Using Gene Expression Profiling to Move Beyond *MYC/BCL2* Rearrangements in High-Grade Lymphoma

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Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma, comprising approximately 40% of all lymphomas. It is highly heterogeneous, with 13 subtypes now being designated as specific entities.¹ The rest remains in the DLBCL not otherwise specified (NOS) group, which constitutes most DLBCLs.

With current immunochemotherapy (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone), overall survival is greater than 60% for patients with DLBCL-NOS. Many attempts have been made at finding clinical parameters or biomarkers that can predict survival. The International Prognostic Index² has been a robust prognosticator, and several modifications seem to have improved its prognostic power.³ Although useful clinically, this prognosticator is not based on biologic mechanisms, and many exceptions exist for each risk group. Gene expression analysis led to the identification of germinal center B-cell-like (GCB) and activated B-cell-like (ABC) DLBCL,⁴ which allows the division of the majority of patients with DLBCL-NOS into two biologically distinct categories. In particular, the GCB subset generally has a better prognosis than that of the ABC subset, although clinical heterogeneity within each is apparent. In the search for additional useful prognostic biomarkers, many markers have been examined, and a number of them have been reported to be predictive of clinical outcome, including *MYC* arrangement, *TP53* mutation, Epstein-Barr virus infection, CD5 expression, CD30 expression, *BCL2* rearrangement or expression, MHC class II expression, and others.⁵⁻¹¹ However, reports often contradict with regard to the prognostic value of these single markers, which makes confidence in their predictive power difficult.

When patients with both *MYC* and *BCL2* rearrangements were examined, the consensus findings indicated that their double-hit lymphomas are associated with significantly worse survival¹² compared with non-double-hit lymphomas. On the basis of these findings, the current WHO classification¹ moved all aggressive B-cell lymphomas (immunomorphologically DLBCL or intermediate between DLBCL and Burkitt lymphoma) with these rearrangements into a separate category: high-grade B-cell lymphomas with *MYC* and *BCL2* and/or *BCL6* rearrangements. *BCL6* rearrangement

has been added to this double-hit category on the basis of less-compelling evidence. Of note, although *MYC/BCL2* double-hit lymphomas are practically all in the GCB-DLBCL subset, *MYC/BCL6* cases come from both GCB- and ABC-DLBCLs. In addition, a number of studies have indicated that patients with high expression of both *MYC* and *BCL2* proteins (as assessed by immunohistochemistry) but without the corresponding translocations (double-expressor lymphomas) have a poorer outcome.^{13,14} However, other studies have failed to find a significant difference. These double-expressor cases have not been assigned a special status in the current WHO classification. Nevertheless, intense clinical interest lies in patients with double-hit and double-expressor lymphomas.

In the articles that accompany this editorial, investigators from two groups have used gene expression signatures to identify high-risk patients with DLBCL. The report by Sha et al¹⁵ used a Burkitt lymphoma-like signature,¹⁶ whereas Ennishi et al¹⁷ used a signature derived from genes differentially expressed between *MYC/BCL2* double-hit and non-double-hit GCB-DLBCLs. With use of their respective signatures, these investigators were able to identify most double-hit lymphomas as expected as well as many non-double-hit lymphomas, which actually constituted half or more of the patients identified. These findings suggest that many of these patients harbored genetic or even epigenetic alterations that produced similar gene expression changes in the tumor cells, as recognized by the respective signatures. This should not come as a surprise because the *MYC/BCL2* double-hit concept is an oversimplification. First, activation of an oncogene or oncogenic pathway can be brought about by multiple mechanisms. *MYC*, for example, can be upregulated through translocation and gene amplification, but aside from structural alterations, *MYC* expression or activity can be enhanced through transcriptional and post-transcriptional mechanisms. Thus, mutation of *MYC* T58 is a well-defined change that prevents the phosphorylation of the threonine residue and subsequent ubiquitination by FBXW7 and proteosomal degradation of *MYC*.¹⁸ Alternatively, *MYC* may be transcriptionally amplified through the activation of

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Author affiliations and support information (if applicable) appear at the end of this article.

