Comment on Collinge et al, page 2196

Defining double-hit lymphoma in the clinic

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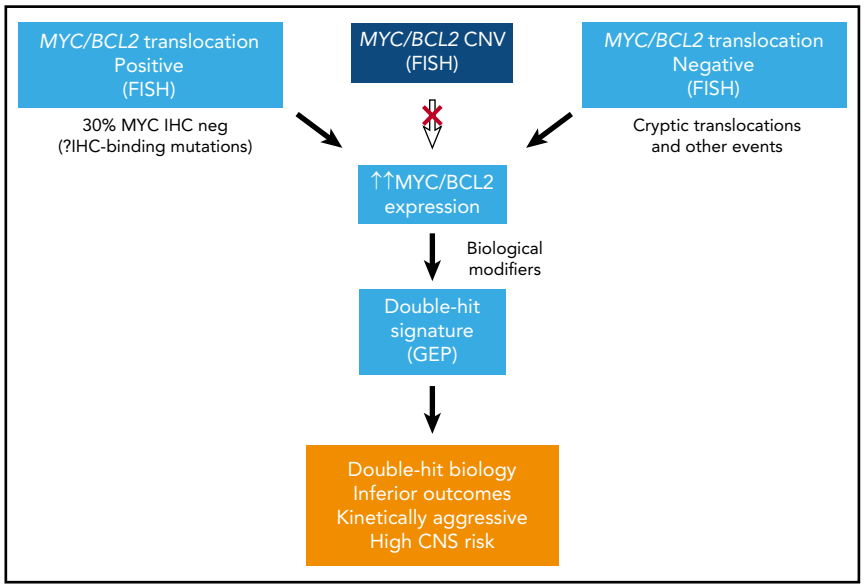
High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements (also called double-hit [or triple-hit] diffuse large B-cell lymphoma [DLBCL]) is a distinct biological entity associated with kinetically aggressive disease, high rates of central nervous system involvement, and inferior clinical outcomes. In this issue of *Blood*, Collinge et al build on their previous work and further dissect the definition of this entity through the lens of the diagnostic tools currently available to pathologists.[1]

In 2020, cell morphology as assessed by the light microscope, for better or for worse, retains its place in the diagnostic armory used to stratify lymphoma into biologically and prognostically relevant groups. After initial subcategorization by microscopic features, there is now an ever-expanding array of downstream diagnostic tools and technologies that are increasingly relied upon to provide further biological insight in order to inform patient management (see figure). High-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements is a recently recognized entity by the World Health Organization (WHO) whose definition is reliant on these technologies and hostage to their limitations.[2] Although this entity is currently defined by the presence of structural variants involving *MYC* and *BCL2*$^{+/-}$ *BCL6* (typically detected by fluorescence in situ hybridization [FISH]), it is clear that the "true" double-hit lymphoma biological entity extends far beyond those with detectable abnormalities by conventional FISH testing. A major step toward biological bedrock was the development of a specific gene expression signature that could identify double-hit lymphoma biology, which revealed that ~50% of patients with the clinicobiological features of double-hit lymphoma was unrecognized by conventional FISH testing.[3] Although this may be related in part to the technical performance and discordance of commonly used FISH probes,[4] subsequent whole-genome sequencing studies have shown that ~30% of these patients harbor translocations of *MYC*/*BCL2*/*BCL6* loci that are cryptic to FISH.[5] However, despite the improved pickup rate when using high-resolution massively parallel sequencing technologies, there is still a significant proportion of patients that belong to the true biological double-hit lymphoma subgroup as defined by gene expression profiling, that do not harbor classical translocations of these loci.



Identifying double-hit lymphoma biology using diagnostic tools. CNS, central nervous system; CNV, copy number variation; GEP, gene expression profiling.

This study by Collinge et al investigates other potential pathways to double-hit lymphoma biology. Using a gene expression signature developed on a cohort of high-grade B-cell lymphoma with *BCL2* rearrangements, double or triple hit, to define true biological double-hit lymphoma, the authors first show that copy number gains of *MYC* (present in ~20% to 25% of patients) do not routinely lead to *MYC* overexpression and are not equivalent to *MYC* translocations in that they do not lead to double-hit lymphoma biology. Likewise, copy number gains of *BCL2* (despite their association with overexpression) were also shown to be nonequivalent to *BCL2* translocations with regard to double-hit lymphoma biology. Further study is required to determine whether the biological basis of this observation relates to a specific requirement of the regulatory region of the partner chromosome (immunoglobulin locus or otherwise), and furthermore, how different *MYC*/*BCL2* translocation partners may also affect the biological outcome. In addition, the impact of *BCL6* genomic abnormalities as well as any effect of histological features, such as blastoid morphology, also remains to be elucidated. However, regardless of the underlying mechanism, these findings indicate that we have more or less reached the limit of FISH to identify cases of true biological double-hit lymphoma, which falls significantly short of identifying the entire population of interest.

