

Inferior survival in high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements is not associated with *MYC/IG* gene rearrangements

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ABSTRACT

High-grade B-cell lymphomas with *MYC* and *BCL2* and/or *BCL6* rearrangements (double-/triple-hit lymphoma) have an aggressive clinical course. We investigated the prognostic value of transformation from low-grade lymphoma, cytological features (high grade *versus* large cell), *MYC* rearrangement partners (immunoglobulin *versus* nonimmunoglobulin gene), and treatment. We evaluated 100 adults with double-/triple-hit lymphoma, reviewing cytological features; cell of origin; and rearrangements of *MYC*, *BCL2*, and *BCL6* using *MYC*, *BCL2*, and *BCL6* break-apart and *IGH/MYC*, *IGL/MYC*, *IGK/MYC*, and *IGH/BCL2* dual-fusion interphase fluorescence *in situ* hybridization probes. Outcome analysis was restricted to patients with lymphoma, *de novo* or at transformation, who received anthracycline-based chemotherapy. Among them, 60% had high-grade cytological features; 91% had a germinal center B-cell phenotype, and 60% had a *MYC/IG* rearrangement. Germinal center B-cell phenotype was associated with *BCL2* rearrangements ($P < 0.001$). Mean (95% confidence interval) 5-year overall survival was 49% (37%-64%). Transformation from previously treated and untreated low-grade lymphoma was associated with inferior overall survival (hazard ratio, 2.99; $P = 0.008$). Patients with high-grade cytological features showed a non-significant tendency to inferior outcome (hazard ratio, 2.32; $P = 0.09$). No association was observed between *MYC* rearrangement partner and overall survival (hazard ratio, 1.00; $P = 0.99$). Compared with patients receiving rituximab, cyclophosphamide, doxorubicin, and vincristine (R-CHOP) and dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (EPOCH-R), patients receiving rituximab, cyclophosphamide, vincristine, doxorubicin, methotrexate/ifosfamide, etoposide, and cytarabine (R-CODOX-M/IVAC) had a non-significant tendency to better overall survival (hazard ratio, 0.37; $P = 0.10$). In conclusion, high-grade B-cell lymphomas with *MYC* and *BCL2* and/or *BCL6* rearrangements had heterogeneous outcomes and *MYC/IG* rearrangements were not associated with inferior overall survival.

Introduction

The diagnosis of 'high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements' (double-/triple-hit lymphoma, DH/THL) was established in the 2016 revision of the World Health Organization (WHO) classification of lymphoid neoplasms.¹ This category includes all large B-cell lymphomas with



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rearrangements of *MYC* and *BCL2* or *BCL6*, or both, besides those that meet criteria for follicular or lymphoblastic lymphoma. It encompasses some cases previously called 'B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) (BCLU)', in accordance with the 2008 WHO classification.² These cases were considered clinically aggressive but were difficult to diagnose because of vague diagnostic criteria.

By morphological evaluation, DH/THL may show a cytological spectrum. The spectrum can range from (i) monotonous medium-sized cells with round nuclei, finely dispersed chromatin, and starry sky appearance, resembling BL, to (ii) intermediate-sized cells with slight pleomorphism and slightly irregular nuclear contours, resembling BCLU,² to (iii) large lymphoid cells with round to irregular nuclear contours, variably sized nucleoli, and varying amounts of cytoplasm, resembling DLBCL. Experts have recommended that during evaluation, details of the morphological appearance be added in a comment to the record because of the potential prognostic importance of morphological characteristics.¹ Yet, few studies have specifically addressed morphological appearance as a prognostic indicator.

MYC is a powerful transcriptional factor that helps to drive the cell from G_{0/1} phase to S phase and promotes cell proliferation and growth, DNA replication, and protein biosynthesis. It was identified initially as the molecular target of the 8q24 rearrangement characteristic of BL but was subsequently identified in various B-cell lymphomas, including 5% to 15% of DLBCL and 30% to 60% of high-grade B-cell lymphomas.³⁻⁵ The *MYC* rearrangement partner in BL is almost invariably an immunoglobulin (*IG*) gene (*IGH*, *IGK*, or *IGL*), whereas in DLBCL and high-grade B-cell lymphoma it is a non-*IG* gene in about 40% of cases.⁵⁻⁹ Common non-*IG* *MYC* partners include lymphomagenesis-related genes, such as *BCL6*, *BCL11A*, *PAX5*, and *IKAROS*.¹⁰ In *IG*/*MYC* rearrangements, *MYC* is juxtaposed to an *IG* enhancer, usually resulting in pronounced amplification of *MYC* protein expression, whereas *MYC* expression and *MYC* transcript levels are often less robust in the clinical setting of non-*IG*/*MYC* rearrangements.^{5,9} The prognostic significance of the *MYC* partner gene is controversial. Some groups found that a non-*IG* *MYC* partner was a survival advantage,^{5,6,8} while other groups observed no significant difference between *IG* and non-*IG* partner cases.^{9,11}

DH/THL was established as a new diagnostic category in part because of its aggressive clinical behavior. However, most DH/THL cases have a *BCL2* rearrangement (i.e., *MYC/BCL2* or *MYC/BCL2/BCL6*). The clinical behavior of those lacking the *BCL2* rearrangement (i.e., *MYC/BCL6* cases) is not well understood because a limited number are available for analysis. At present, the prognostic significance of *MYC/BCL6* in this context is controversial, with different groups identifying superior outcome,^{5,12} no difference in outcome,¹³ or inferior outcome.^{9,14,15} However, fewer than 100 cases have been described in the literature.

