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ORIGINAL REPORT

Concurrent Expression of MYC and BCL2 in Diffuse Large B-Cell Lymphoma Treated With Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone

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ABSTRACT

Purpose
Diffuse large B-cell lymphoma (DLBCL) is curable in 60% of patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). *MYC* translocations, with or without *BCL2* translocations, have been associated with inferior survival in DLBCL. We investigated whether expression of MYC protein, with or without BCL2 protein expression, could risk-stratify patients at diagnosis.

Patients and Methods
We determined the correlation between presence of MYC and BCL2 proteins by immunohistochemistry (IHC) with survival in two independent cohorts of patients with DLBCL treated with R-CHOP. We further determined if MYC protein expression correlated with high *MYC* mRNA and/or presence of *MYC* translocation.

Results
In the training cohort (n = 167), MYC and BCL2 proteins were detected in 29% and 44% of patients, respectively. Concurrent expression (MYC positive/BCL2 positive) was present in 21% of patients. MYC protein correlated with presence of high *MYC* mRNA and *MYC* translocation (both $P < .001$), but the latter was less frequent (both 11%). MYC protein expression was only associated with inferior overall and progression-free survival when BCL2 protein was coexpressed ($P < .001$). Importantly, the poor prognostic effect of MYC positive/BCL2 positive was validated in an independent cohort of 140 patients with DLBCL and remained significant ($P < .05$) after adjusting for presence of high-risk features in a multivariable model that included elevated international prognostic index score, activated B-cell molecular subtype, and presence of concurrent *MYC* and *BCL2* translocations.

Conclusion
Assessment of MYC and BCL2 expression by IHC represents a robust, rapid, and inexpensive approach to risk-stratify patients with DLBCL at diagnosis.

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin's lymphoma and is curable in more than 60% of patients treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP).[1] The best available clinical tool to risk-stratify patients with DLBCL at diagnosis is the International Prognostic Index (IPI); however, there remains marked heterogeneity in clinical outcomes within each risk group, and IPI variables do not provide insight into the underlying tumor biology. Gene expression profiling (GEP) can group DLBCL into prognostically different molecular subtypes based on cell-of-origin (COO) gene signatures, where the activated B-cell (ABC) type is associated with inferior overall survival (OS) compared with the germinal center B-cell (GCB) type.[2,3] GEP is not available in most clinical laboratories; thus, immunohistochemical

algorithms, such as the one proposed by Choi et al,[4] have been developed assigning a COO subtype based on the expression of COO-related proteins.[5,6] Unfortunately, the accuracy with which these algorithms correctly classify COO subtype or predict OS is variable among laboratories.[4,6,7]

Alterations in oncogenes and tumor suppressor genes can drive the pathogenesis of DLBCL.[8,9] Two such oncogenes are *MYC* and *BCL2*, key regulators of cellular proliferation and apoptosis, respectively.[10,11] Deregulation of *MYC* and *BCL2* can result from chromosomal translocation or gene amplification, but it may also occur by other mechanisms, such as transcriptional upregulation downstream of NFκB pathway signaling.[10,12,13]

The presence of *MYC* translocation and high *MYC* mRNA expression have recently been associated with poor OS in patients with DLBCL treated with R-CHOP, raising questions about optimal management of these high-risk patients.[14-16] However, many of these patients with *MYC*-positive DLBCL also coexpress high levels of BCL2 protein, which may be a confounding factor in this disease, given that the presence of concurrent *MYC* and *BCL2* translocations—so-called double hits (DHITs)—are associated with a dismal outcome despite high-dose chemotherapy.[14-19] Fluorescence in situ hybridization (FISH) has been useful at identifying *MYC* translocations but has failed to identify altered MYC expression by other mechanisms and is not available in all clinical laboratories. Recently, a novel monoclonal antibody that targets the *N*-terminus of the MYC protein was shown to provide sensitive and specific staining of nuclear MYC in paraffin embedded tissue, including DLBCL.[20-22]

Herein, we demonstrate that MYC protein expression by IHC represents a rapid and inexpensive marker to identify MYC overexpression in DLBCL, including patients harboring *MYC* translocations, and that the prognostic significance of *MYC* deregulation in R-CHOP–treated patients with DLBCL depends on its coexpression with BCL2 protein.

PATIENTS AND METHODS

Patient Population

We used pretreatment tumor biopsies taken from two independent cohorts of patients diagnosed with de novo DLBCL according to WHO classification (2008) criteria.[1] Patients were initially selected because they were linked to clinical information, including baseline characteristics and outcome, were HIV negative, and were treated with curative intent with R-CHOP therapy (with or without radiation). Ethical approval was granted by the research ethics board of each institution, in accordance with the Declaration of Helsinki.