Immunohistochemistry (IHC) staining for *MYC* has previously been investigated as a means of triaging those patients in whom FISH testing is warranted to detect *MYC* translocations. Although this approach was used by diagnostic laboratories initially, it has since become clear that a significant proportion of patients harboring *MYC* translocations (30% in the

current study) are "negative" for MYC staining by IHC. One mechanism of false-negative staining (ie, negative MYC IHC in the presence of increased *MYC* expression) that has been reported in this work is the presence of a relatively rare (2% to 3%) germline polymorphism, NM_002467.4:c.77A>G; p.(Asn26Ser) (referred to in this work as MYC-N11S), that disrupts the binding epitope of a commonly used IHC clone (Y69). It is also highly likely that other mutations (both germline and acquired through aberrant somatic hypermutation of *MYC*) will result in false-negative MYC IHC staining in a proportion of cases and further reinforces that IHC cannot be routinely relied upon to triage cases prior to FISH testing when pursuing a diagnosis of double-hit lymphoma.

The limitations of FISH and IHC in this context for identifying double-hit biology raise the question of whether gene expression profiling should be routinely used clinically to identify this important subtype. There is little doubt that gene expression profiling is a powerful method for accurate identification of double-hit lymphoma (as well as Burkitt lymphoma, primary mediastinal B-cell lymphoma, DLBCL cell of origin, T-cell lymphoma subtyping, and others); however, up until now this technique has not been widely implemented into routine diagnostic hematopathology practice. Although the reasons for this may be related to technical (instrumentation, bioinformatics support, availability of appropriate tissue) or financial considerations, it may equally be related to the lack of an evidence-based clinical intervention to improve outcomes based on the results of these specific signatures. Regardless, there is little doubt that the prerequisite for improving outcomes in patients with DLBCL (and hematological malignancy more broadly) is to accurately establish the patient's diagnosis with appropriate respect to conventional WHO criteria as well as the molecular and biological phenotype. In 2020, the light microscope, one of the great survivors in the pantheon of scientific instruments, retains its relevance; however, there is clearly further work to do to optimize and effectively implement downstream technologies to fully capture double-hit lymphoma.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

REFERENCES

1. Collinge B, Ben-Neriah S, Chong L, et al. The impact of *MYC* and *BCL2* structural variants in tumors of DLBCL morphology and mechanisms of false-negative MYC IHC. *Blood*. 2021; 137(16):2196-2208.
2. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: International Agency for Research on Cancer; 2017.
3. Ennishi D, Jiang A, Boyle M, et al. Double-hit gene expression signature defines a distinct subgroup of germinal center B-cell-like diffuse large B-cell lymphoma. *J Clin Oncol*. 2019; 37(3):190-201.
4. Chong LC, Ben-Neriah S, Slack GW, et al. High-resolution architecture and partner genes of *MYC* rearrangements in lymphoma with DLBCL morphology. *Blood Adv*. 2018;2(20): 2755-2765.
5. Hilton LK, Tang J, Ben-Neriah S, et al. The double-hit signature identifies double-hit diffuse large B-cell lymphoma with genetic events cryptic to FISH. *Blood*. 2019;134(18):1528-1532.

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THROMBOSIS AND HEMOSTASIS

Comment on Fassel et al, page 2221

A novel "vascular" thrombophilia

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In this issue of *Blood*, Fassel et al[1] report a higher frequency of reduced plasminogen-activating capacity by peripheral blood mononuclear cells (PBMCs) from patients with thrombosis and a positive family history, but not a known inherited thrombophilia, compared with either patients without a family history of thrombosis or patients with a family history accompanied by an inherited thrombophilia (see figure). Deficient cell surface plasmin generation by PBMCs, used in this study as a surrogate for endothelial cells, was caused by decreased expression of annexin A2. This report extends previous studies, primarily in animal models, suggesting an important role for annexin A2–mediated plasmin generation on the vascular wall in the maintenance of blood fluidity and suggests that deficient annexin A2 expression may represent a novel variant of thrombophilia that is independent of altered levels and/or mutations in circulating coagulation proteins or their inhibitors.

Annexin A2 is 1 of a family of 12 annexins and is highly conserved among mammalian species. It has been associated with a remarkable array of biologic functions, most of which depend on its membrane binding and organizing properties. Annexin A2 is expressed in many cell types, including endothelial cells, monocytes, macrophages, trophoblasts, and tumor cells, among others.[2] In endothelial cells, the majority of annexin A2 is present in the cytoplasm, existing largely as a 36-kDa monomer, with only about 4% translocated to the external plasma membrane in the form of a heterotetramer with S100A10 (p11). The mechanism by which annexin A2 is translocated to the cell surface is not well understood; translocation is stimulated by thrombin, hypoxia, and other cell activators or stressors and is dependent on phosphorylation of Y^{23} by a pp60src-like kinase. Translocation also requires expression of p11, which is stabilized by binding to annexin A2.[3]

By binding both tissue plasminogen activator (t-PA) and plasminogen, the annexin A2•p11 heterotetramer mediates activation of plasminogen by t-PA.[4] t-PA binds to an LCKLSL sequence in the N-terminal tail of annexin A2, whereas plasminogen binds to a C-terminal lysine (K308) of annexin A2, thought to be generated by partial annexin A2 proteolysis[5] and/or the C-terminal lysine of p11.[6] Through binding both reactants, annexin A2 lowers the K_M and increases the catalytic efficiency of t-PA–mediated plasminogen activation by ~60-fold[4]; in the context of endothelial cells, this leads to increased vascular wall fibrinolytic activity.