The reported median overall survival (OS) for DH/THL in different series range from 4.5 to 34 months.^{6,10,13,16-26} Patients were treated primarily with rituximab, cyclophosphamide, doxorubicin, and vincristine (R-CHOP)²⁷; dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab

(EPOCH-R)²⁸; rituximab, cyclophosphamide, vincristine, doxorubicin, and dexamethasone (R-hyper CVAD); methotrexate; cytarabine²⁵; and rituximab, cyclophosphamide, vincristine, doxorubicin, and methotrexate/rituximab, ifosfamide, etoposide, and cytarabine (R-CODOX-M/IVAC)²⁹ with or without an autologous stem cell transplant in first complete remission. The median progression-free survival and OS were not improved in some series,^{15,23,25} but were improved in one series.³⁰ Autologous stem cell transplantation in the relapse setting is associated with poor outcomes.³¹⁻³³ Few studies have addressed the prognostic significance of transformation of low-grade lymphoma to DH/THL.³⁴

In the light of the controversy surrounding these issues, the present study investigated the prognostic significance of several of these parameters, including morphological evaluation, *IG*/*MYC* versus non-*IG* rearrangement partner, presence or absence of a *BCL2* rearrangement, transformation from low-grade lymphoma, and therapeutic regimens in diagnostic cases of DH/THL at the Mayo Clinic in Rochester, Minnesota. To our knowledge, this study represents the largest single-institution study of these characteristics among contemporary DH/THL patients.

Methods

The Mayo Clinic Institutional Review Board approved this study and all patients provided consent. Strengthening the Reporting of Observational Studies in Epidemiology reporting guidelines were followed. Cases were identified through review of Mayo Clinic patients in the Mayo Clinic Lymphoma Database (1998-2015) and the Lymphoma Specialized Program of Research Excellence Molecular Epidemiology Resource (2002-2015). Five cases were identified from the Molecular Epidemiology Resource through fluorescence *in situ* hybridization (FISH) performed for other studies.³⁵ The therapeutic regimens were R-CHOP, dose-adjusted EPOCH-R, R-CODOX-M/IVAC, R-hyper-CVAD, methotrexate, cytarabine, and non-anthracycline-based treatment. Additional information regarding case identification and case criteria is detailed in the *Online Supplementary Appendix*.

All cases were diagnosed initially by a Mayo Clinic hematopathologist as either DLBCL or BCLU according to the 2008 WHO criteria;² all were reclassified as DH/THL according to the 2016 WHO criteria.¹ Morphological re-review to assess high-grade versus large-cell histological characteristics was performed by four Mayo Clinic hematopathologists (ALF, PJK, WRM, and EDM). Definitions of high-grade and large-cell cytological features are detailed in the *Online Supplementary Appendix*. For outcome analysis, only cases with a consensus re-review diagnosis were used.

Cell of origin was determined according to the Hans classifier.³⁶ Immunohistochemical methods and criteria are detailed in the *Online Supplementary Appendix*. Interphase FISH was performed on either paraffin sections of tissue specimens or smears of bone marrow aspirate specimens according to previously described methods^{37,38} using break-apart probes for *MYC* and *BCL6*; dual-fusion FISH probes for *IGH*/*MYC*, *IGL*/*MYC*, and *IGK*/*MYC*; and either a *BCL2* break-apart probe or an *IGH*/*BCL2* dual-fusion FISH probe. Further details are provided in the *Online Supplementary Appendix*. Because of tissue limitations, not all probe sets were performed in all cases. Specifically, in some cases, the *MYC* rearrangement partner could not be

identified, and in other cases, the *BCL6* rearrangement status was unknown. Cases with concurrent *MYC* and *BCL2* rearrangements, concurrent *MYC* and *BCL6* rearrangements, and concurrent *MYC*, *BCL2*, and *BCL6* rearrangements are referred to as *MYC/BCL2*, *MYC/BCL6*, and *MYC/BCL2/BCL6*, respectively.

Clinical outcome analysis was limited to patients with DH/THL characteristics identified at initial diagnosis or at transformation from previously diagnosed low-grade lymphoma and who received an anthracycline-based chemotherapy regimen at DH/THL diagnosis. Previous therapies for the 11 patients with prior low-grade lymphoma are as follows: none (n=2); radiation therapy only (n=2); bendamustine and rituximab (B-R) only (n=2); single-agent rituximab (n=1); B-R followed by ibrutinomab consolidation (n=1); R-CHOP with maintenance rituximab (n=1); seven prior therapies, including rituximab, cyclophosphamide, vincristine, and prednisone (R-CVP), CHOP, ifosfamide, carboplatin, and etoposide (ICE), and B-R (n=1); and four prior therapies, including R-CVP and B-R (n=1). Patients with recurrent B-cell lymphoma with high-grade or large-cell histological features were excluded from this analysis because the *MYC/BCL2/BCL6* rearrangement status of the initial biopsy was unknown.

OS was defined as the time from DH/THL diagnosis to death of any cause or to last follow-up. Event-free survival (EFS) was defined as the time from diagnosis to progression, relapse, re-treatment after initial chemotherapy, or death of any cause. EFS12 was defined as event-free status at 12 months after diagnosis. EFS12 was used as an endpoint because of limited follow-up and because progression or relapse occurred primarily in the first 12 months after diagnosis.³⁹ OS and EFS were evaluated with Kaplan-Meier curves and Cox proportional hazards models. All analyses were performed using statistical software (SAS version 9.4; SAS Institute Inc.) and R 3.3.1 (R Project for Statistical Computing). Statistical significance was defined by *P* values less than 0.05.

