



# "Double hit" lymphoma or secondary MYC translocation lymphoma?

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## Abstract

*Chromosomal translocations that juxtapose different genes required for proliferation and differentiation are frequently associated with hematologic neoplasms. A term "double hit" lymphoma refers to a group of mature B-cell malignancies that harbor MYC rearrangement accompanied with another translocation commonly found in lymphomas (i.e. IGH/BCL2). A complex karyotype with multiple abnormalities and very aggressive course of disease are additional characteristics of this group. The response to currently available treatment regimens is unsatisfying, thus reported overall survival of these patients is usually very short. Today, the precise mechanisms of "double hit" lymphoma development are still unclear, although several possible pathways have been proposed in the literature. Similar oncogenic chain of events was also observed in another common hematological malignant neoplasm – multiple myeloma. MYC translocation as a secondary pathogenic phenomenon has been demonstrated in multiple myeloma, as well as complex cytogenetics and very aggressive course of the disease. Therefore, a secondary translocation involving MYC gene might be a potential marker of aggressive neoplasms emerging from different cells of origin, and united under the umbrella of "MYC-plus" malignancies. That concept might, in the future, result in a novel therapy, targeting this distinct, but not unique cytogenetic aberration.*

## WHO CLASSIFICATION OF LYMPHOMAS

A commonly used term 'lymphoma' portrays not a single disease, but more than 40 biologically, morphologically and clinically different entities within the category of malignant neoplasms of lymphoid lineage. Classifications are an essential part of modern medicine, offering a consensus on terminology and disease definitions to be used interdisciplinary both in research and clinical practice. Evolution of lymphoma classifications includes numerous attempts from descriptive schemes, relying on morphology (in the Rappaport classification) to strictly clinically oriented stratifications proposed by hematologists (as in the Working Formulation), usually without significant international acceptance (1). The 3<sup>rd</sup> edition of The World Health Organization (WHO) Classification of Tumours of the Haematopoietic and Lymphoid Tissues that was published in 2001 was regarded as a milestone, because it was the first classification consistently to be used worldwide (2).

The WHO classification divides lymphomas primarily into Hodgkin lymphomas and non-Hodgkin lymphomas. Non-Hodgkin lymphomas are further stratified according to the stage of differentiation, as

TABLE 1

Aggressive B-cell lymphomas in the WHO Classification (2008).

# Variants of mantle cell lymphoma
Diffuse large B-cell lymphoma, not otherwise specified
Diffuse large B-cell lymphoma subtypes <ul style="list-style-type: none"> <li>• <i>T-cell/histiocyte-rich large B-cell lymphoma</i></li> <li>• <i>Primary DLBCL of the CNS</i></li> <li>• <i>Primary cutaneous DLBCL, leg type</i></li> <li>• <i>EBV-positive DLBCL of the elderly</i></li> </ul>
Primary mediastinal (thymic) large B-cell lymphoma
Intravascular large B-cell lymphoma
DLBCL associated with chronic inflammation
ALK-positive large B-cell lymphoma
§ Lymphomatoid granulomatosis
Plasmablastic lymphoma
Primary effusion lymphoma
Large B-cell lymphoma arising in HHV-8-associated multicentric Castleman disease
Burkitt lymphoma
* B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma
* B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

# Aggressive variants include blastoid and pleomorphic mantle cell lymphoma

§ In most patients the disease is aggressive (grade 3), although patients with grade 1 and 2 lesions follow a more indolent clinical course

\* Borderline (grey zone categories)

well as B or T cell (NK cell) lineage (precursor lymphoid neoplasms and mature B-cell and T/NK-cell neoplasms). The principles of classification of lymphoid neoplasms include a multiparameter approach to disease definition: they are based on morphology, immunophenotype and genetics, as well as clinical features of disease entities. Furthermore, lymphomas are usually classified according to their presumed normal counterpart, although some of them, especially the aggressive forms, lack obvious normal counterparts. The currently used 4<sup>th</sup> edition of the WHO classification brought important changes based mainly on new genetic information; redefined consensus guidelines for some diseases, introduced new entities, subtypes and variants of lymphomas, provisional categories, and importantly borderline (grey zone) categories. In this review we will focus on a particular subgroup of mature lymphoid neoplasms with B-cell immunophenotype and aggressive clinical behavior, "double hit" lymphomas (3).