Accepted on November 12, 2018 and published at [jco.org](https://doi.org/10.1200/JCO.18.01910) on December 3, 2018; DOI <https://doi.org/10.1200/JCO.18.01910>

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enhancer/superenhancer elements.^{19,20} Alterations in other genes may augment the effects of *MYC* in lieu of or in addition to abnormalities that affect *MYC* or its regulation. Thus, deletion of *BIM* may counteract induction of apoptosis in inappropriate oncogene activation.^{21,22} Given that amplification of the microRNA cluster miR17~92, a direct *MYC* target, is fairly common in GCB-DCBCL,²³ this genetic abnormality can amplify the function of *MYC*. Therefore, examination of a tumor just for *MYC* rearrangement will miss many potentially important changes that can affect *MYC* activity. Second, genetic changes other than *MYC* and *BCL2* could confer poor survival but are not evaluated. *TP53* mutation could be considered a double hit with *MYC* instead of *BCL2*,^{24,25} but again, this usually is not measured. Therefore, it is important that these high-risk signature-positive tumors be comprehensively characterized to determine the underlying lesions that contribute to the signature and the unfavorable clinical and biologic behavior.

The two reported signatures that identify the high-risk group among patients with DLBCL were independently derived. However, similarities exist in the gene expression profiles of the lymphomas identified. There is an enrichment in gene expression signatures associated with centroblasts or intermediate zone GCB cells, with low expression of the centrocyte signature. *MYC* and *E2F* signatures are enriched as expected, and MHC class I and II expression is low. Stromal signatures associated with inflammation and T-cell infiltration are low. The mutational landscape is similar to that observed in GCB-DLBCL, particularly in patients in cluster 3 or EZB mutations group as recently described,^{26,27} although differences exist. Mutations of *TCF3* and *ID3* that are common in Burkitt lymphoma are infrequent in these patients. Although the reported signatures likely were identifying a largely overlapping group of lymphomas, the extent of overlap and how these signatures can be used in the clinical setting by other groups are unclear. To their credit, Ennishi et al¹⁷ constructed a diagnostic signature for these high-risk patients that can be performed on the NanoString (Seattle, WA) platform. However, it would be of interest to perform additional analyses of the data from these two series to harmonize and optimize the signatures and translate that to a platform that others can use in the clinical setting.

The identification of this high-risk group also raises the question of how these patients should be managed. The management of double-hit lymphoma has been recently reviewed,²⁸ but how the rest of the high-risk patients should

be managed is unclear. Perhaps they could be treated as having a double-hit lymphoma on the basis of the similarity in gene expression profile. It is important to identify and include these patients in future clinical trials. Their tumors should be comprehensively characterized for correlative analysis to determine the molecular lesions that underlie their biology and response to treatment. Of note, some Burkitt lymphomas may exhibit gene expression signatures that overlap with these high-grade signatures and should be carefully excluded.

Sha et al¹⁵ and Ennishi et al¹⁷ mainly focused on the GCB-DLBCL subset and the *MYC/BCL2* double-hit group. However, genetically defined high-risk groups also exist within ABC-DLBCL. In a study on copy number abnormalities in DLBCL, trisomy 3 or deletions that involve *CDKN2A/B* defined patients with poorer outcomes.²³ Two recent mutational analysis also identified high-risk mutation profiles (cluster 5 of Chapuy et al²⁶ and the MCD group of Schmitz et al²⁷) characterized by *MYD88 L265P* and *CD79B* mutations within the ABC-DLBCL subset that may be useful in the stratification of these patients.

Most of the biomarker studies reported examined tumor cell intrinsic abnormalities, such as mutations, copy number alterations, translocations, and expression of certain biomolecules. Host/tumor interaction is an important component in tumor biology and a determinant of the prognosis of the patients. Lenz et al²⁹ reported on stromal signatures in DLBCL that are prognostic. Mutations and/or downregulation MHC class I or II expression provide an example of the interaction between cell intrinsic changes and host immune response.^{11,30} Induction of the expression of PD-L1 in tumor cells and/or cells in the tumor microenvironment is another example. In future investigations, this important aspect of tumor biology should be integrated into prognostic models and evaluation of response to therapy, particularly immunomodulatory therapy and cellular therapy.

Although the identification of this high-risk group of patients with tumor gene expression signature similar to *MYC/BCL2* double hit lymphomas is valuable, heterogeneity still exists in survival within this group, which probably reflects biologic differences that need to be deciphered. Additional molecular information may provide insight into how best to target at least some of these tumors. With advances in technology, greater understanding in the biology of lymphoma, and increasing availability of new agents and approaches in treatment, further refinement in tumor classification is anticipated to be linked to effective targeted therapy in the near future.

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AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

Disclosures provided by the author and data availability statement (if applicable) are available with this article at DOI <https://doi.org/10.1200/JCO.18.01910>.

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AUTHOR'S DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

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Patents, Royalties, Other Intellectual Property: Patent on diagnostic algorithm on GCB/ABC-type DLBCL, patent on diagnostic algorithm for peripheral T-cell lymphoma