

# B-cell lymphomas with concurrent *MYC* and *BCL2* abnormalities other than translocations behave similarly to *MYC/BCL2* double-hit lymphomas

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Large B-cell lymphomas with *IGH@BCL2* and *MYC* rearrangement, known as double-hit lymphoma (DHL), are clinically aggressive neoplasms with a poor prognosis. Some large B-cell lymphomas have concurrent abnormalities of *MYC* and *BCL2* other than coexistent translocations. Little is known about patients with these lymphomas designated here as atypical DHL. We studied 40 patients of atypical DHL including 21 men and 19 women, with a median age of 60 years. Nine (23%) patients had a history of B-cell non-Hodgkin lymphoma. There were 30 diffuse large B-cell lymphoma (DLBCL), 7 B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma, and 3 DLBCL with coexistent follicular lymphoma. CD10, *BCL2*, and *MYC* were expressed in 28/39 (72%), 33/35 (94%), and 14/20 (70%) cases, respectively. Patients were treated with standard ( $n=14$ ) or more aggressive chemotherapy regimens ( $n=17$ ). We compared the atypical DHL group with 76 patients with DHL and 35 patients with DLBCL lacking *MYC* and *BCL2* abnormalities. The clinicopathologic features and therapies were similar between patients with atypical and typical DHL. The overall survival of patients with atypical double-hit lymphoma was similar to that of patients with double-hit lymphoma ( $P=0.47$ ) and significantly worse than that of patients with DLBCL with normal *MYC* and *BCL2* ( $P=0.02$ ). There were some minor differences. Cases of atypical double-hit lymphoma more often have DLBCL morphology ( $P<0.01$ ), less frequently expressed CD10 ( $P<0.01$ ), and patients less often had an elevated serum lactate dehydrogenase level ( $P=0.01$ ). In aggregate, these results support expanding the category of *MYC/BCL2* DHL to include large B-cell lymphomas with coexistent *MYC* and *BCL2* abnormalities other than concurrent translocations.

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Double-hit lymphoma (DHL) is broadly defined as an aggressive B-cell lymphoma with a *MYC* translocation combined with an additional translocation involving other genes, such as *BCL2*, *BCL3*, *BCL6*, or *CCND1*.<sup>1</sup> The most common type of double-hit lymphoma carries *MYC/8q24* rearrangement and

*IGH@BCL2/t(14;18)(q32;q21)*, commonly referred to in the literature as double-hit B-cell lymphoma, *MYC/BCL2*, or *BCL2 + /MYC +* DHL. *MYC*, an oncogene located at 8q24, functions as a transcription factor controlling the expression of many genes, directly or indirectly, and is essential for many cellular physiologic functions.<sup>2,3</sup> *BCL2*, located at 18q21, encodes a protein that inhibits apoptosis and also is involved in physiological DNA repair. The cooperating effects of *MYC* and *BCL2* in lymphomagenesis have been shown in many studies.<sup>4–14</sup>

Numerous case series of *MYC/BCL2* double-hit lymphoma are reported in the literature.<sup>15–24</sup> *MYC/BCL2* DHLs represent ~10% of all lymphomas that otherwise resemble diffuse large B-cell lymphoma

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(DLBCL).<sup>10</sup> Although different studies have focused on different aspects or details of double-hit lymphoma, all studies suggest that patients with these lymphomas commonly have an aggressive clinical course characterized by advanced stage disease, extranodal involvement, and high serum lactate dehydrogenase levels. Central nervous system and bone marrow involvement are common and the International Prognostic Index score (IPI score) is often high-intermediate or high. Most cases of double-hit lymphoma have morphologic features of DLBCL or B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma. Virtually, all cases have a germinal center B-cell immunophenotype and a high proliferation rate. Despite a variety of therapeutic approaches that have been used to date, patients with DHL have a poor prognosis with a median overall survival (OS) of <2 years.

In daily practice we also have encountered cases of large B-cell lymphoma in which concurrent *MYC* and *BCL2* abnormalities are present, but translocations involving *MYC*, *BCL2*, or both genes are not identified. These cases can be grouped into three types: (1) *MYC* translocation coexisting with extra copies of *BCL2*, but no evidence of t(14;18)(q32;q21)/*IGH@BCL2*; (2) t(14;18)(q32;q21)/*IGH@BCL2* coexisting with extra copies of *MYC*, but without evidence of *MYC* translocation; and (3) extra copies of *MYC* and *BCL2* without translocations involving either gene. For the purpose of this study, we have designated these tumors as atypical DHL in contrast with conventionally defined (hereafter referred to as typical) double-hit lymphoma.

Rarely, single or very few atypical DHL cases have been included in *MYC/BCL2* DHL case series that have been reported.<sup>15,20,22,23</sup> To our knowledge, however, little is known about atypical DHL and there is no large study in the literature that has focused on atypical *MYC/BCL2* double-hit lymphoma. In this study, we assess the clinicopathologic, immunophenotypic, and cytogenetic features of a large group of patients with atypical double-hit lymphoma, and compare this patient group to a group of patients who had typical double-hit lymphoma as well as a group of patients who had DLBCL not associated with either *MYC* or t(14;18)(q32;q21)/*IGH@BCL2*.

## Materials and methods

### Case Selection

A total of 151 cases of large B-cell lymphoma with *MYC* and *BCL2* status tested by fluorescence *in situ* hybridization (FISH) are included in this study. These cases are derived from two institutions spanning the time interval of 1 January 2006 through 31 December 2013. Most of these cases were recognized in recent years after large B-cell lymphomas were

screened routinely for *MYC* and *BCL2* rearrangements by FISH testing.