### Aggressive B-cell lymphomas

In previous classifications most aggressive B-cell neoplasms were conveniently designated as diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL) or Burkitt-like lymphoma (atypical Burkitt lymphoma). In the current WHO classification the complexity of this subgroup is reflected in a long list of entities and disease variants (Table 1), and the diagnosis of Burkitt-like lymphoma is abolished. Interestingly, some of them are defined by specific genetic abnormalities (e.g. ALK-positive large B-cell lymphoma), and others by viral agents

contributing to B-cell transformation (e.g. primary effusion lymphoma, large B-cell lymphoma arising in HHV-8-associated multicentric Castleman disease) or even age (EBV-positive DLBCL of the elderly). It also introduced two new, controversial categories of grey zone lymphomas, both defined by overlapping morphological, immunophenotypic and clinical features, with majority of "double hit" lymphomas currently classified as B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma. Some of these aggressive malignancies of B-cell origin share striking morphologic similarities (Figure 1), as well as genetic aberrations involving *MYC* gene, and will be further discussed in more details.

### Mantle cell lymphoma

Mantle cell lymphoma (MCL) is a B-cell lymphoma composed of monomorphous small to medium-sized lymphoid cells with irregular nuclei and a translocation involving the *cyclin D1* gene (3). Postulated normal counterpart of the tumor cell is a mature B-cell of inner mantle zone (usually of naïve pre-GC type). It usually affects middle-aged to older patients with a median age of approximately 60 (4). Microscopically, several architectural patterns of MCL are observed, such as diffuse, nodular, mantle zone, and rarely marginal zone pattern. Morphologic variants include the small cell variant with a more indolent course and blastoid/pleomorphic variants, usually with high proliferation fraction, dispersed chromatin pattern and associated with an aggressive clinical behavior (5). Immunohistochemically tumor cells

typically express CD20, BCL2 and CD5, as well as cyclin D1, although rare cases can be CD5- and cyclin D1-negative. The t(11;14)(q13;q32) is present in almost all cases and is considered the primary genetic event, although some MCL with no evidence of such translocation have been reported (6). Some of them harbor a t(2;12)(p12;p13) translocation involving *cyclin D2* and kappa light chain gene (*IgK*) (7).

**Diffuse large B-cell lymphoma**

For a long time DLBCL was a "wastebasket" diagnosis comprising myriad of morphologically similar, but pathogenetically and clinically different disease entities. Today it is divided in many variants, subgroups and subtypes, largest of which is DLBCL – not otherwise specified (NOS). It is a tumor defined by diffuse proliferation of large B-cells. Even DLBCL-NOS is morphologically and immunophenotypically a heterogenous category of lymphomas, divided in several variants (Table 2) (3).

Postulated normal counterparts are mature B-cells of either germinal center or post-germinal center stage of differentiation.

Most patients present with a rapidly enlarging mass and the disease affects nodal and extranodal sites. It usually occurs in adults and the frequency increases with age. The tumor cells are positive for B-cell markers (CD20, CD19, CD79A), and some cases demonstrate aberrant expression of CD5 or CD43 (8, 9). Expression of CD10, BCL6, and MUM1/IRF4 is variable, and is the basis of immunohistochemical stratification into GCB and non-GCB subgroup (prognostic value of such subdivision is a matter of ongoing debate because of poor reproducibility of immunohistochemical staining analysis) (10, 11). On the other hand, gene expression profiling (GEP) allows a reproducible identification of two prognostically different subgroups (GCB and ABC), with apparently different cells of origin (12-14). Approximately 10% of DLBCL harbor a *MYC* translocation, linked with an unfavorable outcome (15, 16). DLBCL is an aggressive lymphoma, usually resulting in death within 1 or 2 years without treatment (17).

**TABLE 2**

DLBCL, NOS – variants and subgroups.

