



## Copy number aberrations in B-cell lymphoma: A call for consideration in prognosis determination and therapy



In 2016, the World Health Organization (WHO) published an updated classification of hematological malignancies [1]. One of the changes in this new set of classification is the addition of the category "High-grade B-cell lymphoma (HGBCL), with *MYC* and *BCL2* and/or *BCL6* translocations." This category encompasses the so-called "double-hit" and "triple-hit" lymphomas (DHL and THL, respectively). The former has rearrangements involving *MYC* (8q24) and *BCL2* (18q21) or *BCL6* (3q27), while the latter has rearrangements involving all three genes (Fig. 1). The classification of DHL and THL from other B-cell lymphomas is also of clinical consequence, as they are known to be more aggressive and less responsive to the standard therapy. Overall, the utilization of clinically significant cytogenetic abnormalities in lymphoma classification provides an objective method by which lymphomas can be categorized to better predict prognosis and guide therapy. Although this is a tremendous step, the reality may be more complex than it first appears. One complication is that *MYC*, *BCL2*, and *BCL6* rearrangements are not the only clinically significant genetic changes. In addition to translocations, studies have shown that other abnormalities involving these genes may also have clinical implications [2–4]. One such abnormality that can be detected by fluorescent in situ hybridization (FISH) is copy number aberration (CNA).

The normal human genome carries two copies of each gene. When a rearrangement occurs, a gene can be translocated to a more active locus, leading to its overexpression. Such is the case with the t(8;14) (q24;q32) *IGH/MYC* translocation. However, protein expression can also be increased by the presence of extra copies of the gene in question. Thus, overexpression of *MYC*, *BCL2*, and/or *BCL6* proteins can be achieved through either translocations of the respective genes, the presence of extra copies of the respective genes, or a combination of the two. Li et al. designated tumors with the two latter abnormalities as "atypical DHL" and showed that the clinical and pathologic features of patients with atypical DHL are similar to those with typical DHL [2]. Similar results were seen by Lu et al. who showed that patients with both *MYC* and *BCL2* CNAs had similar outcomes to those with DHL [3]. Valera et al. further categorize CNAs into gene gain (3–4 copies) and gene amplification ( $\geq 5$  copies) [4]. They showed that patients with *MYC* amplification had outcomes similar to that of patients with DHL/THL, while those with *MYC* gain were clinically and pathologically more similar to those with unaltered *MYC*.

Further complicating the landscape is the matter of *MYC*, *BCL2*, and/or *BCL6* protein overexpression. Lymphomas in which overexpression of the *MYC* and *BCL2/BCL6* proteins are present are termed "double-expressor" lymphomas (DELs). The WHO defines overexpression as  $> 40\%$  of *MYC*-expressing cells and  $> 50\%$  of *BCL2*-expressing cells by immunohistochemistry (IHC) [1]. Studies have shown that there is a subset of patients with the double-expressor phenotype who do not harbor the underlying translocations [5]. Conversely, there are also tumors that have the double translocations but do not

demonstrate protein overexpression by IHC [5]. Several studies show that DELs have a worse outcome than non-overexpressing diffuse large B-cell lymphoma (DLBCL), but better than DHLs [1]. Thus, currently, the WHO considers double overexpression of *MYC* and *BCL2* proteins without rearrangements as a prognostic indicator, but not a separate category [1].

Collectively, each of these existing studies holds a small piece of the puzzle. There are many more questions to be answered, such as whether a distinction should be made between gene gain and gene amplification, and whether the presence of *MYC* and *BCL2* and/or *BCL6* CNAs should be treated as DHL. The relationship between CNA and protein expression also remains to be fully elucidated. Existing studies are constrained by the limited number of cases and the lack of uniformity in terminology and definitions. In putting the puzzle pieces together, a common ground must first be established.

In our practice, we report amplification of *MYC*, *BCL2*, or *BCL6* when five or more ( $\geq 5$ ) copies are present (Fig. 2A). Cases in which there are three or four signals are designated as a gene gain (Fig. 2B). We often encounter cases of *MYC* rearrangement with concurrent *BCL2* gain or amplification. These patients do not meet the criteria for DHL. However, in our anecdotal experience, their disease progression is more similar to those with DHL rather than patients with DLBCL, not otherwise specified (NOS). It is also important to note that because CNA can have significant clinical implications, a complete cytogenetic workup requires copy number analysis as well as detection of rearrangement. For this purpose, it is best to provide a fresh sample of the tumor. Analysis of archival paraffin-embedded tissues by FISH is wrought with challenges, such as reduced probe penetration and excessive background tissue fluorescence. Nonetheless, rearrangements can still be reliably detected. Copy number analysis by archival FISH, however, is highly problematic. In addition to the aforementioned challenges, the issue of cell overlap makes it, in many cases, exceedingly difficult to determine copy number with sufficient certainty.