The cases in this study were divided into three categories: typical double-hit lymphoma, atypical double-hit lymphoma, and DLBCL without *MYC* and *BCL2* abnormalities as shown by FISH. Typical double-hit lymphoma cases included all cases of large B-cell lymphoma that met the classical definition of *MYC/BCL2* double-hit lymphoma, namely rearrangements of both *MYC* and *BCL2*. Atypical double-hit lymphoma cases included all tumors with concurrent *MYC* and *BCL2* abnormalities other than coexistent translocations and were further subcategorized into three groups: (1) *MYC* translocation coexisting with extra copies of *BCL2*, but lacking evidence of t(14;18)(q32;q21)/*IGH@BCL2*; (2) t(14;18)(q32;q21)/*IGH@BCL2* coexisting with extra copies of *MYC*, but without evidence of *MYC* translocation; and (3) extra copies of *MYC* and *BCL2* without translocations involving either gene. A total of 40 patients with atypical double-hit lymphoma were identified as the study group. These patients and their tumors were compared with 76 cases of typical double-hit lymphoma and 35 cases of DLBCL with no *MYC* and *BCL2* abnormalities.

Corresponding medical records were reviewed to obtain clinical information, including a history of lymphoma, number and sites of involvement, Ann Arbor stage, IPI score, treatment regimens, response to therapy, and OS. Morphologic, immunophenotypic, and cytogenetic data were reviewed. All cases were classified according to the current World Health Organization (WHO) scheme, which includes the provisional category B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma.<sup>25</sup>

### Immunophenotypic Methods

Immunohistochemical studies were performed using formalin-fixed, paraffin-embedded tissue sections either at the time of diagnosis or retrospectively for this study. The panel of monoclonal antibodies included reagents specific for CD3, CD20 (Ventana Medical Systems, Tucson, AZ, USA); CD5, CD10, BCL2, BCL6, MUM1 (Leica Microsystems, Buffalo Grove, IL, USA); Ki-67 (MIB-1) (DAKO, Carpinteria, CA, USA); and *MYC* (Epitomics, Burlingame, CA, USA). The cutoffs for positivity for CD10 and BCL6 were both 30% and the cutoff for MUM1 was 60% as have been used in an earlier study.<sup>26</sup> The cutoffs for a positive *MYC* or *BCL2* results were  $\geq 40\%$  or  $\geq 50\%$  of cells, respectively, as used by others.<sup>27,28</sup>

Flow cytometry immunophenotypic analysis was performed using standard multicolor analysis, mostly eight color. Analysis was performed using either a FACScanto II or FACSCalibur cytometer (Becton-Dickinson Biosciences, San Jose, CA, USA) as described previously.<sup>20</sup> Lymphocytes were gated for analysis using side scatter versus forward scatter,

and CD45 expression versus side scatter. The panel of antibodies included CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD23, CD33, CD38, and immunoglobulin  $\kappa$ - and  $\lambda$ -light chains. All antibodies were obtained from Becton-Dickinson Biosciences.

### Conventional Cytogenetics and FISH

Conventional G-band karyotype analysis was performed on five cases using cell suspensions of lymph node, extranodal tissue biopsy, or bone marrow aspirate specimens. The karyotypes were reported according to the 2013 International System for Human Cytogenetic Nomenclature.<sup>29</sup>

FISH analysis was performed on all cases using LSI MYC dual-color break-apart and LSI IGH@BCL2 dual-color, dual fusion probes (Abbott Laboratories, Des Plaines, IL, USA). For bone marrow aspirate specimens, FISH was performed by using a freshly dropped slide from a harvested bone marrow or a G-banded slide for metaphase mapping according to the manufacturer's instructions. For formalin-fixed, paraffin-embedded tissue samples, FISH was performed on 4- $\mu$ m-thick tissue sections and fixed onto slides according to the manufacturer's protocol. The signals from 200 nuclei were analyzed.

The cutoffs for considering a tumor sample positive for MYC and BCL2 by FISH were generated by assessing 20 normal controls and choosing cutoffs two s.d. above the mean. These cutoffs were different in bone marrow smears versus fixed, paraffin-embedded tissue sections and were also slightly different between the laboratories of the two institutions. However, these cutoffs were low (all  $\leq 5\%$ ) and all case in this study designated as positive for MYC or BCL2 had these abnormalities in  $> 25\%$  of nuclei assessed.

### Statistical Analysis

OS was calculated from the date of diagnosis to the date of death or last follow-up. Patient survival was analyzed using the Kaplan–Meier method and compared using the log rank test (GraphPad Prism 5 software). The Fisher exact test was used to compare differences between the atypical and typical double-hit lymphoma groups. A *P*-value of  $< 0.05$  was considered statistically significant.