The training set consisted of 167 patients who were further selected based on the availability of both fresh frozen and formalin-fixed paraffin-embedded (FFPE) tissue, provided from 10 international institutions. A consensus diagnosis of DLBCL was confirmed by a panel of expert pathologists. A subset of these patients were previously reported by Lenz et al[3] (n = 158), Savage et al[14] (n = 49), Iqbal et al[23] (n = 167), and Choi et al[4] (n = 68). DLBCL molecular subtype (GCB, ABC, and unclassifiable) and molecular Burkitt's lymphoma, if present in DLBCL patient cases with high *MYC* expression, were assigned by GEP according to previously published protocols.[3,24,25]

The validation set consisted of 140 patients from the British Columbia Cancer Agency (BCCA) who were selected based on availability of FFPE tissue only. Nine patients were included in the study by Savage et al[14]; COO for these patients was assigned by IHC according to the Choi et al[4] algorithm.

IHC Analysis

Cores of FFPE tissue were used to construct tissue microarrays at each participating institution. In both data sets, staining was performed on the Ventana platform (Roche, Basel, Switzerland) using routine staining protocols and the following antibodies: BCL2, clone 124 (Dako, Carpinteria, CA); MYC, Y69 (Epitomics, Burlingame, CA) and Ki67 (Dako; Appendix, online only). Protein expression was recorded in 10% increments as the percentage of positive tumor cells. Training set data were analyzed using X-Tile statistical software (http://www.tissuearray.org/rimmlab) to determine the optimum survival cut points for dichotomizing expression of MYC protein (≥ 40%), BCL2 protein (≥ 50%), and Ki67 index (≥ 90%).[26] These cut points correspond to the maximum χ^2 value of the Mantel-Cox test for OS between groups above and below the cut-point threshold.[26] These same cut points were then carried through to the validation set.

The reproducibility of BCL2 and MYC protein expression by IHC was determined by comparing the results obtained by three different pathologists. In the training set, BCL2 expression in approximately half of the patients was scored by three pathologists at the University of Nebraska Medical Center (W.C.C., P.N.M., D.D.W.), with no disagreement; thus, the latter half were evaluated by a single pathologist (P.N.M.).[23] MYC and Ki67 expression were determined by a single pathologist (G.W.S.) at the BCCA. In the validation set, three pathologists at the BCCA (G.W.S., K.L.T., R.D.G.) scored patient cases for BCL2, MYC, and Ki67 protein expression using the same antibodies and thresholds described for the training set. Concordance was achieved when all three pathologists assigned the same positive or negative expression value for a patient. Discordant patient cases were evaluated by all three pathologists at a multiheaded microscope to reach a consensus score.

FISH

Translocations involving *BCL2* and *MYC* were identified by interphase FISH in FFPE tissue in tissue microarrays from both data sets using commercial dual-color break-apart probes from Abbott Molecular (Abbott Park, IL) according to previously described methods.[14] Patient cases with break-apart signals in > 5% of nuclei were considered positive for the presence of a translocation. In the training and validation cohorts, FISH experiments were successful in 167 and 123 patient cases using the *MYC* probe and in 157 and 120 using the *BCL2* probe, respectively.

GEP

GEP was performed on fresh frozen tissue from the training set using Affymetrix HG U133 Plus 2.0 arrays (Affymetrix, Santa Clara, CA).[3] *MYC* mRNA expression was determined using $\log_2$ normalized expression values of probe set 202431_s_at and dichotomized into low versus high expression using a cutoff threshold determined by X-Tile (high, > 9.4).[26]

Statistical Analysis

Progression-free survival (PFS; PFS event, progression or death resulting from any cause) and OS (OS event, death resulting from any cause) were measured from the date of pathologic diagnosis and estimated using the Kaplan-Meier method. Differences were assessed using the log-rank test. The Cox proportional hazards model was used to determine hazard ratios (HRs) and CIs and whether variables were independent of the IPI by multivariate analysis. The χ^2 test, Fisher's exact test, and Pearson correlation were used to determine association and correlation between variables. These statistical analyses were performed using SPSS (version 20.0; SPSS, Chicago, IL) and STATA software (version 8.2; STATA, College Station, TX).