Results

Morphological and tumor characteristics

The study involved 100 patients (male to female ratio, 64:36) with the median (range) age at DH/THL diagnosis of 61 (29-87) years. Sixty-seven patients had DH/THL identified at initial diagnosis; 22, at the time of transformation of previously diagnosed low-grade lymphoma; and 11, from a recurrent specimen in previously diagnosed lymphoma with large-cell or high-grade morphological features for which original diagnostic material was not studied (Table 1). Slides were available for consensus review in 72 cases, and in 65 cases a consensus diagnosis was reached. Of these, 39 patients (60%) had high-grade morphological features (Figure 1A) and 26 (40%) had large-cell morphological features (Figure 1B). According to the Hans classifier, the phenotype of 91 cases (91%) was germinal center B-cell (GCB); six (6%), non-GCB; and three (3%), unknown. In immunohistochemistry analysis, 37 of 43 cases (86%) met criteria for *MYC* positivity, 76 of 84 (90%) expressed *BCL2*, and 30 of 37 (81%) expressed both (called *double expressers*).

MYC rearrangement partner (*IG* versus non-*IG* gene) and *BCL2/BCL6* rearrangement status

The *MYC* rearrangement partner was an *IG* gene in 52 cases (39 *IGH*, 6 *IGK*, and 7 *IGL*), a non-*IG* gene in 35

cases, and unknown in 13 cases (Figure 2A). Fifty-nine cases were *MYC/BCL2*; 13, *MYC/BCL6*; 20, *MYC/BCL2/BCL6*; and 8, *MYC/BCL2* (unknown *BCL6*) (Figure 2B). The *MYC* rearrangement partner (*IG* versus non-*IG* gene) was not associated with morphological features (*P*=0.96), cell of origin (*P*=0.18), or *BCL2* rearrangement status (*P*=0.27) (Table 2). However, *MYC* expression by immunohistochemistry was significantly more common in *IG/MYC* than in non-*IG/MYC* cases (95% versus 74%; *P*=0.049). GCB phenotype was present in 100% of the 85 cases with *BCL2* rearrangements [*MYC/BCL2*, *MYC/BCL2/BCL6*, and *MYC/BCL2* (unknown *BCL6*); collectively referred to as DH-*BCL2/THL*], and in six (50%) of the 12 *MYC/BCL6* cases

Table 1. Clinicopathological features, genetic characteristics, and therapeutic regimens of all patients compared with those of the outcome analysis cohort.

Variable	All patients ^a (n=100)	Outcome analysis cohort ^a (n=70)
Age, years		
<60	47 (47)	34 (49)
≥60	53 (53)	36 (51)
Sex		
Male	64 (64)	41 (59)
Female	36 (36)	29 (41)
Timing of diagnosis		
<i>De novo</i>	67 (67)	59 (84)
Transformation	22 (22)	11 (16)
Recurrence	11 (11)	0 (0)
Morphologic review, central	(n=65)	(n=43)
Large cell	26 (40)	19 (43)
High grade	39 (60)	24 (56)
COO per Hans classifier	(n=97)	(n=69)
GCB	91 (94)	64 (93)
Non-GCB	6 (6)	5 (7)
<i>MYC</i> immunohistochemistry	(n=43)	(n=29)
Positive	37 (86)	26 (90)
Negative	6 (14)	3 (10)
% <i>MYC</i> +, range	20-90	0-90
% <i>MYC</i> +, median	70	50
<i>MYC</i> FISH: rearrangement partner (n=87)		(n=61)
<i>IG</i> gene	52 (60)	35 (57)
Non- <i>IG</i> gene	35 (40)	26 (43)
<i>BCL2</i> and <i>BCL6</i> FISH: rearrangement status		
<i>MYC/BCL2</i>	59 (59)	39 (56)
<i>MYC/BCL6</i>	13 (13)	11 (16)
<i>MYC/BCL2/BCL6</i>	20 (20)	14 (20)
<i>MYC/BCL2</i> (<i>BCL6</i> unknown)	8 (8)	6 (9)
Therapy		
R-CHOP	36 (36)	32 (46)
R-EPOCH	17 (17)	17 (24)
R-CODOX-M/IVAC	17 (17)	15 (21)
R-hyper-CVAD	6 (6)	6 (9)
Platinum-based salvage	10 (10)	0 (0)
Other/none/unavailable	14 (14)	0 (0)

COO: cell of origin; FISH: fluorescence *in situ* hybridization; GCB: germinal center B cell; *IG*: immunoglobulin; hyper-CVAD: cyclophosphamide, vincristine, doxorubicin, and dexamethasone; R-CHOP: rituximab, cyclophosphamide, doxorubicin, and vincristine; R-CODOX-M/IVAC: rituximab, cyclophosphamide, vincristine, doxorubicin, and high-dose methotrexate alternating with rituximab, ifosfamide, etoposide, and high-dose cytarabine; R-EPOCH: rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin. ^aValues are presented as number (%) of patients unless specified otherwise.

($P < 0.001$). All 13 *MYC/BCL6* cases were *de novo* diagnoses, whereas 38% of *DH-BCL2/THL* cases were either transformed ($n=22$) or relapsed ($n=11$) ($P=0.03$). No association was observed between morphological features and *BCL2* rearrangement status ($P=0.16$).

Therapy, clinical characteristics, and outcome

The therapeutic regimens were R-CHOP ($n=36$); dose-adjusted EPOCH-R ($n=17$); R-CODOX-M/IVAC ($n=17$); R-hyper-CVAD, methotrexate, and cytarabine ($n=6$); platinum-based salvage ($n=10$); and non-anthracycline-based treatment ($n=14$). Survival was analyzed for 70 patients receiving anthracycline-based curative-intent therapy at diagnosis of *DH/THL* (Table 1). The median (interquartile range) age of these patients was 61 (29-82) years; 41 (59%) were males. Fifty-nine patients had *de novo* *DH/THL* and 11 had transformation of a previously diagnosed low-grade lymphoma. For treatment, 32 patients

received R-CHOP; 17, dose-adjusted EPOCH-R; 15, R-CODOX-M/IVAC; and 6, R-hyper-CVAD with methotrexate and cytarabine. Twelve patients (17%) received consolidation with autologous stem cell transplantation. At a median (range) follow-up of 21 (1-87) months, EFS12 was 46% (Figure 3A) and median OS was 22 months (95% CI: 13 months - unreached) (Figure 3B). At a 5-year follow-up, the OS rate was 49% (95% CI: 37%-64%).