## Results

### Atypical MYC/BCL2 Double-Hit Lymphoma

**Clinical characteristics.** We identified 40 patients with atypical DHL, 39 adults and 1 child. There were 21 men and 19 women with a median age of 60 years (range, 6–82 years). Twenty-nine (73%) patients presented with *de novo* disease, nine (23%) patients had a history or concurrent low-grade

follicular lymphoma or other B-cell non-Hodgkin lymphoma, and two patients did not have a detailed history available for review. Serum LDH results were available for 29 patients, and 16 (55%) patients had an elevated level. Two patients were initially diagnosed by examination of bone marrow specimens, whereas all others were initially diagnosed in lymph node or other tissue site biopsy specimens. Fourteen of 27 (52%) patients had 2 or more extranodal sites of disease. The involved extranodal sites included the bone marrow, central nervous system, abdomen, gastrointestinal tract (small intestine or colon), liver, spleen, lung, pleural fluid, testis, ovaries, skin, and thyroid gland. Bone marrow involvement was present in 8 of 20 (40%) patients. Only 11 patients underwent spinal tap and cerebrospinal fluid analysis and 1 was involved by lymphoma. Twenty-two of 31 (76%) patients had advanced Ann Arbor stage (III/IV) disease, and 18 of 30 (60%) patients had a high-intermediate or high IPI score (Table 1).

**Morphological and immunophenotypic features.** The atypical DHL cases were classified as follows: 30 (75%) DLBCL, 7 (18%) B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma, and 3 (8%) follicular lymphoma associated with DLBCL. The grade of the follicular lymphoma component in these cases was grade 1, grade 3A, and grade 3B in 1 case each (Table 2, Figures 1 and 2).

All atypical DHLs were of B-cell lineage, positive for one or more pan-B-cell antigens and negative for pan-T-cell antigens. CD10 was positive in 28 of 39 (72%), BCL6 in 25 of 27 (93%), MUM1 in 5 of 16 (31%, all negative for CD10), and BCL2 in 33 of 35 (95%) cases. MYC expression was observed in 14 of 20 (70%) cases by using a 40% cutoff,<sup>28,30</sup> or 17 of 20 (85%) cases by using 10% as cutoff as reported by a recent study.<sup>31</sup> On the basis of these results and by

**Table 1** Comparison of clinical features of atypical and typical MYC/BCL2 DHL

Features	Atypical MYC/BCL2 DHL	MYC/BCL2 DHL
Age (years), median (range)	60 (6–82)	61 (18–87)
Age $> 60$ (years)	53% (21/41)	55% (42/76)
Male:female	21:19	47:29
Previous FL/B-NHL	23% (9/40)	27% (20/72)
BM positive	40% (8/20)	55% (38/69)
CNS positive	9% (1/11)	20% (7/35)
Extranodal sites $\geq 2$	52% (14/27)	58% (39/67)
Elevated serum LDH*	55% (16/29)	79% (52/66)
Stage III or IV	71% (22/31)	84% (62/74)
High IPI score	60% (18/30)	76% (54/71)

Abbreviations: BM, bone marrow; B-NHL, B-cell non-Hodgkin lymphoma; CNS, central nervous system; DHL, double-hit lymphoma; FL, follicular lymphoma; IPI, International Prognostic Index; LDH, lactate dehydrogenase.

\**P* = 0.01.

**Table 2** Comparison of pathologic features of atypical and typical *MYC/BCL2* DHL

Features	Atypical <i>MYC/BCL2</i> DHL	<i>MYC/BCL2</i> DHL
<i>Morphology</i> *:		
DLBCL	75% (30/40)	47% (36/76)
BCLU	18% (7/40)	46% (35/76)
Other	8% (3/40)	7% (5/76)
<i>Immunophenotype</i>		
CD10*	72% (28/39)	99% (71/72)
BCL6	93% (25/27)	95% (36/38)
BCL2 (>50%)	94% (33/35)	90% (61/68)
MYC (>40%)	70% (14/20)	75% (9/12)
MYC/BCL2 Coexpress	55% (11/20)	67% (8/12)
Ki67	20–99% (≥70% in 28/32)	20–99% (≥70% in 60/69)
Complex karyotype	100% (5/5)	100% (27/27)

Abbreviations: BCLU, B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma; DHL, double-hit lymphoma; DLBCL, diffuse large B-cell lymphoma.

\* $P < 0.01$ .

using the Hans algorithm,<sup>32</sup> the 30 cases of DLBCL can be further subclassified as: 21 germinal center B-cell (GCB) type, 5 non-GCB type, and 4 unclassifiable due to incomplete data. The Ki67 proliferation rate in these lymphomas was variable, ranging from 20% up to nearly 100% (Table 2, Figures 1 and 2), with 28 of 32 (88%) cases having a proliferation rate >70%. Eleven of 20 (55%) cases were positive for both *MYC* and *BCL2* by immunohistochemistry.

**Cytogenetic characteristics.** Conventional cytogenetic analysis was performed on five atypical DHLs. Each of these lymphomas had a complex karyotype with >3 numerical and/or structural aberrations (Table 3). Three cases had t(14;18)(q32;q21) and two cases had t(8;14)(q24;q32). No cases carried both translocations. FISH analysis showed 3 cases with *MYC* translocation and extra copies of *BCL2*, 22 cases with t(14;18)/*IGH@BCL2* coexisting with extra copies of *MYC*, and 15 cases having extra copies of both *MYC* and *BCL2*, but no translocations involving either gene. The *MYC* copy number ranged from 3 to 8 and *BCL2* copy number ranged from 3 to 7. In all three cases with *MYC* translocation, *BCL6* was assessed by FISH and no case had a *BCL6* translocation.