RESULTS

MYC and BCL2 Expression in DLBCL

Within the training set, MYC protein was expressed in one third of patients with DLBCL (n = 48 of 167; 29%). In contrast, *MYC* translocations and high *MYC* mRNA expression were detected in only 18 (11%) of 167 and 19 (11%) of 167 patients, respectively, with nine overlapping both groups. MYC protein expression was exclusively nuclear in all cases (Figs 1A to 1C) and was reproducible among pathologists (concordance 94%). Incidence of MYC protein expression (52 of 140; 37%) and *MYC* translocation (16 of 123; 13%) in the

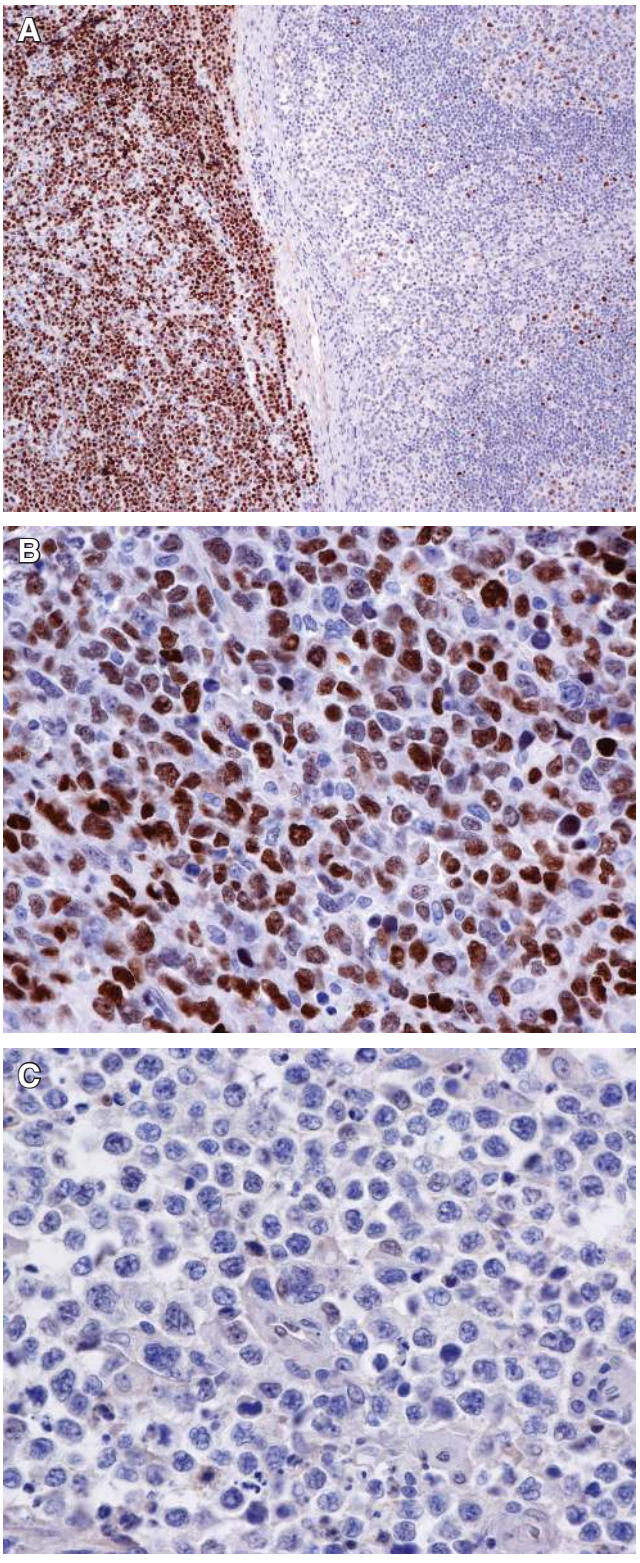

Fig 1. Representative immunohistochemical analysis of MYC protein expression in diffuse large B-cell lymphoma (DLBCL). (A) Control: partial lymph node involvement by Burkitt's lymphoma (left) and follicular hyperplasia (right); there is bright nuclear staining of MYC protein in Burkitt's lymphoma cells, compared with < 5% of benign germinal center B cells stained for MYC (right). (B) MYC protein–positive DLBCL. (C) MYC protein–negative DLBCL.

validation set was similar to that in the training set (Table 1). When looking at MYC protein expression in the combined data sets, there was a wide distribution of MYC protein expression across samples (Appendix Fig A1, online only). The percentage of cells expressing MYC protein was significantly greater in patient cases with translocations than without translocations (mean percent positive cells, 61%; range, 20 to 100 *v* 29%; range, 0 to 100, respectively; $P < .001$). Although MYC protein expression correlated with a high proliferation rate ($r = 0.41$; $P < .001$), MYC protein expression, at our predetermined 40% threshold, was more sensitive than Ki67 at identifying patients with *MYC* translocations (25 of 34; 74% *v* 7 of 34; 21%). Alterations involving *MYC* were present in both the ABC and GCB subtypes of DLBCL, whether defined by GEP (training set) or by IHC (validation set; Table 2).