Patients with *DH/THL* at transformation of previously diagnosed low-grade lymphoma ($n=11$) had poor outcomes [median OS, 10.8 months; hazard ratio (HR), 2.99; 95% CI: 1.33-6.71; EFS12, 10%] - inferior to the outcomes of patients with *de novo* *DH/THL* (median OS, 22 months; $P=0.008$; EFS12, 52%) (Figure 4A). However, seven of the 11 patients had received prior immunochemotherapy for their low-grade lymphoma. Patients with high-grade morphological features on a con-

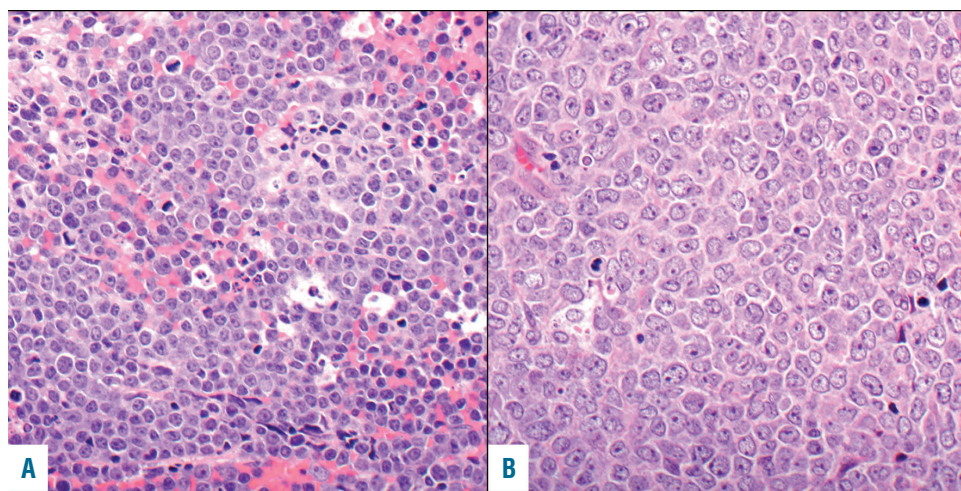


Figure 1. Comparison of high-grade and large-cell cytologic characteristics. (A) Double-hit/triple-hit lymphoma (*DH/THL*) with a high-grade morphological pattern (hematoxylin-eosin, original magnification $\times 400$). (B) *DH/THL* with large-cell morphological characteristics (hematoxylin-eosin, original magnification $\times 400$).

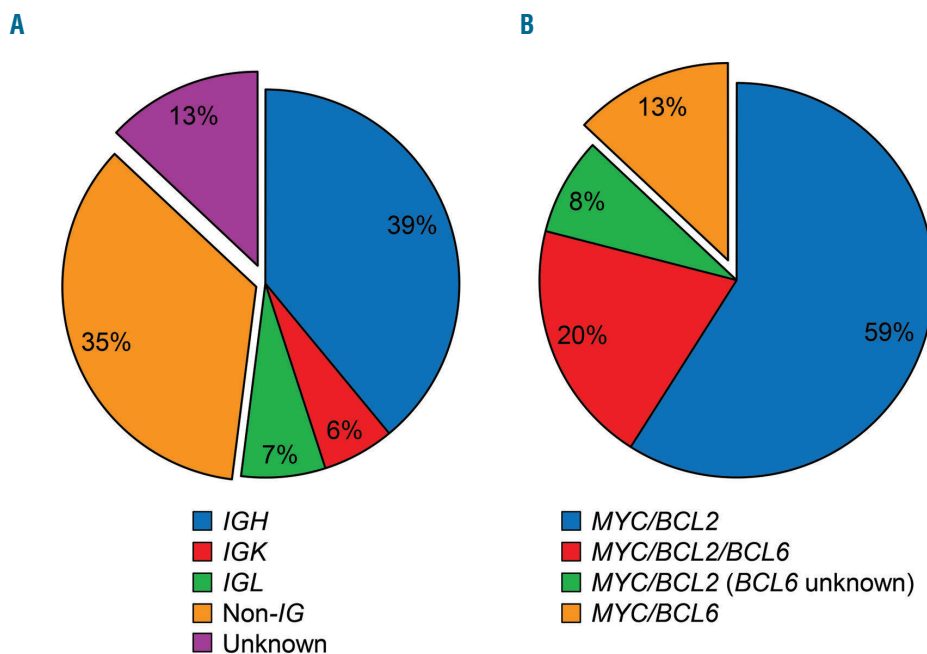


Figure 2. MYC rearrangement partners and *BCL2* and *BCL6* rearrangement status. (A) *MYC* rearrangement partners in double-hit/triple-hit lymphoma (*DH/THL*) by interphase dual-fluorescence *in situ* hybridization (FISH). Cases with an *IG/MYC* rearrangement (*IGH/MYC*, *IGK/MYC*, or *IGL/MYC*) are contiguous. (B) *BCL2* and *BCL6* rearrangement status in *DH/THL* by interphase FISH. All cases had a *MYC* rearrangement. *BCL2*-rearranged cases are contiguous whereas *MYC/BCL6* cases are not contiguous.

sensus pathology review (n=24) showed a nonsignificant tendency to inferior outcome (median OS, 13.5 months; HR, 2.32; 95% CI: 0.88-6.12; EFS12, 37%) compared with that of patients with large-cell morphological features (n=19) (median OS, unreached; P=0.09; EFS12, 57%) (Figure 4B). Patients with *MYC/BCL6* (*BCL2* rearrangement-negative) tumors showed a nonsignificant tendency to better outcomes (n=11) (median OS, unreached; HR, 0.42; 95% CI: 0.13-1.40; EFS12, 64%) than those of patients with *BCL2* rearrangement (DH-*BCL2*/THL; n=59; median OS, 21.7 months; P=0.16; EFS12, 42%) (Figure 4C). There was no association between *MYC* partner (*IG* gene versus non-*IG* gene) and OS (for *IG*, HR, 1.00; 95% CI: 0.48-2.09; P=0.99) (Figure 4D).

