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Clinical significance of MYC expression and/or “high grade” morphology in non-Burkitt, diffuse aggressive B-cell lymphomas: A SWOG S9704 correlative study

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Abstract

The clinicopathologic findings in Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) may show significant overlap and *MYC* abnormalities, found in all BL, also occur in a subset of DLBCL. The 2008 WHO classification introduced the category of “B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL” (BCLU) in recognition of this overlap, but the clinical significance of BCLU (i.e., “high grade”) morphology and the relationship between BCLU morphology and *MYC* abnormalities remains unclear. In this study, we identified 260 cases of non-Burkitt, diffuse aggressive B-cell lymphomas from SWOG S9704, a phase 3 randomized study of standard immunochemotherapy versus autologous stem cell transplant. 31 cases (12%) showed BCLU morphology and 229 (88%) showed typical DLBCL morphology. 27/198 (14%) were positive for *MYC* by immunohistochemistry. BCLU morphology was associated with an increased incidence of *MYC* expression, but otherwise was not associated with distinct clinicopathologic features or significantly decreased survival. *MYC* positive cases were morphologically and phenotypically heterogeneous and were associated with poor progression-free and overall survival in multivariate analysis. These findings confirm that BCLU does not represent a distinct clinicopathologic entity, and demonstrate that BCLU morphology alone does not significantly impact survival compared to typical DLBCL. In contrast, *MYC* protein expression is a poor prognostic factor that may be associated with either BCLU or DLBCL morphology, and *MYC* immunohistochemistry is suggested for routine prognostic evaluation.

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MYC; Immunohistochemistry; B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL; diffuse large B-cell lymphoma

INTRODUCTION

Non-Hodgkin lymphomas that show some but not all of the morphologic, phenotypic, or cytogenetic features that define Burkitt lymphoma (BL) have proven controversial and difficult to categorize throughout the history of lymphoma classification schemes.^{1–9} The 2008 revision to the WHO classification introduced a new category for B-cell lymphoma, unclassified, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (hereafter abbreviated as “BCLU”).¹⁰ Morphologically, BCLU are relatively monomorphic with intermediate sized to large cells, often with a “starry sky” growth pattern. Some cases may show dispersed chromatin. Collectively, these morphologic findings have sometimes been designated “high grade” features because they create a differential diagnosis that includes BL and/or lymphoblastic lymphoma.^{11,12} Cases with typical DLBCL cytology are specifically excluded from the category of BCLU, even if there is a high proliferation fraction or MYC translocation.¹⁰ Overall, the category of BCLU remains somewhat vaguely defined and is acknowledged to be heterogeneous, limiting the clinical utility of this diagnostic category in routine practice. While at least subset of BCLU are “double hit” lymphomas with both *MYC* and *BCL2* translocations that are associated with a very poor prognosis,^{6,13–16} the clinical features of other cases meeting current criteria for BCLU remain uncertain.

Recently, a new monoclonal antibody to MYC has allowed for the evaluation of MYC protein expression by immunohistochemistry (IHC) in routine formalin-fixed, paraffin embedded tissues.^{17–19} Studies in diffuse large B-cell lymphoma (DLBCL) have shown that *MYC* dysregulation is more common than previously appreciated and that only a subset of MYC dysregulated DLBCL contains *MYC* translocations.^{17,19–21} The spectrum of clinicopathologic features associated with MYC dysregulation in diffuse aggressive B-cell lymphomas is not yet clear. In this study, we examine the clinical significance of and relationship between BCLU (i.e., “high grade”) morphologic features and MYC protein expression in a series of DLBCL and BCLU identified from SWOG S9704, a phase III randomized trial of standard chemotherapy versus autologous stem cell transplant (ASCT) in advanced stage, aggressive non-Hodgkin lymphomas.

MATERIALS AND METHODS

SWOG S9704 Trial Design

Details of the SWOG S9704 protocol are reported elsewhere.^{22,23} Briefly, eligibility criteria included de novo diffuse aggressive, non-Hodgkin lymphomas including working formulation categories D through H and J (i.e., follicular large cell, diffuse small cleaved cell, diffuse mixed small and large cell, diffuse large cell, large cell immunoblastic, and small non-cleaved cell), with bulky stage II–IV disease and high-intermediate or high IPI

score. Transformed lymphomas and mantle cell lymphomas were excluded. 370 eligible patients were enrolled. Patients were treated with 5 cycles of cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) or CHOP-rituximab (R) with responders randomized between CHOP±R × 1 followed by ASCT or CHOP±R × 3. The randomized patients were assigned to arms using a dynamic allocation scheme at the time of randomization.

