

# Cytogenetics and molecular cytogenetics in diffuse large B-cell lymphoma (DLBCL)

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**Background.** Diffuse large B-cell lymphoma (DLBCL) accounts for up to 40% of all non-Hodgkin's lymphomas diagnosed in the western hemisphere. Determination of the gene expression profile has confirmed the physiological heterogeneity of the disease and defined three molecular prognostic subgroups – germinal center B-cell-like (GCB), activated B-cell-like (ABC) and primary mediastinal B-cell lymphoma (PMBL) – with different gene expression and prognosis.

**Methods and Results.** This review covers current knowledge on the most frequent recurrent cytogenetic and molecular cytogenetic aberrations in molecular DLBCL subgroups.

**Conclusions.** Cytogenetic and molecular cytogenetic techniques used to determine nonrandom chromosomal aberrations in patients with DLBCL have revealed the incidence of frequent cytogenetic aberrations in the subgroups reported, suggesting their potential use for more accurate prognostic stratification of DLBCL, contributing to personalized selection of the most effective therapy.

**Key words:** diffuse large B-cell lymphoma, prognostic subgroups, chromosomal aberrations, molecular cytogenetics, arrayCGH

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## INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma (NHLs) worldwide, accounting for up to 40% of all NHLs diagnosed in the adult population of the western hemisphere<sup>1</sup>. It is a diffusely growing tumor of large B cells with characteristically high mitotic activity.

Significant morphological, clinical and biological heterogeneity of DLBCL confirms the coexistence of several subtypes of the disease with a different clinical course. Based on gene expression analysis, two and later three basic prognostic subgroups of DLBCL were defined<sup>1-3</sup>.

Whereas the germinal center B-cell-like (GCB) DLBCL subtype is characterized by expression of genes typically expressed in germinal center centroblasts, the activated B-cell-like (ABC) DLBCL subgroup is derived from post-germinal B cells expressing genes characteristic for *in vitro* activated B cells<sup>2</sup>. The third prognostic subgroup is primary mediastinal B-cell lymphoma (PMBL) with gene expression completely different from those in the previous two subtypes and similar to gene expression in nodular sclerosing Hodgkin's lymphoma<sup>3</sup>.

By defining these subgroups, DLBCL was confirmed to comprise several distinct subtypes of tumors caused by different pathogenetic mechanisms. Cytogenetic and molecular analyses in patients with DLBCL showed that tumor cells carry non-random chromosomal aberrations, most frequently chromosomal translocations, deletions, amplifications, as well as gene alterations including aberrant somatic hypermutations.

This review summarizes the known facts about cytogenetic and molecular cytogenetic aberrations and their prognostic role in patients with DLBCL.

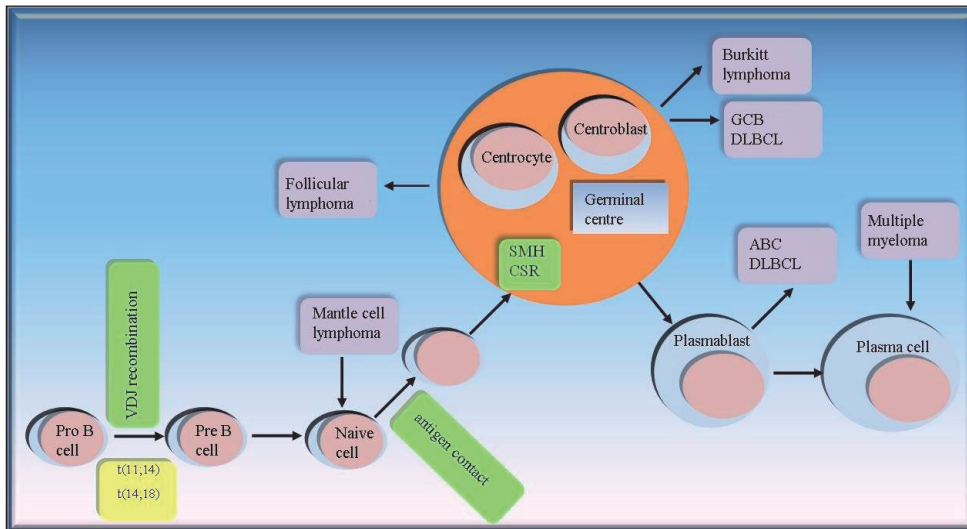
## PATHOGENESIS OF DLBCL

As in other types of cancers, the pathogenesis of DLBCL is a multistep process involving accumulation of numerous genetic and molecular changes leading to a selective growth advantage of a malignant clone.

B-cell lymphomas arise at various stages of B cell development. To induce a specific immune response, a cell goes through several mutation and selection processes during its development, including V(D)J recombination, somatic hypermutation and immunoglobulin class switching. However, it is these posttranslational modifications that increase the risk for a genetic aberration such as recurrent translocation, a triggering event in malignant transformation (Fig. 1) (ref.<sup>4</sup>).