**Treatment.** Detailed therapy data were available for 31 of 40 patients with atypical DHL. All 31 patients were treated with combination chemotherapy (Table 4): 14 (45%) with rituximab, cyclophosphamide, daunorubicin, vincristine, and prednisone (R-CHOP), and 17 (55%) with more aggressive regimens. Seven of 17 patients received rituximab plus hyper-fractionated cyclophosphamide, vincristine,

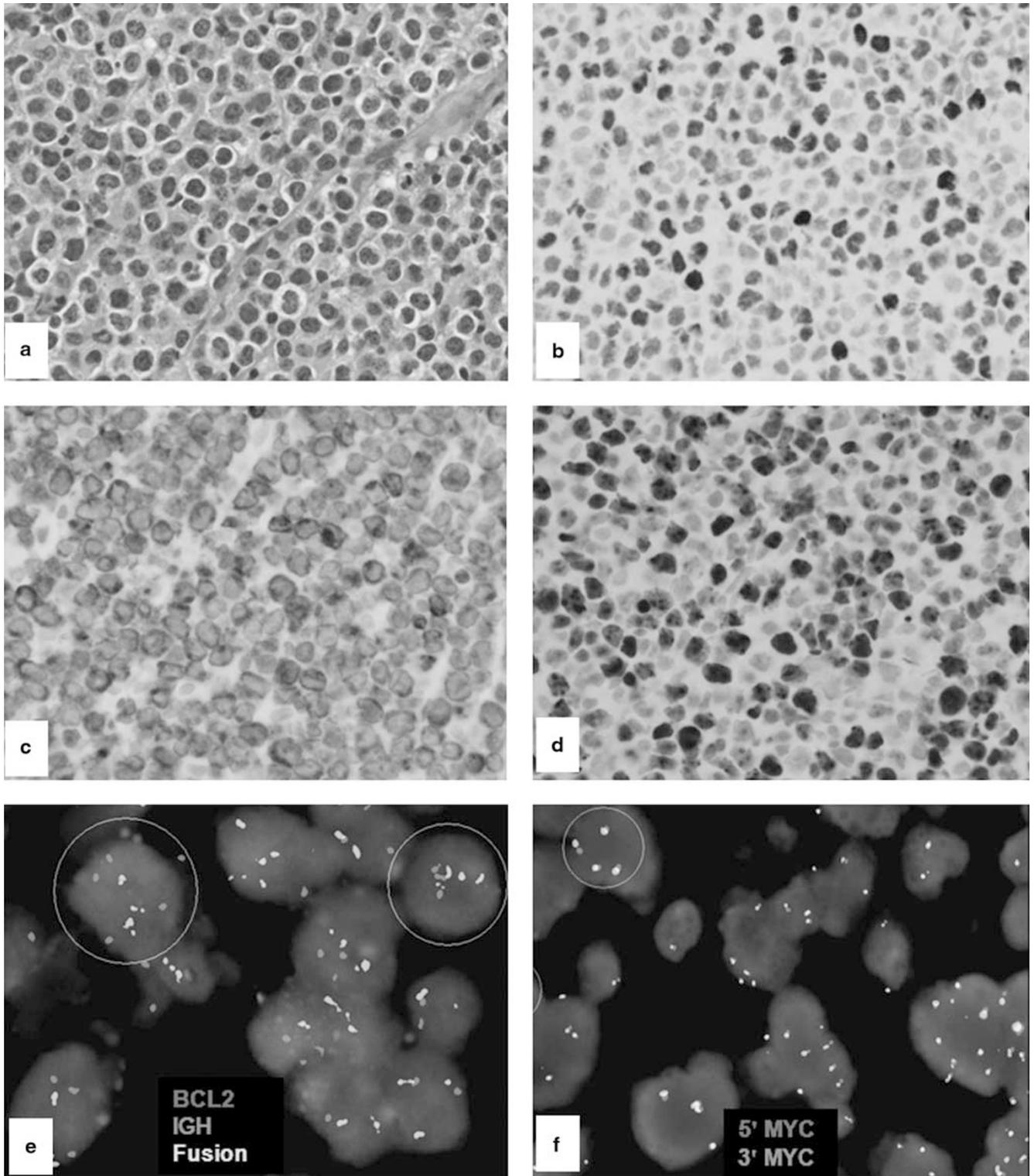
doxorubicin, dexamethasone alternating (R-hyper-CVAD), whereas 10 patients received rituximab plus one of the following regimens: EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin), ESHAP (etoposide, methylprednisolone (solumedrol), high-dose cytarabine (ara-C) and cisplatin), or RICE (rituximab, ifosfamide, carboplatin, and etoposide). Overall, there was no statistical significant difference in OS between patients who were treated with R-CHOP versus those treated with R-hyper-CVAD or other regimens ( $P = 0.67$ ). Five of 31 patients also received hematopoietic stem cell transplant, including 4 autologous and 1 allogeneic transplant.

**Survival of patients with atypical double-hit lymphoma.** Patients with tumors that had *MYC* amplification (defined as copy number  $\geq 5$ <sup>33,34</sup>) were compared with tumors that had three to four copies of *MYC*, and there is no statistically significant difference in their OS ( $P = 0.54$ ). Similarly, patients with tumors that had *BCL2* amplification versus lower copy number showed no statistically significant difference of OS ( $P = 0.47$ ). The OS of patients with the three subtypes of atypical double-hit lymphoma defined by FISH were further analyzed and there was no statistically significant difference in OS ( $P = 0.27$ ). Although the numbers for patients with each type of atypical double-hit lymphoma were small, based on these results we combined all patients with atypical double-hit lymphoma into a single group for comparison with patients with typical double-hit lymphoma and patients with DLBCL.