Compared with patients receiving all other therapies, patients treated with R-CODOX-M/IVAC had superior EFS12 (72% versus 39%, P=0.04) and showed a nonsignificant tendency to improved OS (HR, 0.37; 95% CI, 0.11-1.23; P=0.10) (Figure 5). However, patient selection bias may confound this outcome because the patients were significantly younger (P<0.001) than the patients who received other anthracycline-based regimens. Nevertheless, outcomes stayed consistent although not significant for EFS12 (HR, 0.27; 95% CI: 0.07-1.16; P=0.08) and for OS (HR, 0.40; 95% CI: 0.11-1.51; P=0.18), after adjustment for age in multivariable logistic and Cox models, respectively.

Discussion

The diagnosis of ‘high-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements’ (DH/THL) was established in the 2016 revision of the WHO classification of lymphoid neoplasms, in part to acknowledge the prognostic significance of the *MYC* and *BCL2* or *MYC* and *BCL6* rearrangements, or both, in B-cell lymphomas with large-cell or high-grade morphological features. However, our study shows that within this umbrel-

la category, several subgroups have distinctive features and varying clinical outcomes. Strengths of this single-institution study include thorough pathology review, comprehensive analyses of genetic features and cell of origin, and availability of key clinical, treatment, and outcome data. The main limitations of this retrospective study include missing *MYC* rearrangement data for some cases, potential for selection bias in treatment choice given to the patient, inclusion of cases over an 11-year time frame during which clinical management evolved rapidly, and small sample size for some analyses. Although there is likely some bias toward selection of cases with high-grade cytological features in the early specimens, this bias should not have been present in the 57 cases that were identified after our institution began performing interphase FISH to identify DH/THL in all B-cell lymphomas with either large-cell or high-grade histological features. Of the specimens with histological consensus re-review, 16/20 (80%) of the early cases but only 23/45 (51%) of the later cases had a morphological diagnosis of high-grade B-cell lymphoma, suggesting that the selection bias present in the early cases had been mitigated in the later group.

Our data suggest several important observations. First, patients with DH/THL at transformation of previously diagnosed low-grade lymphoma had a dismal outcome with a median OS of 10.8 months and EFS12 of 10%. In light of the small number of cases and potential confounding effect of the prior immunochemotherapy for some patients, this observation should be interpreted with caution. However, it raises an interesting biological question that may warrant investigation in future studies. Few previous studies have addressed this, although a report of two cases of transformation of low-grade follicular lymphoma to DH/THL described an aggressive clinical course.³⁴ In DH/THL, induction failure occurs early and is worse among patients aged 60 years or older. Nevertheless, the OS for the entire cohort at 5 years was 49%, suggestive of long-term cure in a subset of patients.

Table 2. Correlations between clinicopathological and genetic characteristics in *de novo*, transformed, and recurrent double-hit/triple-hit lymphoma.^a

Characteristic	All patients	Morphological evaluation		Cell of origin		MYC partner		BCL2		Double expresser ^b	
		High grade	Large cell	GCB	Non-GCB	IG gene	Non-IG gene	Rearranged	Not Rearranged	Yes	No
All patients		39 (60)	26 (40)	91 (94)	6 (6)	52 (60)	35 (40)	87 (87)	13 (13)	30 (81)	7 (19)
Timing of diagnosis											
<i>De novo</i>	67 (67)	20 (51)	19 (49)	59 (91)	6 (9)	34 (60)	23 (40)	54 (81)	13 (19)	20 (87)	3 (13)
Transformed	22 (22)	10 (63)	6 (38)	21 (100)	0 (0) ^c	12 (60)	8 (40)	22 (100)	0 (0) ^c	8 (73)	3 (27)
Recurrent	11 (11)	9 (90)	1 (10) ^d	11 (100)	0 (0)	6 (60)	4 (40)	11 (100)	0 (0)	2 (67)	1 (33)
Morphological evaluation											
High grade	39 (60)			36 (95)	2 (5)	20 (53)	18 (48)	35 (90)	4 (10)	16 (80)	4 (20)
Large cell	26 (40)			22 (88)	3 (12)	13 (52)	12 (48)	20 (77)	6 (23)	10 (83)	2 (17)
Cell of origin											
GCB	91 (94)					48 (62)	30 (38)	85 (93)	6 (7)	28 (82)	6 (18)
Non-GCB	6 (6)					2 (33)	4 (67)	0 (0)	6 (100) ^e	2 (67)	1 (33)
MYC partner											
IG gene	52 (60)							46 (88)	4 (12)	18 (84)	3 (16)
Non-IG gene	35 (40)							28 (80)	7 (20)	12 (75)	4 (25)
BCL2											
Rearranged										28 (85)	5 (15)
Not rearranged										2 (50)	2 (50)

GCB: germinal center B cell; IG: immunoglobulin. ^aValues are presented as number (%) of patients. ^bExpression of both MYC and BCL2. ^cP=0.01 to <0.05. ^dP=0.05 to 0.10. ^eP≤0.001.