Morphology review

Central morphologic review was performed using the standard protocols of the SWOG lymphoma pathology committee. Specifically, all cases were reviewed by a lead hematopathologist (JRC) using 2008 WHO criteria. If the initial review diagnosis differed from the submitting diagnosis, the case was also reviewed by a second hematopathologist (RRT) to arrive at a consensus diagnosis. In one case, a third hematopathologist (LR) was consulted to arrive at a consensus diagnosis. Cases of T-cell neoplasms, follicular lymphoma and mantle cell lymphoma (107 cases total) were excluded. Three cases meeting 2008WHO criteria for Burkitt lymphoma (appropriate morphology, CD10 positive, BCL2 negative, Ki67>95% and MYC FISH positive) were also excluded, yielding a final cohort of 260 cases. The distinction between BCLU and typical DLBCL was established by morphology alone, independent of phenotype or *MYC* status.

Tissue microarrays and immunohistochemistry

Tissue microarrays (TMA) containing 2mm cores in duplicate were prepared from all cases with suitable available archived paraffin blocks (n=90) and immunohistochemical stains for CD10, BCL6, MUM1, BCL2, Ki67 and MYC were performed on TMA slides using an automated immunostainer (Ventana Benchmark, Ventana Medical, Tucson, AZ) and CC1 heated antigen retrieval. The following antibodies were employed: CD10 (56C6, 1:5 dilution, Novocastra, Newcastle upon Tyne, UK), BCL6 (PG-B6p, 1:5 dilution, Dako, Carpinteria, CA), MUM1 (MUM1p, 1:20 dilution, Dako), BCL2 (123, predilute, Cell Marque, Rocklin CA), Ki67 (30–9, predilute, Ventana) and MYC (Y69, 1:50 dilution, Epitomics, Burlingame, CA). For cases without paraffin blocks available, or where TMA MYC stains could not be interpreted, MYC IHC was performed using archived whole slides (n=126). In 62 cases, no material was available for MYC IHC. In BCLU cases, additional stains for CD10, BCL6, MUM1, BCL2 and Ki67 were also performed on available whole slides as required to exclude Burkitt lymphoma. For CD10, BCL6, MUM1, and BCL2, a cutoff of 30% tumor cells staining was employed for consideration as a positive result, and GC vs. non-GC phenotype was determined using the Hans algorithm.²⁴ For MYC, a cutoff of 40% nuclear staining was employed, following the criteria of Johnson et al.²¹ This cutoff point has previously been shown in our laboratory to correlate with MYC translocation by FISH (Ly et al²⁵ and unpublished observations).

Fluorescence in situ hybridization (FISH) studies

Interphase FISH studies were performed as previously described in detail²⁶ using dual color, break-apart probes for *MYC* or *BCL2* (Vysis Molecular, Abbot Park, IL). Briefly, 4 µm paraffin sections were baked at 60°C overnight, deparaffinized, and subjected to proteinase

K digestion. Slides were washed and probe applied to each slide with overnight hybridization. Slides were counterstained with DAPI and signals visualized on an Axioskop photomicroscope (Zeiss, Oberkochen, Germany). Signals from 100 to 200 non-overlapping nuclei were counted in each case. Nuclei containing separate red and green signals were scored as abnormal. Using previously described cutoffs based on normal control specimen,²⁶ cases with 10% abnormal nuclei were considered positive for a translocation.

Statistical Considerations

Data were centrally reviewed and evaluated by the SWOG statistical center and the principal investigator according to the criteria established in two international workshops.^{27,28}

Progression-free survival (PFS) was defined as the time from first registration (or randomization depending on the analysis) to the first observation of progressive disease or death due to any cause. Multivariate PFS and OS analyses were performed by Cox regression²⁹ and survival was estimated according to the method of Kaplan and Meier.³⁰ Testing utilized score tests based on the Cox regression model. Analyses to assess the associations of MYC abnormalities or BCLU morphology to patient outcome were also adjusted by IPI factors (Performance Status, Extranodal disease, LDH and Age).

Comparisons in frequencies between groups were calculated with chi-square test statistics.