The three patients with atypical DHL in which *MYC* translocation was identified had poor survival. Although this is a small patient subset, the OS of these patients appeared to be similar to patients with typical DHL ( $P = 0.70$ ). In patients with atypical DHL in which extra copies of *MYC* were identified, the OS of patients in which their tumor carried a *BCL2* translocation was not statistically significantly different from that of patients with tumors associated with extra copies of *BCL2* ( $P = 0.21$ ). These data suggest that non-translocation *BCL2* abnormalities have a negative impact on prognosis similar to *BCL2* translocation in the setting of *MYC* cytogenetic abnormalities (translocation or extra copies).

### Atypical versus Typical *MYC/BCL2* DHL

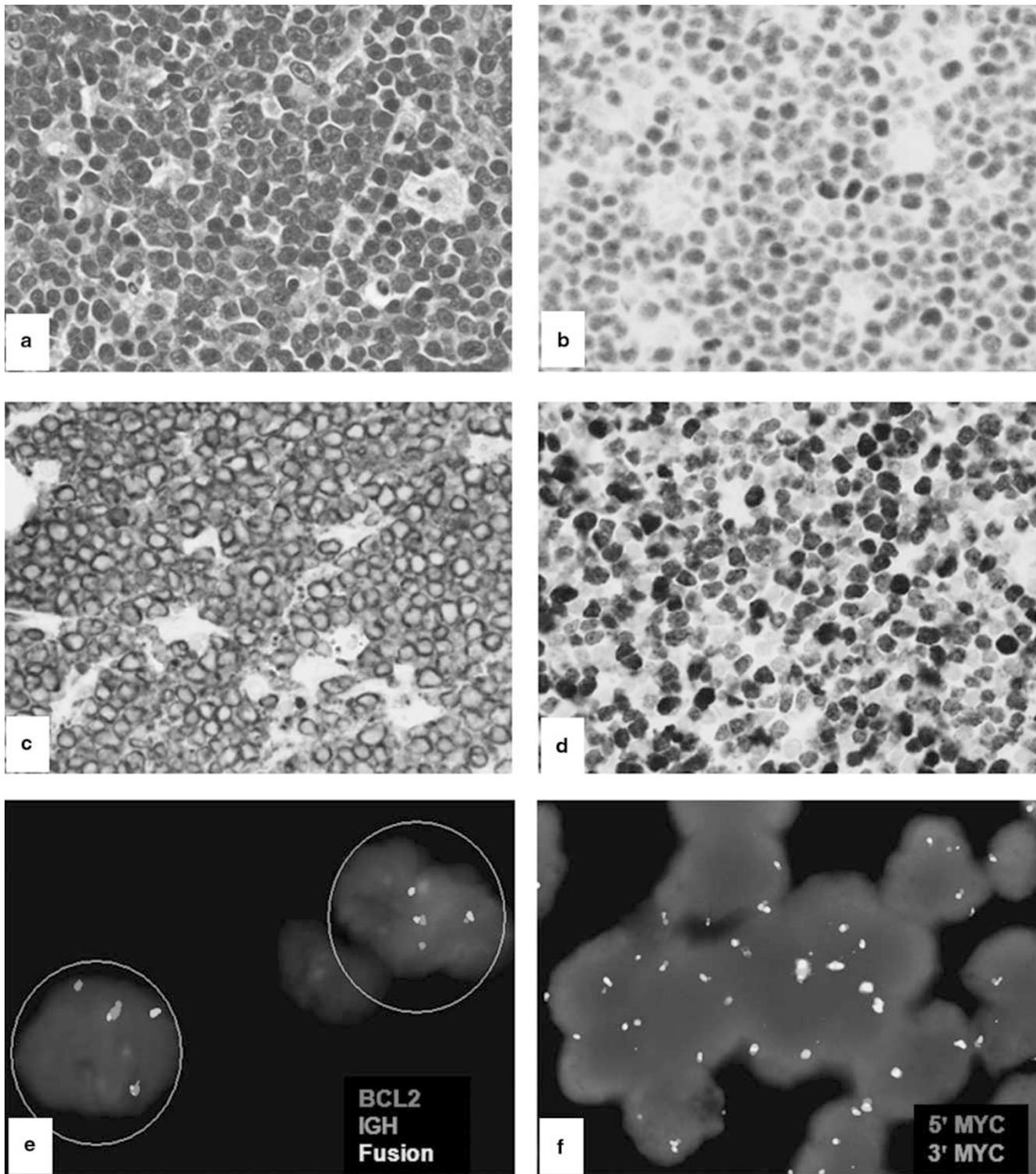
The clinical, morphologic, immunophenotypic, and cytogenetic features, including proliferation rate and karyotypic complexity of patients with atypical DHL ( $n = 40$ ) were very similar to those of patients with typical DHL ( $n = 76$ ) (Tables 1–3). The few statistically significant differences were as follows: atypical double-hit lymphoma tumors more often had DLBCL morphology ( $P < 0.01$ ), were less often CD10 positive ( $P < 0.01$ ), and patients less often had an elevated serum LDH level ( $P = 0.01$ ). Comparison



**Figure 1** An atypical *MYC/BCL2* double-hit lymphoma with diffuse large B-cell lymphoma morphology (a): This lymphoma is positive for both *MYC* (60%) (b) and *BCL2* (100%), (c) and has a Ki67 (d) proliferation rate of ~60% (a–d:  $\times 400$ ); FISH showed extra copies of both *BCL2* (e) and *MYC* (f).

of therapies between the two groups revealed no statistically significant differences ( $P = 0.11$ , Table 4). The OS of patients with atypical DHL was similar to that of patients with typical double-hit lymphoma