BCL2 protein expression was associated with the ABC subtype, and *BCL2* translocation was associated with the GCB subtype ($P = .001$; Table 2). Both were more common in the validation cohort, which included older patients ($P < .05$; Table 1). Indeed, there was an age-related increase in the incidence of BCL2 protein expression, *BCL2* translocation, and ABC subtype, but not *MYC* alterations (Appendix Fig A2, online only). Importantly, BCL2 protein expression by IHC was reproducible among pathologists, with a concordance of more than 91%.

Factors Associated With Clinical Outcome

In univariate analysis, MYC protein expression and *MYC* translocation, alone, were not significantly associated with survival in the training set ($P > .05$). Factors that were associated with OS and PFS included elevated IPI score, ABC subtype, high *MYC* mRNA expression, and BCL2 protein expression (Appendix Table A1, online only). Next, we determined if BCL2 protein expression affected prognosis of MYC deregulation and whether there was an interaction between MYC and BCL2 variables. Presence of *MYC* translocation, high *MYC* mRNA expression, or high MYC protein expression, hereafter referred to as MYC positive, was only associated with inferior OS and PFS when BCL2 protein was coexpressed (BCL2 positive; Figs 2A to 2C; $P < .001$). Indeed, the interaction between these variables suggested that the negative prognostic impact of MYC and BCL2 was amplified when both variables were present, an effect that was consistent across platforms (protein, mRNA, or translocation; Appendix Table A1, online only). For example, the OS HR for coexpression of MYC-positive/BCL2-positive proteins was 3.2, compared with 0.47 for the MYC-positive/BCL2-negative group and 1.6 for the MYC-negative/BCL2-positive group ($P = .001$, $P = .11$, and $P = .17$, respectively). In the validation cohort, coexpression of MYC-positive/BCL2-positive proteins was also associated with inferior OS (HR, 1.6; $P = .17$), but there was no difference in outcome in the remaining patients (Appendix Fig A3, online only). Samples were then stratified into a high-risk group, defined by the presence of MYC-positive/BCL2-positive protein coexpression, and low-risk group, comprising the remaining patients. Presence of MYC-positive/BCL2-positive protein expression was associated with significantly inferior OS and PFS in both training and validation DLBCL cohorts (Figs 3A and 3B; $P < .05$). Importantly, the negative prognostic impact of MYC-positive/BCL2-positive status on OS persisted even after adjusting for IPI and COO in a Cox multivariant model ($P \leq .05$; Appendix Table A2, online only).

Table 1. Clinical Characteristics of Two Cohorts of Patients With DLBCL Treated With R-CHOP

Characteristic	All Patients (N = 307) No.	%	Training Cohort (n = 167) No.	%	Validation Cohort (n = 140) No.	%	P*
Age, years							
Median	63		62		65		
Range	17-92		17-92		19-90		
> 60	179 of 307	58	85 of 167	51	94 of 140	67	**.004**
Ann Arbor stage > II	162 of 296	55	81 of 161	50	81 of 135	60	.107
LDH > upper limit of normal	126 of 297	42	67 of 167	40	59 of 130	45	.364
Extranodal sites ≥ two	50 of 261	19	19 of 126	15	31 of 135	23	.107
ECOG PS > 1†	78 of 282	28	36 of 148	24	42 of 134	31	.190
IPI score‡							**.021**
0 or 1	114 of 288	40	72 of 154	47	42 of 134	31	
2	76 of 288	26	41 of 154	27	35 of 134	26	
3	54 of 288	19	22 of 154	14	32 of 134	24	
4 or 5	44 of 288	15	19 of 154	12	25 of 134	19	
R-CHOP21			159	95	140	100	
R-CHOP14			8	5			.759
Radiation			37	22	29	21	
Cell-of-origin subtype			GEP		IHC§		
ABC	139 of 306	45	70 of 167	42	69 of 139	50	.178
GCB	144 of 306	47	74 of 167	44	70 of 139	50	
Unclassified‖	21 of 306	7	21 of 167	13			
Molecular Burkitt's lymphoma	2 of 306	1	2 of 167	1			
MYC							
MYC translocation	34 of 290	12	18 of 167	11	16 of 123	13	.561
High mRNA expression			19 of 167	11			
High MYC protein expression	100 of 307	33	48 of 167	29	52 of 140	37	.094
BCL2							
BCL2 translocation	68 of 287	24	29 of 157	18	39 of 130	30	**.022**
High BCL2 protein expression	160 of 304	53	73 of 164	44	87 of 140	62	**.002**
Median follow-up time, years¶			3.5		4.7		.503
5-year OS				64		62	

NOTE. Bold font indicates significance.