Of note, progression or relapse occurred almost exclusively within 12 months of diagnosis, suggesting that EFS12 is worth further evaluation as an endpoint for studies of DH/THL.³⁹

Treatment with R-CODOX-M/IVAC may result in superior outcomes for patients younger than 60 years, despite the preponderance of aggressive (high-grade) histological findings within this group. However, selection bias may also have an important role in the superior outcomes for this cohort. A multicenter retrospective analysis evaluated effect of induction regimen and stem cell transplantation on outcomes in DHL, showing that intensive induction therapy was not associated with improved OS.¹⁵ In a meta-analysis of 11 studies and 394 patients, OS was not different across the treatment approaches used in our series.⁴⁰ Sun *et al.* reported a 2-year progres-

sion-free survival rate of 60% and a 2-year OS rate of 82% for 16 patients treated with R-CODOX-M/IVAC followed by hematopoietic stem cell transplantation.³⁰ No randomized clinical trial for this histological subset is currently available.

Second, DH/THL patients with high-grade histological characteristics showed a tendency, although not statistically significant, toward a more aggressive clinical course. Published literature has provided mixed results on this issue. Johnson *et al.*, in their retrospective study of BCLU and DLBCL with concurrent *MYC* and *BCL2* rearrangements identified through karyotypic analysis, showed that BCLU morphological appearance was associated with poorer outcome ($P < 0.001$).⁶ Other investigators have obtained similar results.⁴⁰⁻⁴² Blastoid morphological characteristics have also been associated with adverse clinical

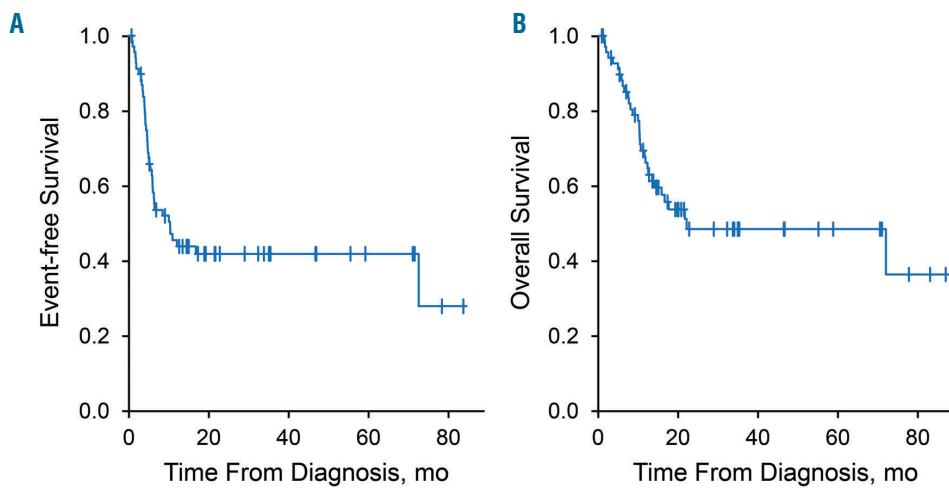


Figure 3. Event-free survival and overall survival of patients receiving anthracycline-based therapy. (A) Event-free survival of the 70 patients receiving anthracycline-based therapy at the time of diagnosis of double-hit/triple-hit lymphoma. (B) Overall survival of the 70 patients.

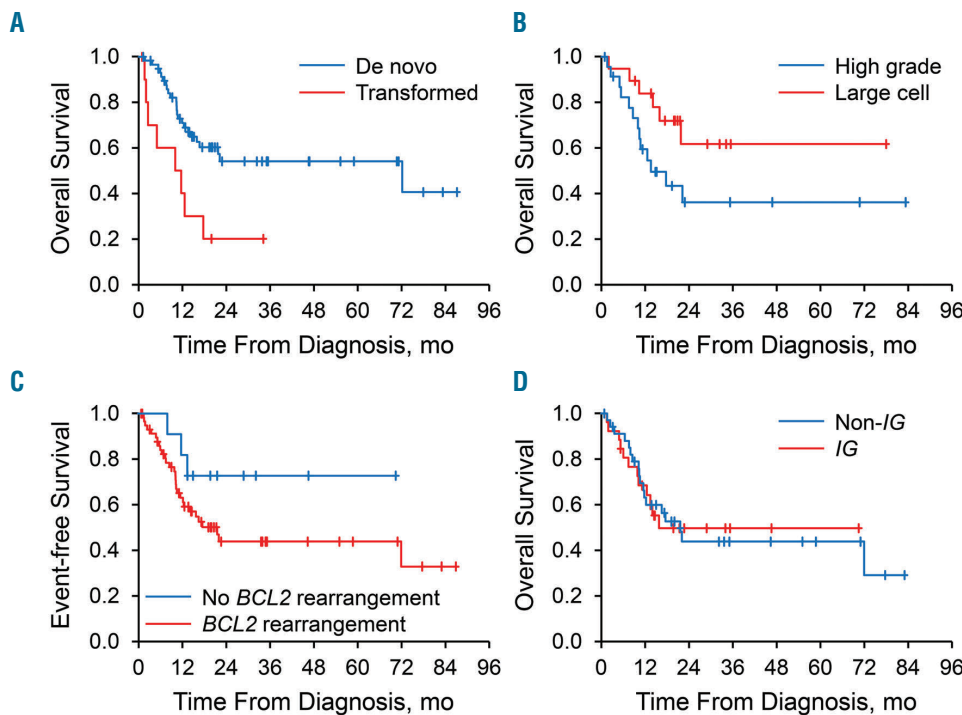


Figure 4. Overall survival according to transformation of previously diagnosed low-grade lymphoma, morphological characteristics, *BCL2* rearrangement status, and *MYC* partner (*IG* gene versus non-*IG* gene). (A) Patients with double-hit/triple-hit lymphoma (DH/THL) at transformation of previously diagnosed low-grade lymphoma had inferior overall survival (OS) compared with patients who had *de novo* DH/THL [hazard ratio (HR) 2.99, $P = 0.007$]. (B) DH/THL patients with high-grade morphological characteristics showed a tendency toward inferior OS compared with patients with large-cell morphological characteristics, but it was not statistically significant (HR 2.32, $P = 0.09$). (C) DH/THL patients with *BCL2* rearrangements [*MYC/BCL2*, *MYC/BCL2/BCL6*, and *MYC/BCL2* (*BCL6* unknown)], so-called DH-*BCL2*/TH, showed a non-significant tendency toward inferior OS compared with those who did not have *BCL2* rearrangements (*MYC/BCL6*) (HR 2.44, $P = 0.16$). (D) No association was observed between *MYC* partner (*IG* versus non-*IG*) and OS ($P = 0.99$).