(Figure 3a), with a 2-year OS rate of 54% in atypical double-hit lymphoma and 49% in typical double-hit lymphoma patients, respectively ( $P = 0.47$ ). Excluding patients with a history of low-grade B-cell



**Figure 2** An atypical *MYC/BCL2* double-hit lymphoma of B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. (a) This lymphoma is positive for both *MYC* (70%), (b and c) *BCL2* (100%), and has a Ki67 (d) proliferation rate of ~90% (a–d:  $\times 400$ ); FISH showed *BCL2* rearrangement (e) and extra copies of *MYC* (f).

lymphoma, there was no difference in OS between patients with *de novo* atypical double-hit lymphoma versus patients with typical double-hit lymphoma (Figures 3b,  $P=0.26$ ).

We also compared patients with atypical double-hit lymphoma ( $n=40$ ) to patients with DLBCL in which there was no evidence of *MYC* or *BCL2* abnormalities shown by FISH ( $n=35$ ). The DLBCL

**Table 3** Conventional cytogenetic findings in atypical *MYC/BCL2* DHL

Case	Karyotype
9	51 ~ 53,XX, + X,add(1)(q42), + 4, + 5,del(5)(q22q35)x2, + 7,add(7)(p22), + 11,add(11)(p15), + 12, inv(13)(q12q32),t(14;18)(q32;q21), + 18[cp6]/46,XX[14]
27	47,XY,del(6)(q21q25), + 8,t(14;18)(q32;q21)[19]/46,XY[1]
28	47X,-X,add(2)(p23),add(3)(q29), t(14;18)(q32;q21),del(17)(p11.2), + 20, + 21, add(21)(p11.2)[4] /46,X,-X, add(1)(q44),add(2)(p23),der(3)inv(3)(p21p25)add(3)(q29), t(14;18)(q32;q21),del(17)(p11.2), + 20[14]
32	72,XX,-Y,der(1)t(1;1)(p34;q21) add(1)(q32), der(1)dup(1)(q21q23) t(1)(1;1)(q23;?), del(3)(p14p21), - 4,ins(5)(q12;?), + 7,t(8;14)(q24.1;q32)x2,inv(11)(q21q23), + 12, + 13, - 15,add(16)(p13.3), + 21, + 21, + mar[3]/73,sl, + 18[5]/73,sl, + 11[2]
33	48,XX,dup(1)(q32q21),t(3;7)(q29;q32),t(8;14)(q24q32),del(13)(q12q22),del(15)(q11.2q15), + 18, + 18[19]/46,XX[1]

**Table 4** Comparison of treatment between atypical and typical *MYC/BCL2* DHL

Treatment	Atypical <i>MYC/BCL2</i> DHL	<i>MYC/BCL2</i> DHL
R-CHOP	45% (14/31)	27% (19/71)
Aggressive Regimen	55% (17/31)	73% (52/71)
SCT	16% (5/31)	20% (14/71)

Abbreviations: DHL, double-hit lymphoma; R-CHOP, rituximab, cyclophosphamide, daunorubicin, vincristine, prednisone; SCT, stem cell transplant.

patient group showed similar ages and gender distribution (Table 5) and received similar therapies when compared with patients with atypical DHL. However, patients with atypical DHL more frequently presented with high stage disease, extranodal involvement, and high IPI score when compared with patients with DLBCL lacking *MYC* and *BCL2* abnormalities (Tables 5,  $P < 0.05$ ). Especially, the OS of patients with atypical DHL was significantly worse than the survival of DLBCL patients (Figures 3c and d,  $P < 0.05$ ).