Abbreviations: ABC, activated B-cell–like; DLBCL, diffuse large B-cell lymphoma; ECOG PS, Eastern Cooperative Oncology Group performance status; GCB, germinal center B-cell–like; GEP, gene expression profiling; IHC, immunohistochemistry; IPI, International Prognostic Index; LDH, lactate dehydrogenase; OS, overall survival; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CHOP14, R-CHOP 14-day cycle; R-CHOP21, R-CHOP 21-day cycle.

*P values are for comparison between training and validation cohorts.

†ECOG PS ranges from 0 to 4, where higher score indicates greater degree of impairment.

‡IPI score ranges from 0 to 5, with 0 indicating absence of prognostic factors and 5 indicating presence of all prognostic factors; IPI score was not calculated if more than one variable was unavailable.

§IHC algorithm according to Choi et al.[4]

‖Unclassified indicates gene expression profiles are intermediate between ABC and GCB.

¶Median follow-up time for living patients was 3.5 years (range, 0.52 to 11.3 years) and 4.7 years (range, 1.0 to 8.0 years) for training and validation sets, respectively.

Concurrent Translocation of MYC *and* BCL2

There were 14 patient cases in the combined data set (14 of 290; 5%) fitting the diagnostic criteria of DLBCL but with concurrent translocation of *MYC* and *BCL2* (ie, DHIT). Given this low incidence, both data sets were pooled for subsequent analyses. These patients had more adverse clinical risk factors, including higher levels of lactate dehydrogenase, worse performance status, and higher IPI scores than other patients with DLBCL ($P < .05$; Appendix Table A3, online only). DHIT samples expressed BCL2 protein (13 of 13); 10 (71%) of 14 samples expressed MYC protein, but only one (7%) of 14 had Ki67 > 90%. As expected, the clinical outcome of patients with DLBCL with DHIT status treated with R-CHOP in this study was extremely poor, with 5-year OS and PFS rates of 27% and 18%, respectively (Fig 3C). However, the outcome of the remaining MYC-positive/BCL2-positive patients (excluding those with DHIT status) was also poor compared with outcome among those who did not express MYC-positive/BCL2-positive proteins (Fig 3C; 5-year OS and PFS, 36% and 32% *v* 71% and 65%, respectively; $P < .05$). Interestingly, two of the three long-term survivors in the DHIT group had < 40% cells in their biopsy expressing MYC protein, suggesting that this may have prognostic relevance, analogous to what has been previously reported for BCL2 protein expression in DHITs,[19] but too few patient cases preclude any meaningful conclusions.

DISCUSSION

Overexpression of MYC protein occurs in 33% of patient cases with DLBCL, of which only one third can be explained by the presence of *MYC* translocation. That MYC protein is expressed in both the GCB

Table 2. Differences in *MYC* and *BCL2* Alterations According to Cell-of-Origin Subtype in Two Cohorts of Patients With DLBCL

	ABC		GCB		Unclassified*		mBL		
Cohort	No.	%	No.	%	No.	%	No.	%	*P*†
MYC									
MYC translocation									
Training‡	6 of 70	9	10 of 74	14	1 of 21	5	1 of 2	50	.346
Validation§	6 of 62	10	10 of 61	16					.268
High mRNA expression									
Training	10 of 70	14	5 of 74	7	2 of 21	10	2 of 2	100	.139
MYC protein expression									
Training	24 of 70	34	17 of 74	23	6 of 21	29	1 of 2	50	.399
Validation	**35 of 69**	**51**	17 of 70	24					**.001**
BCL2									
BCL2 translocation									
Training	4 of 70	6	**23 of 74**	**31**	2 of 11	10	0	0	**< .001**
Validation	11 of 65	17	**28 of 65**	**43**					**.001**
BCL2 protein expression									
Training	**44 of 70**	**63**	22 of 74	30	7 of 21	35	0	0	**< .001**
Validation	**53 of 69**	**77**	34 of 70	49					**.001**
DHIT‖¶	5 of 14	36	9 of 14	64					
MYC and BCL2 protein expression¶	42 of 55	76	13 of 55	24					**< .001**
Absence of concurrent MYC and BCL2 protein expression or DHIT	92 of 235	39	143 of 235	61					

NOTE. Bold font indicates significance.

Abbreviations: ABC, activated B-cell lymphoma; DHIT, double-hit lymphoma; DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell lymphoma; GEP, gene expression profiling; mBL, molecular Burkitt's lymphoma.

*Unclassified indicates gene expression profiles are intermediate between ABC and GCB.

†All *P* values refer to comparison between GCB and ABC subtypes.

‡In training set, cell-of-origin distinctions were assigned according to GEP and signatures previously described by Wright et al[25] and Dave et al.[24]

§In validation set, cell-of-origin distinctions were assigned according to immunohistochemical algorithm reported by Choi et al.[4]

‖DHIT refers to presence of concurrent *MYC* and *BCL2* translocations.