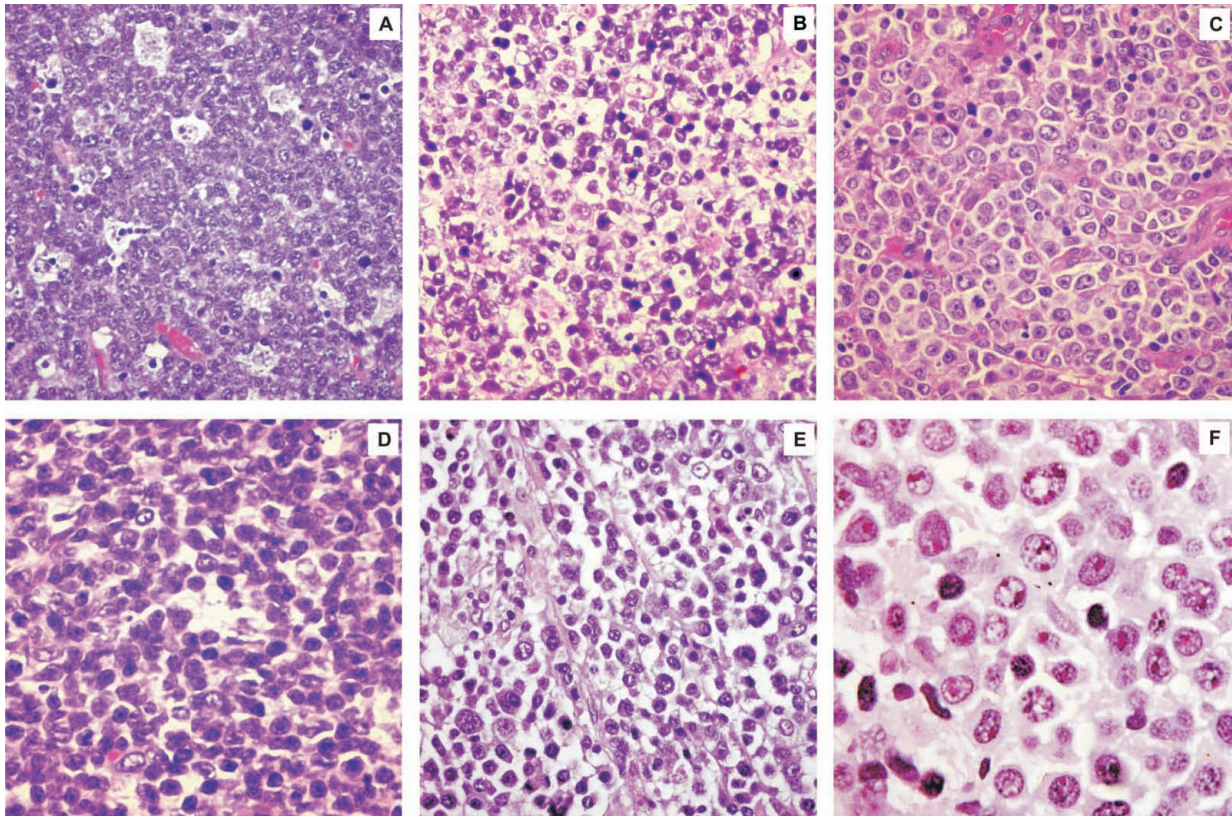
<b>Common morphologic variants</b>
<ul style="list-style-type: none"> <li>• Centroblastic</li> <li>• Immunoblastic</li> <li>• Anaplastic</li> </ul>
<b>Molecular subgroups</b>
<ul style="list-style-type: none"> <li>• GCB (germinal center B-like)</li> <li>• ABC (activated B-cell- like)</li> </ul>
<b>Immunohistochemical subgroups</b>
<ul style="list-style-type: none"> <li>• CD5-positive DLBCL</li> <li>• GCB</li> <li>• Non-GCB</li> </ul>

**Burkitt lymphoma**

This is an aggressive lymphoma that often presents in extranodal sites or as an acute leukemia, characterized by the translocation involving *MYC* gene. There are three main clinical variants of the disease: endemic BL (typically affecting children in equatorial Africa and commonly associated with EBV), sporadic BL (mainly in children and adolescents throughout the world), and immunodeficiency-associated BL (primarily in the setting of HIV infection), showing differences in clinical features, biology, and even morphology (3). The tumor is composed of cohesive proliferation of monotonous, medium-sized cells with finely clumped and dispersed chromatin and deeply basophilic cytoplasm. Postulated cell of origin is a GC or post GC mature B-cell. This lymphoma has an extremely high proliferation index, as well as high fraction of apoptotic cells, with a typical "starry sky" pattern. Tumor cells typically express pan-B markers (CD19, CD20, CD22), almost invariably show diffuse positivity for CD10, BCL6 and are uniformly negative for TdT (marker of immature lymphoid cells expressed in precursor lymphoid neoplasms). BCL2 is usually negative or rarely weakly positive in a minority of tumor cells. *MYC* translocation is present in virtually all BL, although it is not specific, and usually involves the *IGH* gene (3). *MYC* translocation is considered a primary event in BL, and typically no other (or very few) cytogenetic abnormalities are present, depicted by the term "simple karyotype" (18). This is a very aggressive, but potentially curable disease in cases of endemic and sporadic BL.