## CYTOGENETIC AND MOLECULAR CYTOGENETIC METHODS

To determine chromosomal changes in DLBCL, conventional cytogenetics is used with G-banding, a technique often considered the “gold standard” for lymphoma diagnosis. However, the technique is labor-intensive and requires fresh tumor tissue which must be processed within a few hours after extirpation, as well as a high



**Fig. 1.** Schematic diagram of the development of non-Hodgkin's lymphomas at various stages of B cell differentiation. V(D)J is the first critical phase in which the reciprocal translocations  $t(11;14)(q13;q32)$  or  $t(14;18)(q32;q21)$  may occur. The  $t(14;18)(q32;q21)$  translocation is a typical aberration in follicular lymphoma also reported in DLBCL patients. During immunoglobulin class switching, there is an increased risk of DNA breaks and thus chromosomal aberrations frequently detected in NHL patients<sup>4</sup> (adapted by ref.<sup>4</sup>).

level of skills to culture the lymphoma cells, to prepare high-quality metaphases and to analyze very often highly complex rearrangements. Given these problems, conventional cytogenetics is now replaced by other molecular cytogenetic methods such as fluorescent *in situ* hybridization (FISH) (ref.<sup>5</sup>), comparative genomic hybridization (CGH) or array comparative genomic hybridization (arrayCGH) (ref.<sup>6</sup>).

FISH is a rapid, cheap and relatively easy technique that can also be applied to formalin-fixed, paraffin-embedded (FFPE) tissue sections. The method may be used to detect both balanced (mainly translocations) and unbalanced aberrations. Hybridization results are available within three days of sampling. To determine complex changes in the karyotype or origin of marker chromosomes, multiplex FISH (mFISH) may be utilized, with five fluorescent dyes that are mixed in particular ratios to provide a range of shades to distinguish individual chromosomal pairs. Mainly complex abnormalities can be visualized readily as well as marker chromosomes. However, this method requires metaphases and special equipment and interpretative skills.

Whole genome analysis is made possible by the CGH method and the derived array CGH. Both methods utilize DNA isolated from patient tumor tissue and no difficult culturing of cells is needed. While CGH was mainly used in the 1990s<sup>7</sup>, it has recently been replaced by arrayCGH. The latter is based on different fluorescent labeling of reference and tumor DNAs that are subsequently mixed and hybridized to DNA fragments spotted onto a slide. With increasing density of the spotted DNA sequences, high resolution of up to 120kb may be achieved, as compared with the 5-Mb resolution of traditional CGH.

Whole genome analysis may also be carried out by modern analysis of single nucleotide polymorphisms

(SNPs). The technique may not only show unbalanced aberrations in the genome but also determine loss of heterozygosity (LOH)/uniparental disomy (UPD) regions<sup>8</sup>.

For complete assessment of the cancer genome, both balanced and unbalanced aberrations in the patient's genome must be characterized. Since comparative hybridizations are capable of determining unbalanced changes only, the assessment must be complemented with methods used to evaluate balanced changes, in particular FISH.

## GENETIC ABERRATIONS IN DLBCL PROGNOSTIC SUBGROUPS

The gene expression profiles in all three DLBCL molecular subtypes indicate that each of them arises at a different phase of B cell development and differentiation. Cytogenetic studies in this prognostic subgroups showed an incidence of recurrent chromosomal aberrations (Table 1) allowing us to distinguish individual DLBCL subtypes<sup>9</sup>, with recurrent changes being detected in up to 62% of all DLBCL cases<sup>10,11</sup>.