¶Frequencies and *P* values refer to differences between molecular subtypes in combined data sets (training and validation), where patients were stratified according to presence of DHIT or presence of concurrent MYC and BCL2 proteins, which excluded DHITs. Cell-of-origin data were not available in one of 307 patient cases, and BCL2 protein was not available in three of 307 patient cases, one of which was DHIT.

and ABC subtypes suggests an underlying biologic pathway independent of COO subtype. In addition to promoting cell-cycle progression, MYC plays an important role in metabolism, protein synthesis, stem-cell renewal, and mRNA regulation and can induce apoptosis by increasing p53 expression or amplifying apoptotic signaling pathways.[27-30] Given this broad range of biologic activity, it is not surprising that deregulation of *MYC* is oncogenic.[10] MYC protein expression has also been found to occur as a consequence of other genomic events (eg, inactivating *p53*), increased protein stability, activation of upstream signaling pathways (eg, NFκB), desensitization to inhibitory cytokines, and loss of host immunity.[20,31,32] Given that many MYC-positive samples did not have a high proliferation rate in this study, these nonproliferative functions of MYC may contribute to the clinical and biologic attributes of DLBCL, possibly by inducing further DNA damage and genomic instability.[33] Thus, MYC protein expression by IHC in DLBCL may represent a final integrator of all or most of the mechanisms that underlie MYC deregulation.

We found *MYC* and *BCL2* IHC interpretation to be robust, reproducible, and largely unaffected by the vagaries introduced by differing fixation techniques used by the 10 institutions participating in this study. The cutoff scores for many biomarkers have often been arbitrary; in the case of BCL2, they have ranged from $>$ 10% to $>$ 75%, making comparison among studies difficult.[34,35] As such, we defined our thresholds based on rational statistical methods in accordance with recommended guidelines.[36] Our BCL2 threshold compares favorably with that defined by Tzankov et al,[34] who used receiver operator curves to define optimal cutoff scores, but it is lower than the $\geq$ 75% threshold reported by Salles et al,[35] who used a statistical approach similar to that of our study. The latter study investigated IHC markers in 2,451 DLBCL samples within the Lunenburg Lymphoma Biomarker Consortium, many of which were derived from young patients enrolled onto clinical trials, including studies evaluating R-CHOP administered every 14 days.[35] These data suggest that age and treatment may be important factors to consider when defining the optimal thresholds of biomarkers in DLBCL. Indeed, we and others have shown that there are age-related changes in tumor biology and that treatment, such as rituximab, can influence the prognostic impact of several biomarkers in DLBCL, including BCL2.[16,37-42]

We also demonstrated that BCL2 protein expression is the main determinant of clinical outcome in MYC-positive DLBCL. This biologic effect was robust across different platforms (IHC, GEP, and FISH) and was validated in a second independent cohort, which was representative of a population-based registry, suggesting that this biomarker may be relevant even in older patients.[16] Furthermore, coexpression of MYC and BCL2 proteins by IHC remained significant after adjusting for IPI, COO, and presence of DHIT. This is in keeping with the known biologic function of BCL2, which is a potent inhibitor of apoptosis and has been clearly shown to mediate chemotherapy resistance in MYC-positive lymphoma murine models.[43] Although previous studies have demonstrated a negative prognostic effect of *MYC*