outcome.⁴⁵ In contrast, Petrich *et al.*, in their retrospective study of 311 DH/THL patients, reported that histological appearance had no effect on OS; however, theirs was a multicenter study across 23 medical centers without central pathological review.¹⁵ Our cases were derived from a single institution, and outcome analysis was restricted to cases with a consensus re-review diagnosis among four expert hematopathologists, lending support to the validity of this finding.

The clinical significance of high-grade histological characteristics underscores the importance of the revised WHO recommendation to note morphological appearance in all DH/THL cases.¹ Furthermore, because DH/THL cases often show morphological characteristics of DLBCL (40% in this series), a complete interphase FISH analysis to exclude DH/THL is important for all cases with large-cell or high-grade morphological appearance. Also, low MYC expression by immunohistochemistry does not reliably predict absence of a MYC rearrangement. In the present series, 14% of evaluable cases, all with a MYC rearrangement by definition, had fewer than 40% MYC-positive cells by immunohistochemistry. However, these cases were too few to analyze outcome, and it is not known whether absence of MYC expression in DH/THL has a favorable impact on survival.

Third, clinical and pathological differences were reported and depended on whether a BCL2 rearrangement was present. BCL2-rearranged cases [i.e., MYC/BCL2, MYC/BCL2/BCL6, or MYC/BCL2 (BCL6 unknown)] showed heterogeneous presentation as *de novo*, recurrent, or transformation events but were uniformly of GCB type. The latter finding has been previously described.^{4,5,7,9,26} Conversely, MYC/BCL6 cases uniformly developed *de novo* but were equally likely to be GCB or non-GCB type. Thus, all patients with non-GCB DH/THL in our cohort had MYC/BCL6. Although the association between non-GCB subtype and MYC/BCL6 has been reported previously,^{5,9} this is the largest cohort to date to investigate this issue.

Clinically, our 12 MYC/BCL6 cases with outcome analysis showed a nonsignificant tendency to superior OS and EFS12, regardless of whether only the *de novo* cases were evaluated. Previous studies have yielded con-

flicting results. Two studies showed an association between MYC/BCL6 and poor prognosis.^{9,14} In the first study, only six FISH-confirmed cases were evaluated.¹⁴ In the second study, the MYC/BCL6 group, which had older patients with uniformly large-cell histological features, was compared with a MYC/BCL2 group that was younger and had various histological patterns, including low-grade lymphoma.⁹ Two other studies reported improved survival of patients with MYC/BCL6 compared to those with MYC/BCL2 DH/THL, but only seven and four cases, respectively, were studied.^{5,44} Another group showed better survival in *de novo* MYC/BCL6 with large-cell morphological features compared with MYC/BCL2 DH/THL combined with MYC-rearranged single-hit DLBCL.¹² Four additional studies – three included patients with large-cell lymphomas and high-grade histological characteristics^{13,15,25} and one comprised only subjects with large-cell histological features²⁶ – showed no difference in survival between MYC/BCL2 and MYC/BCL6 DH/THL. However, the former two studies also included cases of follicular lymphoma,^{13,15} and the third study included cases with extra intact signals, as well as rearrangements of MYC, BCL2, and BCL6.²⁵ This lack of uniformity may have affected the outcome data. More studies are needed to resolve the issue of the prognostic significance of MYC/BCL6 in DH/THL.

Some groups have suggested that it may not be necessary to perform interphase FISH studies to exclude DH/THL in large-cell or high-grade B-cell lymphomas of non-GCB phenotype. However, if this approach were followed, about one-half of MYC/BCL6 DH/THL would fail to be identified. The MYC/BCL6 cases in our study showed a tendency toward superior OS and EFS12. Nevertheless, in the light of conflicting outcome data in the literature, potential for poor clinical outcome, and suggested benefit from more aggressive therapy in this cohort, we advocate the performance of interphase FISH to exclude DH/THL in all large-cell and high-grade B-cell lymphomas, regardless of their cell of origin.

Fourth, we found no association between MYC partner (*IG* gene versus non-*IG* gene) and clinical outcome. To our knowledge, our study represents the largest (n=87) and most comprehensively studied cohort to date in which