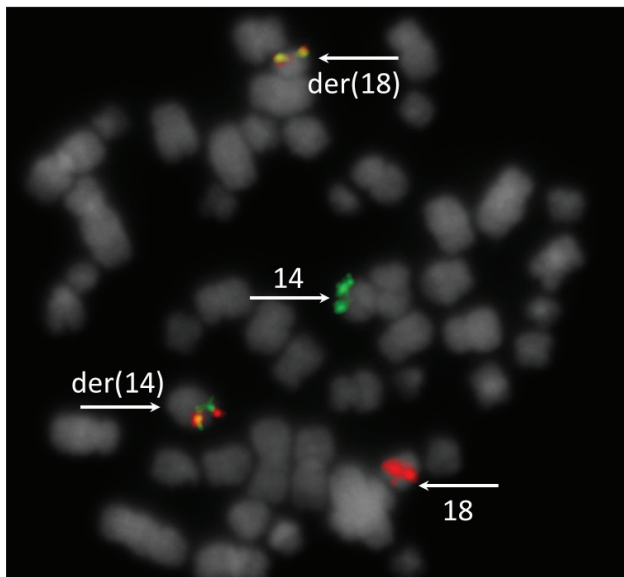
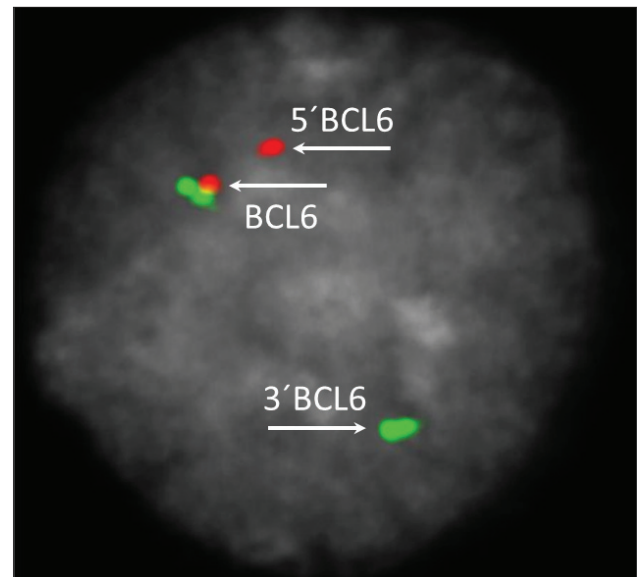
### GCB DLBCL

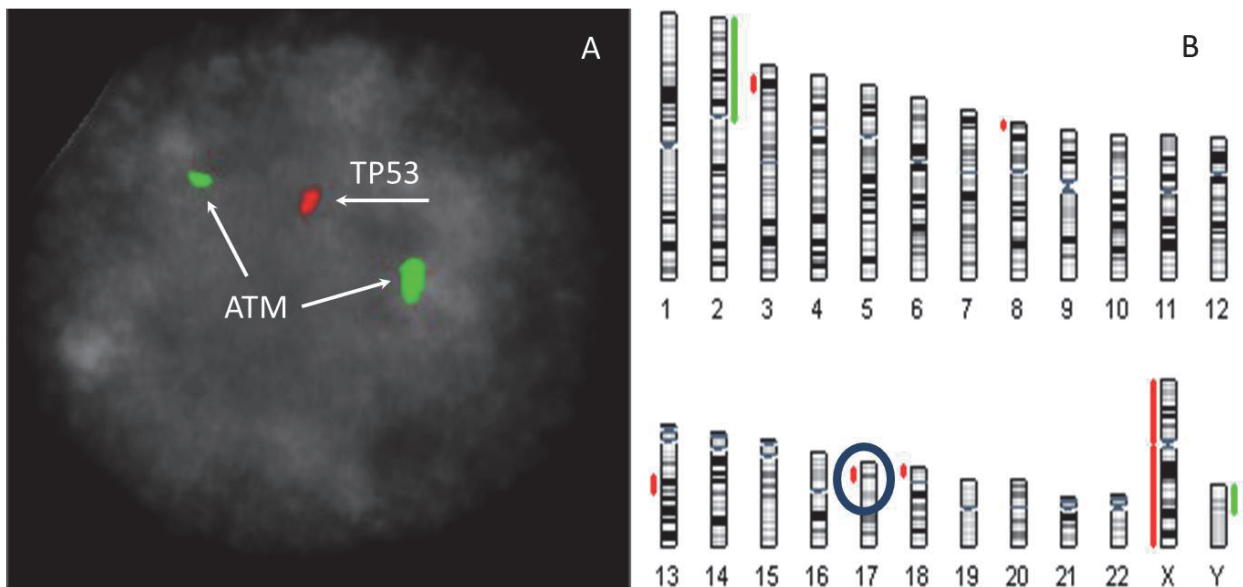
The most common translocation in DLBCL generally, with a frequency of up to 46% (ref.<sup>9</sup>), is  $t(14;18)(q32;q21)$ , with rearrangements of the *BCL2* (B-cell leukemia/lymphoma 2) (18q21) and immunoglobulin heavy chain (*IGH*) genes (14q32). As in follicular lymphoma, the  $t(14;18)$  translocation is routinely detected by FISH with locus-specific probes for the *IGH* and *BCL2* genes (Fig. 2).

