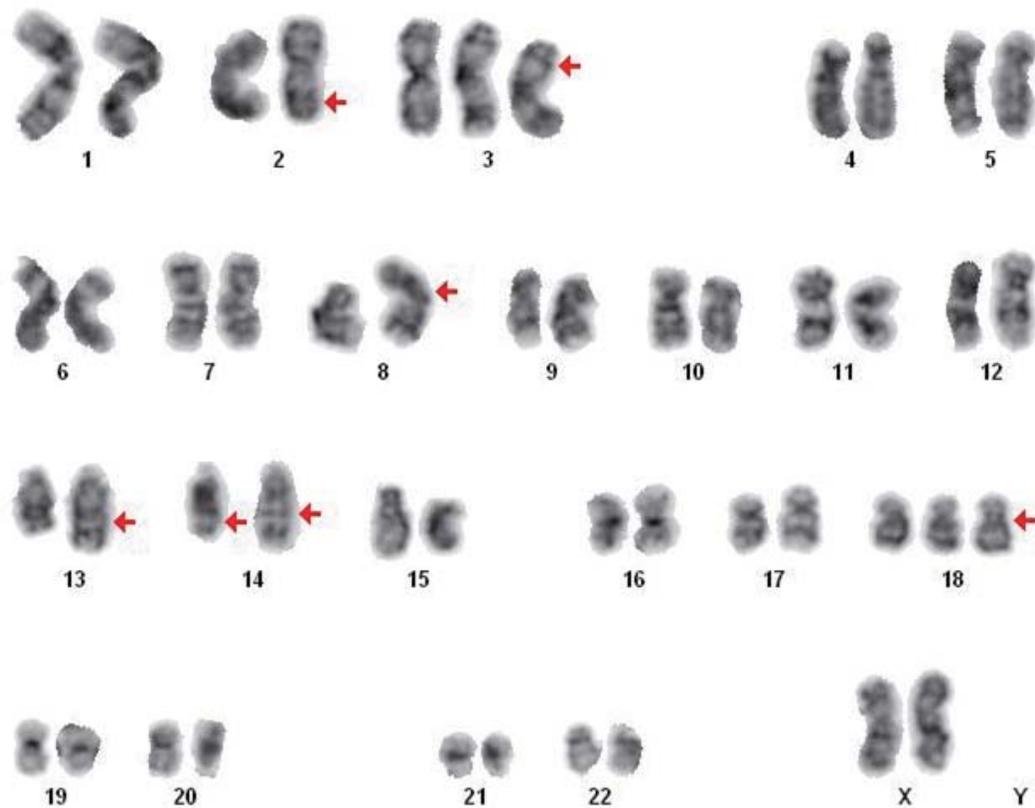
while large cells are monoclonal. We do not thus have flow cytometric evidence of low grade non-Hodgkin lymphoma as a precursor to the marginal zone large B-cell lymphoma. We cannot exclude that in our patient this lymphoma has not occurred through the multistep process and that for example trisomy 3 and trisomy 18 were present in early phases while Burkitt translocation occurred as a later event. The overexpression of *MYC* (trisomy 8q) was either there from the beginning, was acquired as an intermediate step, or along with the occurrence of the *MYC/IGH* translocation. Absence of any other type lymphoma in this patient precludes colonization of marginal zone by large B-cells from primary lymphoma arising elsewhere. This NMZLBCL is thus a primary lymphoma.

Overexpression of *BCL2* antiapoptotic protein in lymphoma cells of our patient is due to the acquired trisomy 18.<sup>19,20</sup> In our patient with aggressive lymphoma, B-cells proliferated rapidly due to the *MYC* overexpression and Burkitt translocation. Malignant B-cells also did not die because there was an overexpression of antiapoptotic protein *BCL2* (trisomy 18). Extrapolating from the literature we speculated that the prognosis was grave<sup>21,22</sup> and indeed the patient died 18 weeks after she noted submandibular mass. The degree of contribution of other chromosomal changes to malignant behavior is not yet fully understood.

In summary, we report the first case of a NMZLBCL and the first with *MYC* rearrangement. It was a very aggressive, lethal lymphoma that exhibited a high proliferation rate, displayed Burkitt translocation t(8;14)(q24;q32), and multiple other chromosomal abnormalities including trisomy 3 (overexpression of *BCL6*), three copies of 8q with an intact q24 cytoband (overexpression of *MYC*), and trisomy 18 (overexpression of *BCL2*). We think that the appropriate name for our patient's lymphoma should be a NMZLBCL (because the large malignant B-cells expand the marginal zones) and that designation diffuse large B-cell lymphoma with an interfollic-



**Fig. 7.** Fluorescence *in situ* hybridization study of the neck lymph node biopsy. Three copies of intact *BCL6* (break apart probe). Three copies of *IGH* and *BCL2* (fusion probes), and three copies of *MALT1* (break apart probe).



**Fig. 8.** Chromosomal analysis demonstrated multiple changes: 48,XX,t(2;13)(q33;q32), +3,der(8;8)(q10;q10)t(8;14)(q24.1;q32), der(14)t(8;14)(q22.3;q32), +18[2]/46,XX=20

ular pattern would not be accurate in our case since malignant large B-cells were located between remnants of follicles and not between the intact follicles. The term diffuse large B-cell lymphoma with an interfollicular pattern of proliferation was introduced in 2008 and patients had favorable prognosis.<sup>23,24</sup> Our patient died within 18 weeks after the first sign of the disease was noted. Because the small B-cells in our patient are polyclonal we presume that this nodal marginal large B-cell lymphoma is *de novo* one and not transformed from a NMZL of usual type. There is a theoretical possibility that

this NMZLBCL originated from diffuse large B-cell lymphoma with an interfollicular pattern which, during its progression colonized marginal zones of previously intact secondary follicles.

In summary, we suggest that in aggressive NMZLs (clinical picture or high proliferation rate of lymphoma cells), FISH analysis at least for *MYC* rearrangement, in addition to chromosome analysis, should be performed, since *MYC* involvement, associated with a more rapid progression of the neoplasia, might warrant a more aggressive treatment.

**CONFLICT OF INTEREST:** Authors declare no conflict of interest.

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