RESULTS

Morphologic review of all 260 cases identified 31 patients (12%) with BCLU (Figure 1A). 24 BCLU cases (77%) showed a starry sky growth pattern. In 21 cases (68%), the tumor was composed of relatively uniform intermediate sized cells, while the remaining 10 (32%) were somewhat more heterogeneous with intermediate sized to large cells. One case with intermediate cell size and starry sky growth pattern also displayed blastoid cytology. The remaining 229 cases (88%) displayed typical DLBCL morphology (Figure 1C). As shown in Table 1, the clinical features at presentation did not vary with morphologic features.

Immunohistochemical stains (IHC) for MYC could be performed in 198 cases (Figure 1 B and D). Positive staining for MYC was identified in 27 cases (14%), while 171 cases (86%) were negative for MYC. As shown in Table 2, clinical features at presentation did not vary with MYC status. FISH studies for MYC translocations could be performed in 69 cases. Overall, MYC translocations were identified in 13/69 cases (19%), including 7/14 (50%) with MYC positivity by IHC and 6/55 (11%) of cases without MYC staining by IHC ($p<0.001$). In 7 cases with MYC translocations, sufficient material was available to assess for concurrent BCL2 translocations. 5/7 (71%) contained BCL2 translocations, while the remaining 2 were negative for BCL2 translocations by FISH but were positive for BCL2 protein expression by IHC.

The pathologic features of cases with BCLU vs typical DLBCL morphology are summarized in Table 3. Cases of BCLU were phenotypically heterogeneous, with a germinal center phenotype identified in 13/27 (48%) of cases. Ki67 staining was higher in cases with BCLU morphology than in DLBCL (median 80% vs. 50%, $p<0.001$). No significant differences were seen between BCLU and DLBCL morphologies with respect to CD10, BCL6, MUM1, or BCL2 staining. MYC positivity by IHC was more frequent in

cases with BCLU morphology (6/21, 29%) than in cases with typical DLBCL morphology (21/177, 12%) ($p=0.035$). *MYC* translocations as identified by FISH were also more common in cases with BCLU morphology (8/24, 33%) than in those with typical DLBCL morphology (8/53, 15%) although this trend did not reach statistical significance ($p=0.068$). 3 of the 5 cases documented to have both *MYC* and *BCL2* translocations showed BCLU morphology.

BCLU cases were further subdivided into two subgroups: 13 cases with uniform intermediate cell size and starry sky growth pattern (i.e., those most resembling Burkitt lymphoma) and 18 other cases. Each of these subgroups were heterogeneous with respect to phenotype and *MYC* status, and no significant differences were found between these subgroups (data not shown).

As shown in Table 4, cases with *MYC* positivity by IHC were also phenotypically heterogeneous with a germinal center phenotype identified in 11/17 cases (65%). *MYC* IHC positive cases were more likely to be positive for CD10 (10/17, 59%) and *BCL2* (21/26, 81%) than were *MYC* IHC negative cases (25/80, 31% for CD10 and 42/79, 53% for *BCL2*, $p=0.032$ and 0.013 , respectively). Ki67 staining was also higher in the *MYC* positive cases (median 80% vs. 60%, $p=0.02$). No significant differences were detected in terms of *BCL6* or MUM1 staining. However, these phenotypic comparisons should be interpreted with caution; due to the need to exclude a true Burkitt lymphoma, a higher proportion of cases with BCLU morphology were assessed for these markers than for cases with typical DLBCL morphology.

The influence of *MYC* IHC status and morphologic features on clinical response, progression free survival (PFS) and overall survival (OS) were assessed in a multivariate model adjusting for IPI score and inclusion of rituximab therapy during induction (Table 5). Both *MYC* positivity by IHC and BCLU morphology were inversely associated with clinical responses as assessed at the end of induction therapy ($p=0.039$ for each comparison). Considering all registered patients, *MYC* IHC positivity was associated with inferior PFS ($p=0.0021$) and OS ($p<0.0001$). When analysis was limited to patients undergoing randomization (Figure 2 A and B and Table 5), this difference remained significant ($p=0.0279$ and $p=0.0016$ for PFS and OS, respectively). The association of *MYC* expression with poor prognosis appeared greater in the no transplant arm, but this difference was not statistically significant after adjusting for treatment arm interactions. In contrast, BCLU morphology showed no significant association with PFS ($p=0.12$) and a trend to inferior OS ($p=0.054$) when considering all patients (including patients going off study prior to randomization). In randomized patients, BCLU morphology showed no association with PFS or OS ($p=0.70$ and 0.40 , respectively) (Figure 2 C and D).