*BCL2* is an anti-apoptotic gene participating in the development and differentiation of B cells and inhibiting apoptosis, programmed cell death. As a result of

**Table 1.** Recurrent cytogenetic aberrations and their frequency in DLBCL molecular subgroups.

Subgroup	Cytogenetic aberration	Involved gene	Incidence (%)
CGB	2p14-p16 gain	<i>REL</i>	17 <sup>10</sup>
	8q24 rearrangement/gain	<i>MYC</i>	22/4.9 <sup>13</sup>
	10q23 loss	<i>PTEN</i>	11 <sup>10</sup>
	12q12 gain	undescribed	21 <sup>9</sup>
	18q21 rearrangement	<i>BCL2</i>	30 <sup>36</sup> -46 <sup>9</sup>
ABC	3q27 rearrangement	<i>BCL6</i>	57 <sup>11</sup>
	3 gain	<i>FOXP1</i>	26 <sup>10</sup>
	6q21 loss	<i>PRDM1</i>	24 <sup>4</sup> -40 <sup>9</sup>
	9p21 loss	<i>INK4a/ARF</i>	30 <sup>10</sup>
	17p loss	<i>TP53</i>	18 <sup>9</sup>
	18q21-q22 gain	<i>BCL2</i>	34 <sup>9</sup>
	18q11.2-23 gain	<i>BCL2 ,NFATC1</i>	54 <sup>17</sup>
	19q13 gain	<i>SPIB</i>	26 <sup>4</sup>
PMBL	2p14-p16 gain	<i>REL</i>	47 <sup>9</sup>
	9p24 gain	<i>JAK2</i>	45 <sup>10</sup> -50 <sup>24</sup>
	12q11-q13 gain	<i>IGF1</i>	19 <sup>24</sup>
	12q24 gain	<i>ELK3, EPS8, IFNG</i>	31 <sup>24</sup>
	16p13 rearrangement	<i>CIITA</i>	38 <sup>23</sup>

**Fig. 2.** Reciprocal translocation t(14;18)(q32;q21) detected by FISH on metaphase chromosomes using the Vysis LSI *IGH/BCL2* Dual Color, Dual Fusion Translocation Probe (Abbott Molecular).**Fig. 3.** Rearrangement of *BCL6* detected by FISH on the interphase cell using the LSI *BCL6* Dual Color, Break Apart Rearrangement Probe (Abbott Molecular) (normal finding of *BCL6* is co-localization of red and green colors; the rearrangement is confirmed by signal splitting).



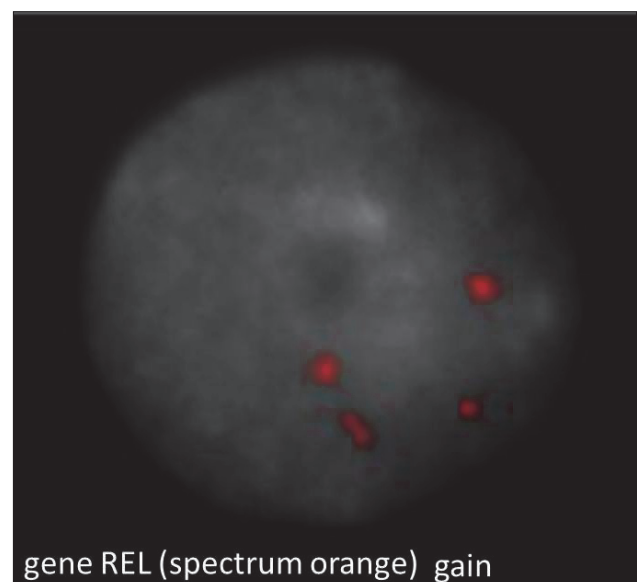
**Fig. 4.** A: The interphase cell with a deletion of the tumor suppressor gene *TP53*. FISH with the locus-specific Vysis *ATM/TP53* Dual Color Probe (Abbott Molecular). The deletion of *TP53* is confirmed by a loss of the red signal. B: ArrayCGH profile (CytoChip Bluegenome) with 17q deletion involving the *TP53* gene.

increased *BCL2* expression and inhibition of apoptosis, cells become “immortal”. Increased *BCL2* expression is a negative prognostic factor associated with poor prognosis and shorter survival of patients on conventional chemotherapy<sup>1,11</sup>. For these patients, new treatment options are sought. Introduction of rituximab (MoAb anti CD20) into the treatment protocol is beneficial for patients in both the GCB and ABC prognostic subgroups. However, the treatment outcome in GCB patients with t(14;18) is not as good as in those from the ABC subgroup<sup>12</sup>.

The second most frequent cytogenetic aberration in the GCB subgroup is translocation leading to rearrangement of the *MYC* gene (8q24), reported in as many as 22% of patients<sup>13</sup>. According to a study by Bea et al.<sup>9</sup>, the most frequent gain region in the GCB subgroup is locus 12q12. The extra copies were detected in up to 21% of patients with GCB DLBCL compared with the ABC and PMBL subgroups with a frequency of only approximately 5%.

Another very frequent cytogenetic change seen in DLBCL is 2p14-p16 gain. In DLBCL patients classified into the GCB subgroup, this aberration was confirmed in as many as 17% of cases. Compared with the PMBL subgroup with a frequency of gain of up to 47%, it must be mentioned that duplication of this region is a rather characteristic marker for stratifying patients into the PMBL subgroup despite the fact that it is one of the most frequent aberrations in GCB DLBCL (ref.<sup>9</sup>).