**B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma – grey zone lymphoma**

This controversial category comprises rare B-cell lymphomas that demonstrate overlapping morphological and genetic features of both DLBCL and BL and particularly aggressive clinical course. They usually affect adults and older individuals. Before the 2008 classification many of them were diagnosed as Burkitt-like lymphoma or atypical Burkitt lymphoma. It is a heterogenous category, incorporating several biologically and pathogenetically different entities, but today the methods to precisely diagnose and define those are still lacking. Nevertheless, the importance of this grey zone lies in the fact that these lymphomas are resistant to currently available treatment regimens, hence these patients should not be included into the well-defined categories of DLBCL or BL. Morphologically these tumors consist of diffuse proliferation of medium and large-sized atypical lymphoid cells, with variable number of cells with typical centroblastic morphology. The variability of cell size and shape is usually greater than in BL, and some cases are morphologically indistinguishable from DLBCL. Tumor cells express B-cell markers, and often share most immunophenotypical features with BL (CD20+, CD10+, BCL6+, BCL2-), although many of them show strong and diffuse expression of BCL2, which is incompatible with the diagnosis of BL. The proliferation index is usually very high. (19).



**Figure 1.** A) Burkitt lymphoma, B) B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma, C) diffuse large B-cell lymphoma-NOS, D) mantle cell lymphoma, E) plasmablastic lymphoma, F) multiple myeloma. A-F hematoxylin-eosin staining; original magnification x400 (A-E) and x630 (F).

These tumors often harbor *MYC* translocations as a secondary oncogenic event, but unlike in BL, the translocation partner is frequently a light-chain or a non-IG gene (20). A complex karyotype with multiple abnormalities is another feature differentiating these lymphomas from BL (21). A subgroup of grey zone lymphomas that has recently received an overwhelming attention in the field of hematopathology are so called "double-hit" lymphomas. They are characterized by dual translocations, involving *MYC* and *BCL2*, or other genes such as *BCL6*, *CCND1*, *BCL3*. They can occur *de novo*, or as a result of follicular or mantle cell lymphoma transformation (22, 23). The diagnosis of "double-hit" lymphoma is challenging because no specific morphological or immunophenotypic criteria have been defined. These patients often have significant extranodal involvement including the bone marrow and CNS, and a very poor prognosis with a median survival of only 0.2-1.5 years (20, 21).

### Plasmablastic lymphoma

This is a lymphoma composed of large cells that morphologically resemble immunoblasts, but demonstrate a plasma cell immunophenotype. The postulated cell of origin is a plasmablast (immature lymphoid cell of B-lineage switched to the plasma cell gene expression program). It usually occurs in the setting of HIV infection,

mainly in adults. The typical sites of involvement are oral cavity, and other extranodal mucosal sites (sinonasal cavity, orbit, gastrointestinal tract) (24). Tumor cells express plasma cell markers CD138, CD38, MUM1/IRF4, EMA, and are usually negative for pan-B markers (CD20, PAX5). *In situ* hybridization for detection of EBV is positive in the majority of plasmablastic lymphomas. This is an aggressive disease with a median survival of 6 to 7 months (25, 26).

### Plasma cell myeloma (multiple myeloma)

This is a plasma cell neoplasm with a wide spectrum of clinical presentations including indolent and highly aggressive forms. It is characterized by a clonal proliferation of mature plasma cells associated with an M-protein in serum and urine, and generalized bone marrow involvement. Postulated normal counterpart is a post-GC plasma cell demonstrating IG class switch and somatic hypermutation. Tumor cells typically express CD138, CD79A, and CD38, but unlike normal plasma cells they are usually CD19 negative and often show aberrant expression of CD56. Cyclin D1 can also be positive in a subset of myelomas (27). Numerous genetic abnormalities include trisomies, hyperdiploidy, deletions and translocations. Complex cytogenetic aberrations are common (28). The most frequent translocations affect *IGH* and partner genes such as *CCND1*, *C-MAF*, *FGFR3/MMSET*,

*CCND3*, and *MAFB* (29). One of the most common structural abnormalities that occurs early in the pathogenesis is monosomy or partial deletion of chromosome 13 (13q14), and it is associated with disease progression (30). *MYC* rearrangement occurs as a secondary event during the pathogenesis (3). Plasma cell myeloma is incurable, with a wide range of survival (from 6 months to 10 years and more) (31).