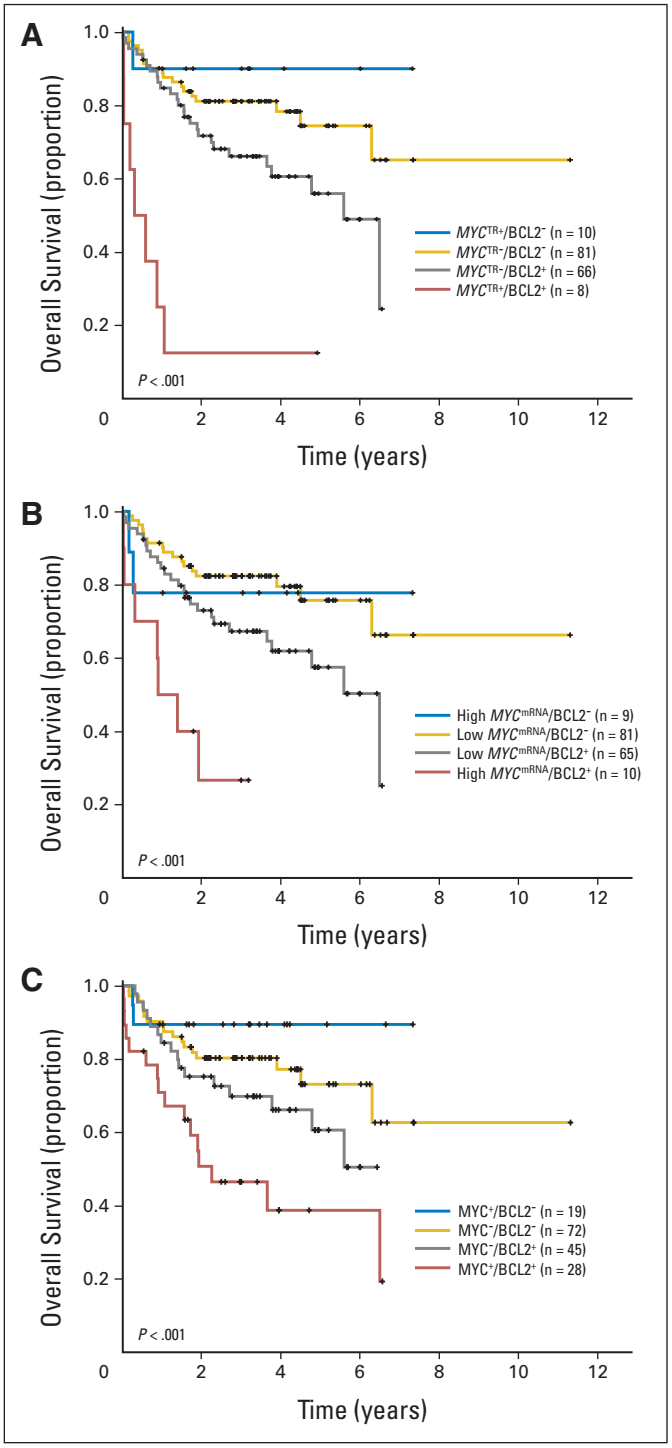

Fig 2. Overall survival (OS) of patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone based on alterations in MYC and BCL2 in the training set. Kaplan-Meier curves represent OS according to (A) presence of *MYC* translocation (TR) and BCL2 protein expression (BCL2+), (B) presence of high *MYC* mRNA expression and BCL2 protein expression, and (C) presence of MYC and BCL2 protein expression. Log-rank $P < .001$ for both OS and progression-free survival. Total evaluable patients for the analyses: (A) n = 165, (B) n = 165, and (C) n = 164.