A recurrent change detected in 11% of patients diagnosed with GCB DLBCL is deletion of the tumor suppressor gene *PTEN* (10q23.3) (ref.<sup>10</sup>). The same frequency is reported in amplification of microRNA miR17-92 on the long arm of chromosome 13, which participates in suppressing the physiological function of *PTEN*. Both aberrations are solely associated with the GCB prognostic subgroups and have not been detected in the ABC or PMBL subtypes of DLBCL (ref.<sup>4,14</sup>).



**Fig. 5.** Gain of the *REL* gene on the interphase cell detected by BAC RP11-498O5 (Bluegenome, Spectrum Orange).

#### ABC DLBCL

Translocation involving the *BCL6* gene (3q27) is the most commonly identified aberration detected in as many as 57% of patients with ABC DLBCL (ref.<sup>11</sup>) (Fig. 3). *BCL6* is a proto-oncogene encoding a transcriptional repressor expressed solely during B cell differentiation in the germinal center (GC) (ref.<sup>1</sup>), participating in formation of the GC. By contrast, its downregulation is necessary for terminal differentiation of B cells into memory or plasma cells. At the same time, *BCL6* suppresses the activity of the tumor suppressor gene *TP53* inducing apoptotic cell death. In case of *BCL6* deregulation in DLBCL patients, there is indefinite expression of this gene and inhibition



of programmed death resulting from DNA damage during somatic hypermutation<sup>15</sup>. Most frequently, translocation t(3;14)(q27;q32) has been detected in DLBCL patients. The translocation partners need not be only immunoglobulin heavy chain genes (*IGH*/14q32). Two variant translocations involving immunoglobulin light chain genes t(3;22)(q27;q11) (*IGL*) and t(2;3)(p12;q27) (*IGK*) have been described. At the same time, more than 20 non-IG translocation partners have been identified, involved in signaling pathways, cell cycle control and genomic stability. The highest number of loci included in translocations were shown to be at chromosomes 3 (3q25, 3q26.33, 3q27.3, 3q29), 6 (6p21, 6q21.1, 6q15) and 12 (12p13.31, 12q12.1, 12q23-q24.1) (Jardin et al.<sup>16</sup>). Patients treated with conventional therapy with *BCL6* rearrangement associated with translocation of genes for immunoglobulin heavy or light chains had a more favorable prognosis than those with translocations involving non-IG genes<sup>17</sup>. An example of poor prognosis of non-IG reciprocal translocation of *BCL6* is t(1;3)(p34;q27), associated with high proliferative activity of tumor cells<sup>15</sup>. A more detailed analysis of an as yet unknown gene in the locus 1q34 involved in translocation is the subject of further studies to elucidate its role during lymphomagenesis<sup>15</sup>. Rearrangements or numerical changes of *BCL6* are routinely demonstrated by FISH on interphase nuclei or metaphase chromosomes as seen in Fig. 3.

Another frequent aberration in the ABC subgroup is trisomy 3 (26%) (ref.<sup>10</sup>). This chromosomal aberration has never been shown in either GCB<sup>9</sup> or PMBL DLBCL (ref.<sup>10</sup>). On chromosome 3, about 1,092 candidate genes have been identified, with *FOXP1* (3p14.1) being the third most frequently overexpressed marker in the group, due to trisomy 3, gain or amplification of the gene itself. DLBCL patients in the ABC prognostic subgroup found to have *FOXP1* gain showed 3.4-5.7-fold higher expression of *FOXP1* mRNA than those in the GCB subgroup<sup>10</sup>.

Gains of long arms of chromosome 18 were described independently in two studies focused on genes involved in the pathogenesis of ABC DLBCL (ref.<sup>9,18</sup>). In 2005, Tawagaa et al.<sup>18</sup> reported a gain of 18q11.2-q23 in as many as 54% of patients in the ABC subgroup<sup>18</sup>. Another very frequent recurrent aberration of the long arms of chromosome 18 is gain of the 18q21-q22 region involving *BCL2* (18q21) (ref.<sup>9</sup>). As a result of duplication/amplification of the long arms of chromosome 18, expression of genes located in the 18q region is deregulated. The two most upregulated genes are *BCL2* (18q21) and *NEATC1* (18q23). It is apparent that both genes are involved in the pathogenesis of ABC DLBCL (ref.<sup>10</sup>). Expression of *BCL2* gives rise to an anti-apoptotic protein participating in programmed cell death. The t(14;18)(q32;q21) translocation is mainly seen in patients in the GCB DLBCL prognostic subgroup and it correlates with the *BCL2* mRNA level. ABC DLBCL is also characterized by a high level of *BCL2* expression due to a gain in the 18q21 suggesting an alternative mechanism of the gene upregulation. Different mechanisms of *BCL2* upregulation are likely to lead to different effects on the clinical course of the disease. Expression of *BCL2* is associated with poor

prognosis in patients in the ABC subtype but not in GCB DLBCL (ref.<sup>12</sup>).