DISCUSSION

Burkitt lymphoma (BL) is a relatively well-defined clinicopathologic entity characterized by typical morphologic features, a characteristic phenotypic profile, and the consistent presence of a *MYC* translocation, generally as part of a simple karyotype.^{6,31–34} While not always completely prototypical, cases displaying all of the classic features of BL are relatively

straightforward to diagnose. In contrast, aggressive lymphomas that display some but not all of the morphologic, phenotypic or genetic features of BL have long created diagnostic challenges.^{3,6–9} Specifically, there is morphologic overlap between BL and diffuse large B-cell lymphoma (DLBCL), and the characteristic phenotypic profile (CD10 positive, BCL2 negative, Ki67>95%) and genotypic hallmark (*MYC* translocation positive) of BL may also be seen in DLBCL. Historically, lymphomas with such overlapping features have been variously, and controversially, classified using a variety of designations including small, non-cleaved, non-Burkitt lymphoma, Burkitt-like lymphoma and/or atypical Burkitt lymphoma.^{1,2,4–6,35} More recently, overlap between BL and DLBCL was further confirmed by gene expression profiling studies showed that molecular signatures of BL could also be found in lymphomas not meeting current criteria as BL, including some with a typical morphologic appearance of DLBCL and some with so-called “double hit” genotypes, containing *MYC* plus *BCL2* and/or *BCL6* translocations.^{36,37} The 2008 revision to the WHO classification, recognizing the longstanding controversy regarding such cases, created a category of “B-cell lymphoma, unclassifiable, with features intermediate between Burkitt lymphoma and diffuse large B-cell lymphoma” (BCLU)^{10,38}. In routine practice, however, considerable diagnostic and therapeutic challenges remain. The current criteria for designation as BCLU remain vague, the prognostic significance of specific morphologic and phenotypic features within BCLU are unknown, and it is not clear how cases diagnosed as BCLU should be treated. This study was therefore undertaken to examine the clinical and pathologic significance of BCLU (i.e., “high grade”) morphologic features and *MYC* protein expression in non-Burkitt lymphomas.

31 (12%) of the cases in this series showed morphology consistent with a diagnosis of BCLU. BCLU cases showed no significant differences in clinical features at presentation compared to typical DLBCL and were heterogeneous with respect to phenotype (48% GC-type, 52% non-GC). The phenotypic profile was assessed via the widely utilized Hans algorithm²⁴ because insufficient material remained for further evaluation with newer immunohistochemical algorithms such as the Choi³⁹ or Tally⁴⁰ methods. Given the largely overlapping results observed with each of these methods, it is likely that these newer algorithms would also demonstrate phenotypic heterogeneity within this series of BCLU. Although *MYC* protein expression and *MYC* translocations were more frequent in BCLU than DLBCL (29% vs 12%, $p=0.035$, and 33% vs 15%, $p=0.068$, respectively), the majority of BCLU cases were negative *MYC* protein and *MYC* translocations. Finally, the presence of BCLU morphology did not significantly influence survival in multivariate analysis. These results show that in this cohort of patients with advanced stage disease, BCLU morphology does not appear to define a clinically, pathologically, or prognostically distinct set of cases.

MYC staining by IHC was identified in 27 cases (14%) overall, a slightly lower rate than the 19–33% incidence reported in several recent large studies of DLBCL.^{13,19,20} This difference may be a consequence of the selection criteria for this transplant study, which yielded a younger cohort of patients than earlier non-transplant studies. In addition, cases meeting Working Formulation criteria for Burkitt lymphoma, which might include either Burkitt lymphoma or BCLU by 2008 WHO criteria, may not have been enrolled on this protocol. Nevertheless, consistent with prior reports,^{19,20,25,41} *MYC* staining by IHC was associated

with *MYC* translocations as detected by FISH ($p < 0.001$), although half of *MYC* IHC positive cases lacked detectable *MYC* translocations and nearly half of cases with *MYC* translocations detected by FISH were negative for *MYC* protein by IHC. The significance of the latter set of cases (*MYC* FISH+ but IHC-) is unclear. Other recent studies^{21,42} also identified a subset of *MYC* translocated DLBCL lacking *MYC* protein expression by IHC. Additional studies, including identification of the *MYC* translocation partner genes, will be required to further clarify the biologic and clinical significance of this subset of cases.