## Discussion

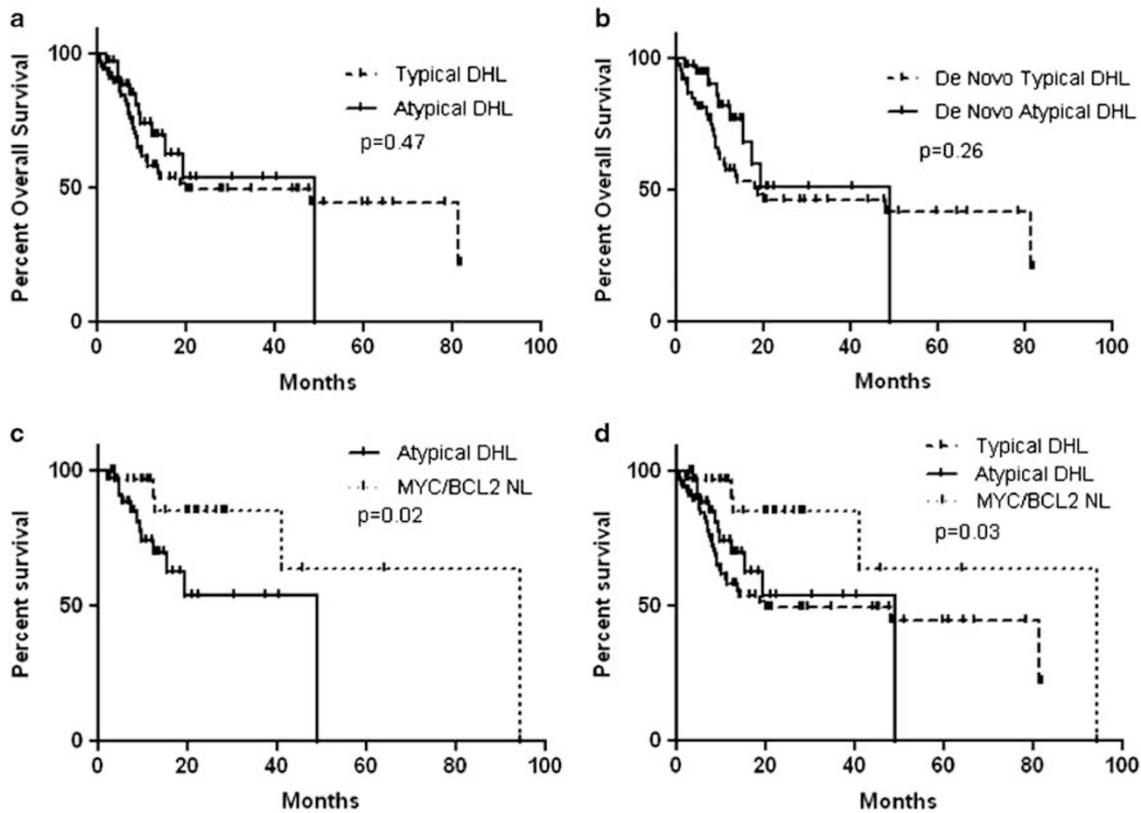
Patients with large B-cell lymphoma with concurrent *IGH@BCL2* and *MYC* rearrangements, known as *MYC/BCL2* double-hit lymphoma (and referred to here as typical double-hit lymphoma), are known to have aggressive clinical course with a poor prognosis. It is also known that some cases of clinically aggressive large B-cell lymphoma have concurrent abnormalities of *MYC* and *BCL2*, but do not have concurrent *MYC* and *BCL2* translocations (designated here as atypical DHL). The clinicopathologic features and prognostic implications of atypical double-hit lymphoma are not well understood and are the focus of this study.

We identified 40 patients with atypical DHL and compared them with 76 patients with typical DHL and 35 patients with DLBCL without *MYC* or *BCL2* abnormalities. Overall, the clinical and pathologic features of patients with atypical and typical DHL were very similar with few statistically significant

differences present. From a clinical perspective, there was only one difference; patients with atypical DHL less often had an elevated serum LDH level ( $P = 0.01$ ). From the pathologic perspective, atypical double-hit lymphoma cases more often showed DLBCL morphology and had a lower frequency of CD10 expression. The OS of patients with atypical DHL was poor and very similar to the OS of patients with typical double-hit lymphoma. Patients with atypical double-hit lymphoma had more aggressive clinical features and a significantly worse OS than patients with DLBCL without *MYC* and *BCL2* abnormalities. In aggregate, these data suggest that atypical double-hit lymphoma is sufficiently distinct from DLBCL and more similar to typical double-hit lymphoma.

In the 2008 WHO classification, lymphoma cases of typical double-hit lymphoma were recognized and thought to commonly fit within the provisional disease category of B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma. This does not appear to be the case for atypical double-hit lymphomas, however, which in this study more often had DLBCL morphology. Another apparent difference is the cell-of-origin classification. Nearly all typical DHLs have a germinal center B-cell origin and carry t(14;18)/*IGH@BCL2*. In contrast, a subset of atypical double-hit lymphomas lacks *IGH@BCL2* and does not express CD10 ( $P < 0.01$ ).

It seems reasonable to hypothesize that cases of atypical DHL are a manifestation of the cooperative effects of *MYC* and *BCL2* overexpression, and therefore affected patients have clinical and pathologic features and a poor prognosis similar to patients with typical double-hit lymphoma. Chromosomal translocations that involve *MYC/8q24* and *BCL2/18q21* are thought to result in upregulation of *MYC* and *BCL2* expression. However, *MYC* and *BCL2* can theoretically be activated through mechanisms other than translocation, and copy number increase is one possible mechanism. Data showing that DLBCL patients with *MYC* and *BCL2* coexpression shown by immunohistochemistry have a poor prognosis in the absence of concurrent *MYC* and *BCL2* translocations indirectly supports this hypothesis.<sup>28,30,34–36</sup> In this study, similar proportions of



**Figure 3** Comparison of overall survival (OS). There is no statistically significant difference in OS between atypical *MYC/BCL2* double-hit lymphoma ( $n = 37$ ) and typical *MYC/BCL2* double-hit lymphoma ( $n = 74$ ) either including (a) or excluding transformed lymphomas (b); however, atypical double-hit lymphoma has a significant worse OS compared with diffuse large B-cell lymphoma without *MYC* and *BCL2* FISH abnormalities (*MYC/BCL2* NL) (c), similar to typical double-hit lymphoma (d).

**Table 5** Comparison of clinical features of patients with atypical *MYC/BCL2* DHL and patients with DLBCL with normal *MYC* and *BCL2*

Features	Atypical <i>MYC/BCL2</i> DHL	<i>MYC/BCL2</i> Normal DLBCL
Age (years), median (range)	60 (6–82)	63 (3–87)
Age > 60 (years)	53% (21/40)	54% (19/35)
Male:female	21:19	20:15
Previous FL/B-NHL	23% (9/40)	9% (3/35)
BM positive	40% (8/20)	16% (4/25)
CNS positive	9% (1/11)	0% (0/14)
Extranodal sites $\geq 2^*$	52% (14/27)	20% (8/40)
Elevated serum LDH	55% (16/29)	45% (14/31)
Stage III or IV*	71% (22/31)	43% (13/30)
High IPI score*	60% (18/30)	32% (9/28)

Abbreviations: BM, bone marrow; B-NHL, B-cell non-Hodgkin lymphoma; CNS, central nervous system; DHL, double-hit lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; IPI, International Prognostic Index; LDH, lactate dehydrogenase.