The ABC molecular subgroup of DLBCL is derived from B cells which differentiate and proceed to the plasma cell stage. The correct course of differentiation is controlled by the *PRDM1* gene (6q21), a transcriptional repressor, whose deletion or inactivating mutation was observed in as many as 40% of patients with ABC DLBCL. Apart from *PRDM1*, more genes located on the long arms of chromosome 6 are involved in the pathogenesis.

The minimally lost regions were in 6q21q22 and 6q25qter (ref.<sup>9</sup>). In 2008, Thelander et al.<sup>19</sup> reported two minimal deleted intervals in DLBCL, 6q14.1q24.1 and 6q23.3qter. A proximal 6q deletion was found mostly in patients after transformation whereas a distal deletion is associated with *de novo* DLBCL (ref.<sup>19</sup>). In addition to *PRDM1*, other candidate genes were identified in the 6q21 locus, *FOXO3A* and the tumor suppressor gene *HACE1* (ref.<sup>19</sup>). Detailed analyses of candidate genes located in the recurrently deleted region 6q are a subject of current studies aiming at determination of all target genes and their role in the pathogenesis of DLBCL (ref.<sup>19</sup>).

A common aberration detected in ABC DLBCL cases is deletion of the *INK4a/ARF* tumor suppressor locus on chromosome 9, involving three tumor suppressors (p16, p15 and p14<sup>ARF</sup>), observed in up to 30% of ABC DLBCL cases, as compared with the GCB (4%) and PMBL (6%) subgroups<sup>10</sup>. ABC DLBCL cases with hemizygous and homozygous loss of this locus had, respectively, 6.9 and 6.8-fold lower levels of *CDKN2A* mRNA than cases without these abnormalities. This finding suggests that cases with hemizygous loss have additional genetic or epigenetic changes that silence *CDKN2A* expression<sup>10</sup>.

A frequent chromosomal aberration found in as many as 26% of ABC DLBCL cases is a gain of the 19q13 region involving the *SPIB* gene, a transcription factor controlling formation of the germinal center. Like *BCL6*, *SPIB* also participates in *PRDM1* inactivation, leading to inhibition of B cell differentiation into the plasma cell stage<sup>4</sup>.

As many as 18% of patients diagnosed with ABC DLBCL were shown to have a deletion of the tumor suppressor gene *TP53* (17p13) (Fig. 4). Inactivation of *TP53* results in uncontrolled cell proliferation and subsequent tumor genome instability. Mutation and/or deletion of *TP53* decreases the overall survival of all DLBCL patients<sup>9</sup>. Also in the era of immunochemotherapy, patients with *de novo* DLBCL with confirmed *TP53* mutations and/or deletions were reported to have a significantly shorter progression-free survival<sup>20</sup>. Deletion of *TP53* may be detected by FISH using a locus-specific probe or by arrayCGH.

## PMBL

The third subtype of DLBCL, that is pathologically, clinically and molecularly distinct from the previous two groups, comprises patients diagnosed with primary mediastinal B-cell lymphoma. PMBL is seen in 2% of patients treated for NHL (ref.<sup>21</sup>). This subtype is more frequent in young patients, in particular women with a median age at diagnosis of 30-35 years<sup>4</sup>. At diagnosis, the tumor is typically located in the mediastinum<sup>22</sup>.

Since PMBL was defined as an individual subgroup within DLBCL, a large number of studies have been published on cytogenetic aberrations in PMBL patients. Most frequently, cytogenetic changes involving *REL*, *PDL1/PDL2*, *JAK2*, *JMJD2C* and *CIITA* were reported<sup>23</sup>.

Patients diagnosed with PMBL typically have gains of the long arms of chromosome 9, in particular of the 9p24 region. This cytogenetic aberration has become a characteristic feature of PMBL patients<sup>23</sup>. Duplication or multiplication of this locus is associated with upregulation of the Janus kinase 2 (*JAK2*) gene. A gain of this gene is observed in up to 50% of patients with PMBL (ref.<sup>24</sup>). Given its high detection rates, *JAK2* has become an important diagnostic and stratification marker in PMBL patients<sup>10</sup>. In the pathogenesis of PMBL, many genes are involved and they cooperate. Compared with the other DLBCL subgroups, high expression of another three genes in the proximity of *JAK2* was shown in PMBL, namely *PD-L1/PD-L2* and *SMARCA2*. The *PD-1* genes (*PD-L1* and *PD-L2*) are located in the 9p24.1 locus. Current research is aimed at confirming the role of these candidate genes in the pathogenesis of PMBL (ref.<sup>25</sup>).