Notably, cases with *MYC* positivity by IHC did not display distinct clinical features at diagnosis, and were phenotypically heterogeneous (65% GC, 35% non-GC phenotype). *MYC* positivity, however, was significantly associated with poor progression free and overall survival in multivariate analysis accounting for IPI and rituximab use. These findings are similar to those recently reported by other investigators using *MYC* IHC with similar cutoffs for interpretation as a positive result,^{17,21} although prior studies have been limited to cases showing typical DLBCL morphology treated with R-CHOP. To our knowledge, this is the first study to evaluate the significance of *MYC* IHC in both DLBCL and BCLU cases and in patients treated with autologous bone marrow transplant. Interestingly, the adverse effect of *MYC* protein expression appeared to be greatest in the standard chemotherapy treatment arm, although this difference did not reach statistical significance. The possibility that autologous bone marrow transplant may improve the outcome of *MYC*+ DLBCL or BCLU should be specifically examined in future clinical trials.

Recent studies have reported that *MYC* protein expression is associated with an unfavorable prognosis in DLBCL only when there is concurrent *BCL2* protein expression, while cases expressing *MYC* protein in the absence of *BCL2* expression show no effect on prognosis.^{17,21,43} In the current study, 81% of *MYC* IHC+ cases coexpressed *BCL2* (Table 4). Long term survival appeared greater in the *MYC*+ *BCL2*- cohort compared to the *MYC*+ *BCL2*+ cohort, but too few cases with a *MYC*+ *BCL2*- phenotype were present for to reach statistically informative results (data not shown).

The findings in this study have important implications for the classification of aggressive B-cell lymphoma including the use of the diagnosis of BCLU in routine practice. As a general guiding principle, the WHO classification has sought to define diagnostic categories that represent distinct clinicopathologic entities. The category of BCLU, when introduced in 2008, was a notable exception to this rule, being explicitly recognized as a heterogeneous “working category” while such cases were further studied.^{10,37} The findings in this report confirm the heterogeneity of BCLU and show that such cases, as morphologically identified, lack distinct clinicopathologic features and do not show significant differences in survival compared to cases with typical DLBCL morphology. These findings suggest that lymphomas displaying BCLU morphology that do not meet current WHO criteria as Burkitt lymphoma might be best considered part of the spectrum of DLBCL, not otherwise specified, rather than being placed into a separate diagnostic category in order to minimize diagnostic confusion and recognize that such cases appear to behave similarly to other DLBCL. *MYC* protein expression, on the other hand, may be seen in cases that display either BCLU or typical DLBCL morphology, and may be associated with either GC or non-GC phenotype. *MYC* positivity is associated with poor survival, but does not otherwise

appear to be associated with distinct clinicopathologic features. MYC positivity should therefore be considered to represent a poor prognostic factor, rather than a defining feature of BCLU or another specific diagnostic entity. Finally, it must be noted that this study was limited to patients with advanced stage disease, and it remains possible that distinct clinicopathologic entities associated with MYC expression and/or high grade morphologic features and early stage disease may exist. Additional studies including both early and advanced stage disease will be required to address this possibility.

In conclusion, this study has confirmed the prognostic significance of MYC positivity by IHC in DLBCL and, for the first time, extends this observation to cases of BCLU. As immunohistochemical staining is widely available and can be performed with greater ease, less cost and shorter turnaround time than FISH studies, MYC IHC is suggested for use in routine clinical practice to assess prognosis in DLBCL. Due to the poor prognosis in MYC positive cases, these patients may be candidates for experimental therapies. Additional studies utilizing MYC IHC to risk-stratify therapies should also be considered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Representative morphologic patterns and MYC expression. Top row: This BCLU (A) displays intermediate cell size and a starry sky growth pattern. Immunohistochemistry showed a non-germinal center phenotype with coexpression of BCL2 and MYC (B). FISH studies were negative for a MYC translocation. Bottom row: This case (C) shows typical DLBCL morphology with a mixture of centroblasts and immunoblasts. Immunohistochemistry showed a germinal center phenotype with coexpression of BCL2 and MYC with variable intensity (D). FISH studies were positive for a MYC translocation.

Figure 2.

Overall survival and progression-free survival amongst randomized patients stratified by MYC immunohistochemistry and morphology.