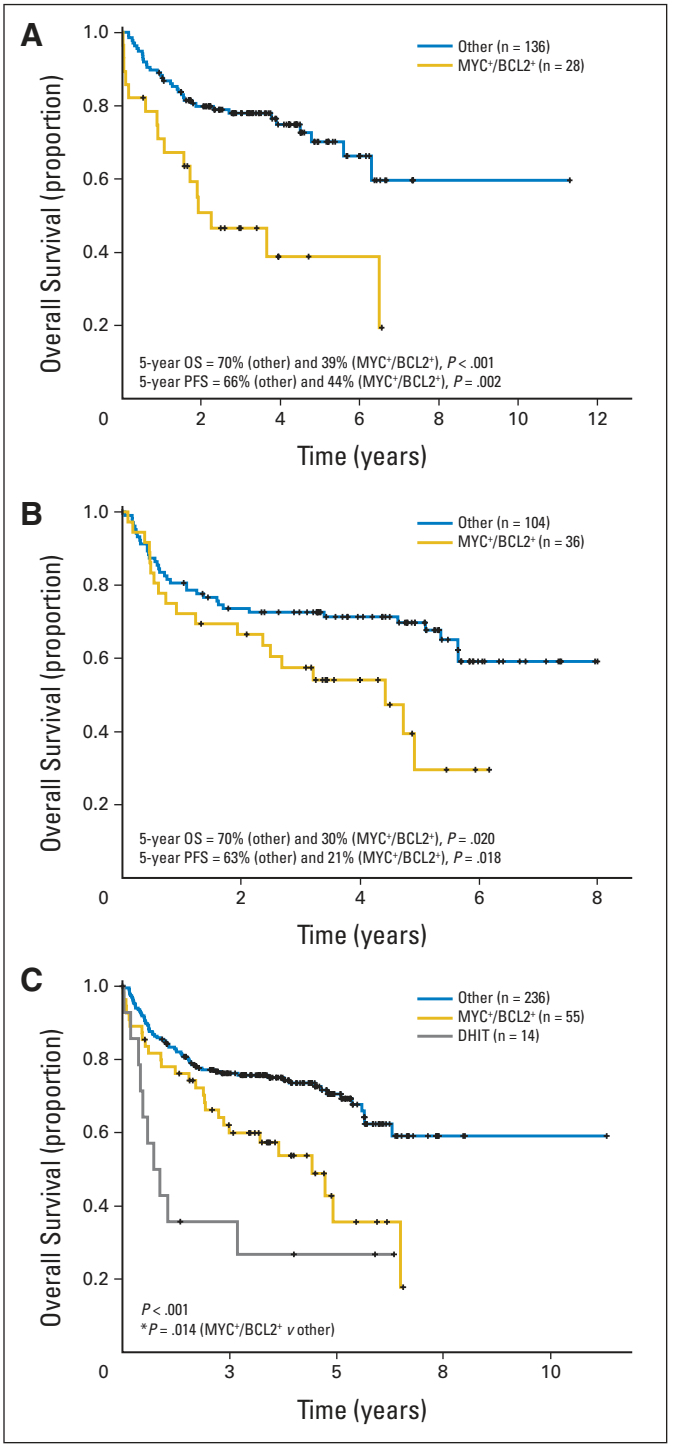

Fig 3. Overall (OS) and progression-free survival (PFS) of patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone according to presence of concurrent expression of MYC and BCL2 proteins (MYC+/BCL2+) and/or presence of concurrent *MYC* and *BCL2* translocations. Kaplan-Meier curves represent OS according to presence of MYC+/BCL2+ in (A) the training cohort (n = 164) and (B) validation cohort (n = 140). (C) Both training and validation cohorts were combined because of the low frequency of double hits (DHITs). Patients were stratified according to presence of DHIT or MYC+/BCL2+ excluding DHITs. Four DHIT patient cases had no MYC protein expression; one had a missing value for BCL2 protein expression.

translocation in DLBCL, a majority of patients' samples were BCL2 protein positive,[14,16] and it is unknown whether those with *MYC* translocations, but lacking BCL2 expression, would share a similar fate. This is an important question if patients with MYC-positive DLBCL are to be considered for more aggressive therapy. Unfortunately, there are no data to support the use of any one regimen for the treatment of MYC-positive/BCL2-positive DLBCL at this time. The results from the MRC/NCRI (Medical Research Council/National Cancer Research Institute) LY10 trial and a recent report from Snurderl et al[18] did not demonstrate a survival advantage in treating patients with DHIT lymphoma with high-dose regimens, most of which would not be well tolerated in older patients.[44,18] The probable role that BCL2 has in this disease suggests that MYC-positive/BCL2-positive tumors may be amenable to chemotherapy regimens that include drugs targeting BCL2, such as BH3 mimetics, a strategy that has been shown to cure a subset of murine MYC-positive/BCL2-positive lymphomas.[45-47]

The primary aim of this study was to determine the role of MYC protein as a prognostic biomarker in DLBCL, not as a potential screening test to identify patients harboring *MYC* translocation. Our results confirm those of two recent studies demonstrating the excellent reproducibility of MYC protein by IHC among pathologists, the high correlation with presence of *MYC* translocation, and the weak correlation with Ki67. MYC protein by IHC in our study had a sensitivity of 74%, which was lower than the 100% reported by the other studies that evaluated it as a screening test for identifying patients with *MYC* translocations. Our study used a lower threshold (40% *v* 50%[21] and 70%[22]), which was set based on the relationship between MYC protein and survival, not the presence of a translocation. Furthermore, the study by Kluk et al[21] included too few patient cases to meaningfully address this issue (n = five translocations of 56 samples of de novo DLBCL), and that by Green et al[22] included patient cases of Burkitt's lymphoma, which raised the baseline incidence of *MYC* translocations to 15%, a level higher than that observed in de novo DLBCL. Taken together, patients with *MYC* translocation are likely to express MYC protein. More importantly, our data suggest that MYC protein expression is more common than *MYC* translocation, and the MYC-positive/BCL2-positive immunophenotype is itself a powerful predictor of survival, independent of presence of *MYC* translocation.

In conclusion, this study has identified a group of clinically high-risk patients with DLBCL who coexpress MYC and BCL2 proteins, a clinical scenario that is more common than patients with lymphomas harboring the DHIT genotype (18% *v* 5%, respectively). Importantly, these biomarkers retained their prognostic significance in an independent cohort that consisted of patients from a population-based registry. Unlike with FISH, assessment of MYC and BCL2 protein expression by IHC represents a rapid, inexpensive, and reproducible technique that could be adopted by pathologists in most clinical centers. These promising results need to be validated prospectively in larger cohorts, using standardized staining and scoring methodologies, before implementation as prognostic biomarkers in clinical practice. Thus, MYC and BCL2 represent relevant biomarkers that should be tested in the context of clinical trials such that more effective therapies can be offered to these high-risk patients.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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