In up to 47% of cases, a gain of the short arms of chromosome 2 was detected, especially of the 2p14-p15 region<sup>9,14</sup>. In lymphoproliferative hematological malignancies, there is often duplication of the *REL* proto-oncogene (2p16) (Fig. 5) encoding a transcription factor of the *NF- $\kappa$ B* family. Most frequently, the duplication is associated with PMBL (ref.<sup>9</sup>).

A recurrent cytogenetic aberrations in PMBL patients are gains of the 12q24 (31%) and 12q11-q13 (19%) regions. These chromosome regions contain *IGF1*, *ELK3*, *EPS8* and *IFNG*, candidate genes associated with malignant transformation<sup>24</sup>. Recent studies have been searching for the role of these genes in DLBCL lymphomagenesis<sup>23</sup>.

So far, genetic studies of DLBCL have confirmed the diagnostic and prognostic impact of recurrent chromosomal aberrations mainly serving as markers for stratification into prognostic subgroups. At the same time, many cytogenetic aberrations are of clinical value for determining the prognosis of DLBCL patients.

## SECONDARY DLBCL

Secondary DLBCL results from transformation from another NHL or chronic lymphocytic leukemia (CLL). Most frequently, DLBCL is transformed from follicular lymphoma (FL) and CLL. Because primary FL transforms to DLBCL in 17% of cases during the course of the disease, the finding of t(14;18) involving *BCL2* is not surprising. In secondary DLBCL arising from FL, a common clonal origin can be demonstrated in most cases<sup>26</sup>. Transformation from CLL is called Richter's syndrome.

### Richter's syndrome

Richter's syndrome (RS), first described in 1928, is transformation of CLL into a more aggressive type of lymphoproliferative disease, frequently DLBCL. Although molecular mechanisms leading to RS have not been elu-

cidated so far, it is apparent that the transformation itself is a clinical-pathological process of an increase in new molecular aberrations, ultimately associated with very poor prognosis<sup>27</sup>.

*De novo* DLBCL has a significantly different profile from the transformed form<sup>28</sup>. The relationship between pathogenesis of *de novo* DLBCL and RS DLBCL has not been explained but it is clear that deregulations of *BCL2* and *BCL6* are associated with *de novo* DLBCL (ref.<sup>28</sup>).

The transformation from CLL is evidenced by cytogenetic aberrations involving a gain of the 13q region (13q13.3qter), including the *MIRHG1* locus<sup>28</sup>. *MIRHG1* is a group of microRNAs involved in lymphomagenesis through interaction with the *MYC* signaling pathway<sup>29</sup> participating in the pathogenesis of DLBCL. Genetic studies have confirmed that in more than 50% of RS cases, there is at least one chromosomal aberration leading to deregulation of the *MYC* signaling pathway<sup>28</sup>.

The *MYC* transcription factor (8q24) participates in regulation of proliferation activity, differentiation and apoptosis. The t(8;14)(q24;q32) translocation was the first recurrent chromosomal aberration described in lymphoproliferative malignancies<sup>30</sup>. Although *MYC* rearrangement is characteristic for Burkitt's lymphoma it has been confirmed in up to 15% of all patients with DLBCL (ref.<sup>31,32</sup>).

The most frequent fusion partner of the *MYC* gene is *IGH* (in up to 85%) (ref.<sup>13</sup>); less frequent are *IGL* a *IGK*, genes for the immunoglobulin light chains. Recent studies have shown that translocation of this gene is significantly associated with poor prognosis in DLBCL patients treated with R-CHOP chemotherapy<sup>11,31</sup>. Amplification of *MYC*, together with other oncogenes (*REL*, *BCL2*, *GLI*, *CDK4* and *MDM2*) has been detected in the advanced form of the disease<sup>33</sup>.

Not only does *MYC* rearrangement have a negative impact on the overall survival. Poor prognosis is also associated with *MYC* copy number gains<sup>13</sup>. Duplication of *MYC* was found in as many as 7% of patients with DLBCL and was never observed as a single aberration. It was always accompanied by other cytogenetic changes including gains in 13q31, 7p/7q, 1q, 9q, 2p16-p15, 12p/12q and 5p/5q and losses in the 8p and 17p regions<sup>34</sup>.

Apart from the *MYC* gene deregulation, other aberrations occur during RS, in particular deletion of the tumor suppressor gene *TP53* and a gain of *MYCN* (ref.<sup>28</sup>).

The resources reporting cytogenetic aberrations occurring during the transformation period suggest that the prognosis of RS DLBCL is very poor, worse in *de novo* DLBCL (ref.<sup>28</sup>).