Taken together, we recommend complete cytogenetic and FISH evaluation on all new cases of HGBCL, which includes analyses of both rearrangements and copy number aberrations of *MYC*, *BCL2*, and *BCL6*. To this end, clinicians are greatly encouraged to provide a fresh sample of the tumor, rather than relying on archival FISH. We also recommend the use of consistent terminology for gene gain and amplification, defined as three to four copies and five or more copies, respectively. Clinicians should also be cautioned that tumors that do not strictly meet the criteria for DHL may nevertheless behave as such if CNAs of *MYC*, *BCL2*, and/or *BCL6* are present.

In conclusion, the use of clinically significant genetic abnormalities in the classification of hematologic malignancy offers the advantage of providing objective criteria that are predictive of the tumor's behavior and response to therapy. However, genetic abnormalities are complex, and it may be difficult to encompass the many existing variations.

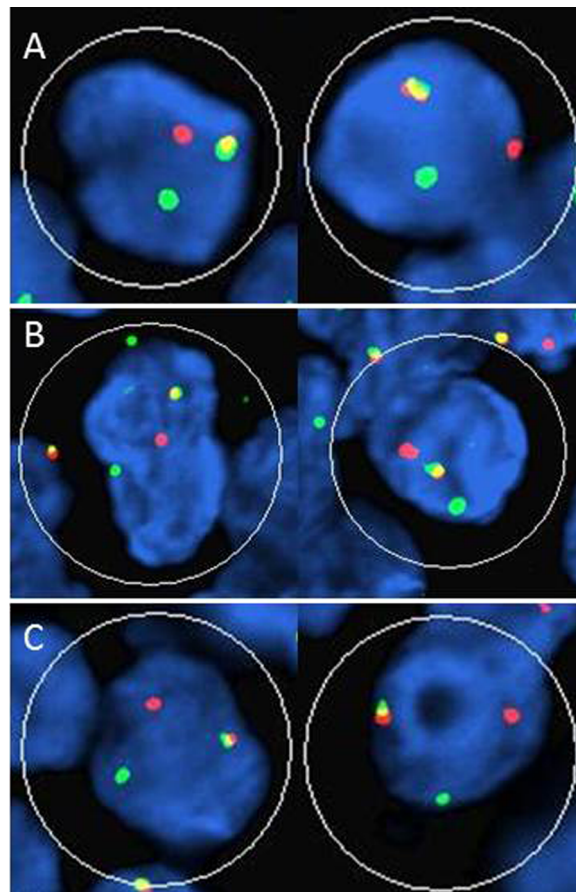
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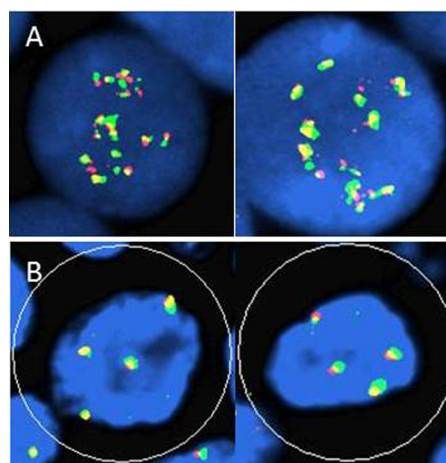
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**Fig. 1.** Nuclear FISH demonstrating a case of triple hit lymphoma. Representative cells show rearrangements of *BCL6* (A), *MYC* (B), and *BCL2* (C) by FISH break-apart probes (all probes obtained from Abbott Molecular, Des Plaines, IL, USA). The yellow fusion signals demonstrate the intact gene. The red and green signals demonstrate the rearrangements. A. nuc ish(*BCL6* × 2)(5′*BCL6* sep 3′*BCL6* × 1); B. nuc ish(*MYC* × 2)(5′*MYC* sep 3′*MYC* × 1); C. nuc ish(*BCL2* × 2)(5′*BCL2* sep 3′*BCL2* × 1).



**Fig. 2.** Nuclear FISH demonstrating copy number aberration showing *MYC* amplification (A) and *MYC* gain (B). A. nuc ish(*MYC* amp); B. nuc ish(*MYC* × 4).

Currently, B-cell lymphomas with *MYC* and *BCL2* and/or *BCL6* abnormalities other than translocations are classified based on their morphology and fall under either HGBL-NOS, DLBCL-NOS, or Burkitt's lymphoma. However, as detailed above, many studies have shown that these tumors behave more similarly to DHL and THL. In order to better predict the prognosis of these tumors and formulate therapy standards, further studies must be conducted. Moreover, to assist in the integration of data from existing and future studies, standard terminologies and definitions must be established.

#### Declarations of interest

None.

#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.lrr.2019.04.004](https://doi.org/10.1016/j.lrr.2019.04.004).

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