\* $P < 0.05$ .

typical and atypical DHLs showed *MYC* and *BCL2* coexpression shown by immunohistochemistry, also supporting the above hypothesis. Of interest, not all cases of typical or atypical DHL showed *MYC* and *BCL2* overexpression. Therefore, *MYC* or *BCL2*

abnormalities in atypical double-hit lymphoma do not always result in overexpression detectable by immunohistochemistry. In the literature, this observation also has been made in a subset of typical double-hit lymphoma. The pathogenesis of those cases needs further study.

Others have described cases of B-cell lymphoma with extra signals of *MYC* or *BCL2* identified by FISH. Yoon *et al*<sup>37</sup> studied 154 DLBCL cases. They reported 14 patients who had tumors with *MYC* rearrangement and 11 patients who had tumors with increased *MYC* copy number. Patients with tumors characterized by extra *MYC* copy number had a poorer prognosis, similar to patients with *MYC* rearrangement, and poorer than patients with DLBCL without *MYC* abnormalities. Mossafa *et al*<sup>33</sup> studied 344 cases of non-Hodgkin lymphoma and identified 16 cases with multiple copies of *MYC* but no rearrangement. These 16 patients had a very poor prognosis: 9 patients died during a median follow-up of 11 months, although these include cases of different types of lymphoma. Testoni *et al*,<sup>36</sup> in a series of 166 cases of DLBCL treated with R-CHOP, identified 17 with extra copies of *MYC* (all  $\leq 4$  copies) but no *MYC* translocations. All of these cases showed high *MYC* mRNA expression by gene expression profiling analysis. However, only patients

with concomitant del(8p) had a poorer overall and progression-free survival. These findings are similar to and further support the results in this study. A recent study has suggested that *MYC* expression, translocation, or amplification (>4 copies) is associated with poorer prognosis in DLBCL patients, whereas tumors with three to four copies of *MYC* do not have a poorer prognosis.<sup>31</sup> However, this study included only three cases with *MYC* amplification (>4 copies). Our results suggest that patients with atypical double-hit lymphoma that had three to four copies of *MYC* showed similar OS to patients with atypical DHL with more than four copies of *MYC*.

In the study by Yoon *et al*,<sup>37</sup> increased *BCL2* copy number was identified in 17 of 145 (12%) cases and was associated with worse prognosis. In the 20 cases of *MYC/BCL2* double-hit lymphoma reported by Snuderl *et al*,<sup>15</sup> one patient had *MYC* rearrangement with extra copies of *BCL2* and had a poor prognosis. Jiang *et al*<sup>38</sup> studied 60 cases of DLBCL and identified 18 cases with *BCL2* gene amplification. All cases with *BCL2* amplification demonstrated overexpression of *BCL2* and were associated with a worse prognosis. Extra *BCL2* copies were more commonly seen in the non-GCB type of DLBCL<sup>37,39</sup> and usually associated with overexpression of *BCL2*,<sup>40</sup> which has been correlated with poorer prognosis in DLBCL in some studies.<sup>31</sup> Overall, these studies directly or indirectly support the hypothesis that extra copies of *MYC* and *BCL2* likely result in *MYC* and *BCL2* overexpression in most cases and confer a poorer prognosis.

This study also has limitations. Our study group is relatively small compared with many studies on large B-cell lymphoma in the literature, although our study group is similar in number to most studies published on double hit-lymphoma. The limited number of cases in each of the three subtypes of atypical double-hit lymphoma also precludes a definitive conclusion that all three subgroups are similarly aggressive. A larger case series is needed to further confirm the results we present. In addition, not all cases in this study were assessed by FISH for *BCL6* rearrangements and one could speculate that some cases in the atypical DHLs group are *MYC/BCL6* double-hit lymphoma. However, *BCL6* was assessed by FISH on the 3 cases had a *MYC* translocation among the 40 cases of atypical double-hit lymphoma and no *BCL6* rearrangement were identified. Therefore, the possibility of *MYC/BCL6* double-hit lymphoma defined using conventional criteria is excluded. Despite these limitations, we believe the clinical presentation of patients with atypical versus typical DHL is similar. Furthermore, the significant difference in behavior between patients with atypical double-hit lymphoma versus patients with DLBCL (DLBCL) lacking *MYC* and *BCL2* abnormalities supports the idea that patients with atypical double-hit lymphoma have a poor prognosis similar to patients with typical DHL.

In conclusion, a small subset of large B-cell lymphomas have concurrent *MYC* and *BCL2* abnormalities other than translocations, designated as atypical *MYC/BCL2* double-hit lymphoma. The clinical and pathologic features of these patients were very similar to patients with typical *MYC/BCL2* DHL in this study. In particular, we show that patients with atypical DHL have aggressive clinical features and a poor prognosis, similar to patients with typical double-hit lymphoma and significantly worse than patients with DLBCL lacking *MYC* and *BCL2* FISH abnormalities. Although information about atypical DHL cases is sparse in the literature, our results suggest that these cases appear to fall within the biologic spectrum of *MYC/BCL2* DHL. We acknowledge that our study size is somewhat small and that additional larger studies are needed to further confirm the findings presented.

## Disclosure/conflict of interest

The authors declare no conflict of interest.

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