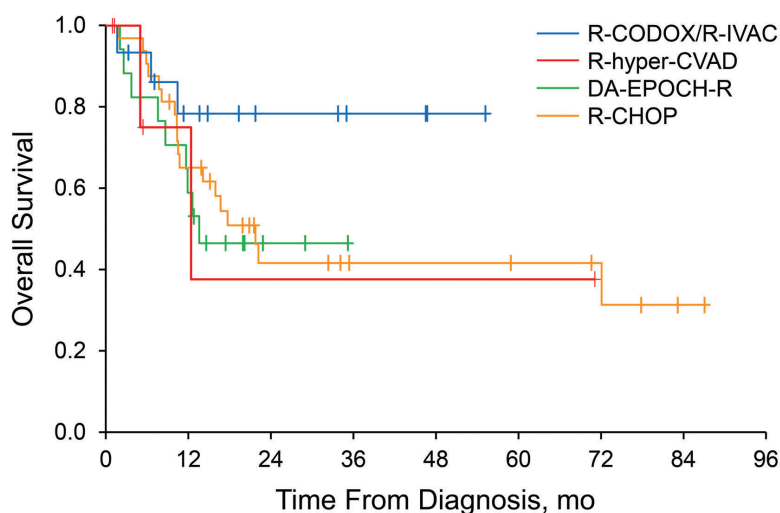


Figure 5. Overall survival according to treatment regimen. Patients treated with R-CODOX-M/R-IVAC had superior overall survival, although the difference was not statistically significant (hazard ratio 0.37, $P=0.10$). DA-EPOCH-R: dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab; R-CHOP: rituximab, cyclophosphamide, doxorubicin, and vincristine; R-CODOX-M/IVAC: rituximab, cyclophosphamide, vincristine, doxorubicin, and high-dose methotrexate alternating with rituximab, ifosfamide, etoposide, and high-dose cytarabine; R-hyper-CVAD: rituximab, cyclophosphamide, vincristine, doxorubicin, and dexamethasone.

this issue has been addressed. Previous studies on this matter have yielded conflicting results. Johnson *et al.*, in a retrospective study of 54 DLBCL and BCLU cases with *MYC/BCL2* identified by abnormal karyotype, showed that a non-*IG/MYC* partner was associated with a favorable clinical outcome.⁶ However, they also found an association between non-*IG/MYC* partner and DLBCL histological appearance ($P < 0.001$). In contrast, we found no such association in our cohort ($P = 0.96$). This difference may, therefore, in part explain the divergent findings of clinical outcome between the two studies. In addition, case identification by abnormal karyotype may have selected for more aggressive disease. Pedersen *et al.* prospectively studied a cohort of 237 DLBCL and BCLU cases, including primary, transformed, and relapsed cases.⁸ They observed that *IG/MYC* rearrangements ($n = 9$) were associated with a worse OS than were non-*IG/MYC* rearrangements ($n = 10$). However, only 19 cases were studied, and this analysis included both *MYC/BCL2* and *MYC* single-hit cases but lacked *MYC/BCL6* cases. In addition, the patients received various treatment regimens, although most included rituximab plus intensive chemotherapy. An additional controversial element was the FISH protocol, because cases that were positive for a *MYC* rearrangement on the basis of a split *MYC* break-apart probe but were negative for *IGH/MYC* fusion were then studied with *IGK* and *IGL* break-apart probes rather than *IGK/MYC* and *IGL/MYC* dual-fusion FISH probes. Cases with concurrent *MYC* and *IGK* or *IGL* split break-apart probes were presumed to represent fusions, likely overestimating the number of *IG/MYC* cases.

Copie-Bergman *et al.* performed interphase FISH using break-apart probes for *MYC*, *BCL2*, and *BCL6* and dual-fusion FISH probes for *IGH/MYC/CEP8*, *IGK/MYC*, and *IGL/MYC* as needed in 574 *de novo* DLBCL cases treated with rituximab–anthracycline-based chemotherapy.⁵ They found that the DH/THL cohort as a whole ($n = 32$) had poorer outcome than the DLBCL cohort that lacked DH/THL rearrangements ($P = 0.046$). However, on evaluating the *IG/MYC* and non-*IG/MYC* partner cases separately, Copie-Bergman *et al.* found that the 12 *IG/MYC* DH/THL cases had a poorer prognosis than the 19 DH/THL cases without non-*IG/MYC* rearrangement. They also observed poorer OS and progression-free survival for the 25 *MYC/BCL2* cases than for the seven *MYC/BCL6* cases, but they did not report the *IG* gene versus non-*IG* gene partners for these cases. Therefore, the *IG* versus non-*IG* gene results may have been confounded by the number of *MYC/BCL2* versus *MYC/BCL6* cases in each group. More than 90% of their DH/THL cases had large-cell histological characteristics (3 were reclassified

as high grade on re-review); by comparison, 60% of our cases had high-grade histological characteristics, which may have contributed to the discrepant findings between the two groups.

Other studies have shown no difference in survival between *IG/MYC* and non-*IG/MYC* groups. Aukema *et al.* studied 80 *MYC* rearrangement-positive B-cell lymphomas, with exclusion of pediatric cases as well as adult cases with a gene expression profile of molecular BL.⁹ Most cases had the histological appearance of DLBCL, although some had other histological patterns, such as BCLU and follicular lymphoma. Similar to our study, Aukema *et al.* found no difference in survival between the *IG/MYC* and non-*IG/MYC* groups, both overall and within the *MYC/BCL2* ($n = 26$) and *MYC/BCL6* ($n = 14$) subgroups. However, various treatment regimens were used, and only some patients received immunotherapy (rituximab). Li *et al.* also found no significant difference in survival in *MYC/BCL2* DH/THL cases with *IG* ($n = 23$) versus non-*IG* ($n = 5$) *MYC* partners.¹¹

More studies are needed to resolve the issue of the prognostic significance of *MYC* rearrangement partner in DH/THL. Our study, as well as those of Aukema *et al.*⁹ and Li *et al.*,¹¹ suggests that it may not be necessary to identify whether the *MYC* gene rearrangement partner is an *IG* gene. Our current approach is to perform FISH on all aggressive B-cell lymphomas. However, in the light of these findings coupled with the association of DH/THL with such features as GCB phenotype, high-grade B-cell lymphoma morphological characteristics, and high *MYC* expression in immunohistochemistry, further risk-benefit analyses of alternative triage strategies are warranted.

In conclusion, in this large, retrospective, single-institution study of DH/THL, although no differences in survival were seen between the *IG/MYC* and non-*IG/MYC* groups, transformation from previously treated and untreated low-grade lymphoma was associated with inferior OS, and there was a trend toward inferior OS in patients with high-grade morphological patterns and the presence of a *BCL2* rearrangement.

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