## DOUBLE HIT, TRIPLE HIT LYMPHOMA

Lymphomas with recurrent aberrations involving more oncogenes and with rearrangement of the *MYC* gene at the same time are called "double-hit" (DH) lymphomas. The most frequent concurrent rearrangements are those of *MYC* and *BCL2*. Other frequently involved genes are *BCL6*, *CCND1* and *BCL3*. Concurrent rearrangement of

three genes, one of them being *MYC*, is referred to as “triple-hit” (TH) lymphoma. DH and TH lymphomas are highly aggressive lymphoproliferative malignancies. Although patients with this diagnosis are treated with high-dose chemotherapy, they have very poor prognosis and their disease progresses rapidly<sup>35</sup>.

## PROGNOSTIC AND THERAPEUTIC VALUES OF GENETIC ANALYSES

Prognostic stratification of DLBCL patients is essential for as objective decision-making about tailored therapy as possible. Clearly defined prognostic factors including genetic aberrations with a confirmed key role<sup>1,9,11-13,16,17,20,27,31,35,38</sup> are today an integral part of comprehensive stratification.

Gene expression profiling has enabled us to define three DLBCL molecular subgroups with different clinical course of the disease. According to original studies in the era of chemotherapy, the 5-year survival significantly confirms the prognostic difference between these subgroups of DLBCL. Among patients with GCB DLBCL, 59% survive. PMBL is less aggressive, with survival rates of about 64%. On the other hand, the most aggressive form is ABC DLBCL, with only 30% (ref.<sup>9</sup>) or 35% of patients<sup>1</sup> surviving at 5 years from diagnosis.

Although the use of expression profiling for determination of prognostic subgroups in DLBCL patients is still not a routine approach, chromosomal aberrations allow us to distinguish individual prognostic subgroups.

A gain in the 3q region is a chromosomal aberration frequently present in patients from the ABC prognostic subgroup, compared with the GCB group in which the aberration has never been reported<sup>9</sup>. This subtype is also frequently characterized by a gain of long arms of chromosome 18, a less frequent aberration in the other subgroups. The above cytogenetic aberrations correlate with a short survival rates in DLBCL patients<sup>9</sup>.

Increased expression of *BCL2* resulting from gene translocation in GCB patients is associated with a shorter survival<sup>1,11</sup>. Similarly, a cytogenetic aberration in the ABC subtype, rearrangement of *BCL6* seen in up to 50% of patients in this prognostic subgroup, means a poor prognosis<sup>37</sup>. Compared to conventional chemotherapy, R-CHOP therapy does not improve the prognosis of patients with *BCL6* rearrangement but it improves response of patients without rearrangement of gene *BCL6* (ref.<sup>37</sup>).

The latest strategy for studying tumor genomes is whole genome sequencing, a method to determine genetic aberrations present mainly in the encoding regions of DLBCL patients' genome. Sequencing combined with SNP array and FISH allows a complete assessment of genetic aberrations and identification of new deregulated genes participating in the pathogenesis of DLBCL (ref.<sup>39</sup>). Advances in more accurate prognostic stratification are particularly important for patients with refractory or relapsing disease. For these patients they may contribute to selection of alternative therapeutic approaches<sup>40</sup>.

## CONCLUSION

Diffuse large B-cell lymphoma is the most frequent type of non-Hodgkin's lymphoma in the adult population of the western hemisphere. Significant morphological, clinical and biological heterogeneity of DLBCL confirms the existence of several subtypes of the disease with different clinical course. The DLBCL subtypes arise at different phases of the B cell with increased risk of genetic aberrations that initiate malignant transformation. Recurrent chromosomal aberrations have been shown repeatedly in individual subgroups and this enables stratification of DLBCL patients into three molecular subtypes.

Cytogenetic and molecular cytogenetic methods are an integral part of routine assessment of tissues infiltrated with tumor cells. High-resolution arrayCGH and SNP analysis contributes to the discovery of new candidate genes of clinical significance. For this reason, these methods are valuable in routine clinical practice since they may help us better understand the pathogenesis of diffuse large B-cell lymphoma.

## ABBREVIATIONS

ABC, Activated B-cell-like; arrayCGH, Array comparative genomic hybridization; CGH, Comparative genomic hybridization; CLL, Chronic lymphocytic leukemia; DH lymphoma, Double hit lymphoma; DLBCL, Diffuse large B-cell lymphoma; FFPE, Formalin-fixed paraffin-embedded; FISH, Fluorescence *in situ* hybridization; FL, Follicular lymphoma; GCB, Germinal center B-cell-like; LOH, Loss of heterozygosity; mFISH, Multicolor fluorescence *in situ* hybridization; NHL, Non-Hodgkin's lymphoma; PMBL, Primary mediastinal B-cell lymphoma; RS, Richter's syndrome; SNP, Single nucleotide polymorphisms; TH lymphoma, Triple hit lymphoma; UPD, Uniparental disomy.

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## CONFLICT OF INTEREST STATEMENT

**Author's conflict of interest disclosure:** The authors stated that there are no conflicts of interest regarding the publication of this article.

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