## Genes involved in the "double hit" concept

### MYC

*MYC* or *c-MYC* (myelocytomatosis viral oncogene homolog) gene located at 8q24.21 was recognized in 1982 as a significant part of the translocation present in Burkitt lymphoma (32, 33). In the following years it was confirmed that *c-MYC* is translocated not only in mouse plasmacytomas, but also in human Burkitt lymphoma cells, with different translocation (most frequently with *IG* partners) constituting a possible reason for observed high level of its transcripts. It was also proposed that aberrant transcription control is one of the characteristics of B-cell neoplastic transformation (34-39). Moreover, it was vaguely suggested that *c-MYC* translocation is, at least in plasmacytoma, "not the only step in induction", but possibly a secondary translocation (40). The first studies of human protein encoded by *c-MYC* suggested that this protein has affinity for both single and double stranded DNA and that it is localized in the nucleus (41, 42). Furthermore, it was shown that *c-MYC* protein has a role in DNA synthesis and that it forms a dimer with MAX protein, resulting in a complex that binds to DNA in a sequence-specific manner, thus regulating activation of various genes and influencing proliferation and differentiation (43-47). Numerous studies were performed and revealed that *c-MYC* gene regulation control works in dual manner: it induces genes involved in activation of translational initiation, cell cycle regulators, DNA repair genes, genes involved in metabolism and stress response, but also represses genes involved in cell growth arrest and cell adhesion (48, 49). Beside direct control of gene expression, *c-MYC* can regulate expression indirectly, through repression of various miRNA (50, 51).

In neoplastic cells genomic alterations of *c-MYC* most frequently involve translocations and amplifications, *MYC* rearrangement being the main aberration associated with the development of Burkitt lymphoma, diffuse large B-cell lymphoma and double-hit lymphoma. In all those entities, *c-MYC* rearrangements mark aggressive tumor behavior (3). In B-cell lymphomas, activation-induced cytidine deaminase (AID) is necessary for chromosomal breaks leading to *IGH/MYC* translocation (t(8;14)(q24;q32)), suggesting that this translocation occurs during the stages of B-cell development in which antibody class switch recombination or somatic hypermutation take place (52-54). t(8;14)(q24;q32) is also found in plasmacytoma/multiple myeloma where it is regarded as a secondary translocation. *IGH* switch recombination and somatic hypermutation mechanism in normal plasma cells and neoplastic

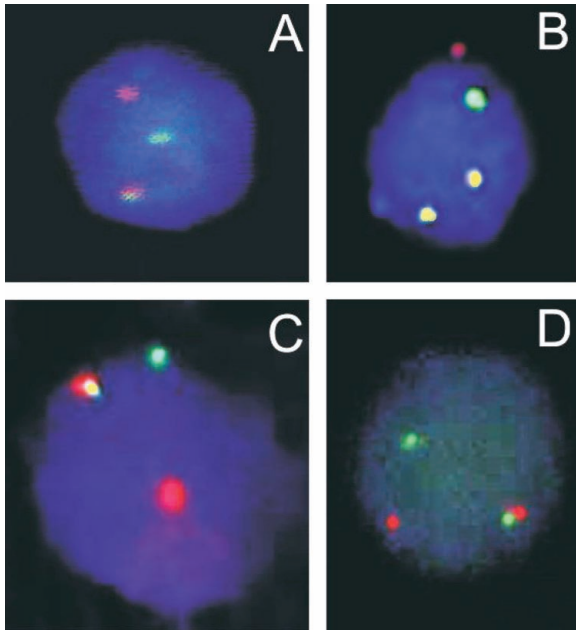
plasma cells are turned off, therefore the mechanism of this translocation in plasma cells differs from the one in B-cell lymphomas. Interestingly, the same translocation is again associated with the unfavorable outcome of the disease (55-57). Moreover, *IG/MYC* translocations are observed as a frequent event in plasmablastic lymphoma, an aggressive lymphoma in which *MYC* activation is believed to hold very important role in disease development (58).

### BCL2

*BCL2* (B-cell CLL/lymphoma 2), located at 18q21, is a gene that encodes the protein located in the inner membrane of mitochondria and serves as an antiapoptotic guardian (59-62). There are two isoforms of *BCL2* protein that differ slightly in hydrophobic binding groove, and therefore have different binding properties responsible for interaction with Bad and Bak proteins, suggesting a role in at least two different antiapoptotic pathways (63). *BCL2* gene was first described in 1984 in follicular lymphoma cells where it forms translocation with the immunoglobulin heavy chain locus on chromosome 14 (64). The same translocation was later found in diffuse large B-cell lymphoma, primary cutaneous marginal zone B-cell lymphoma, histiocytic/dendritic cell sarcomas arising from follicular lymphoma, chronic lymphocytic leukemia, monoclonal B-cell lymphocytosis, small lymphocytic lymphoma and splenic marginal zone lymphoma. In all these entities *IGH/BCL2* is responsible for constitutive expression of *BCL2* protein (65-70). Apart from being an important factor contributing to lymphomagenesis, *IGH/BCL2* was observed in healthy individuals. A clone of normal lymphocytes harboring this translocation can be persistent and more frequently present in elderly individuals (71, 72). The presence of the translocation in healthy individuals, as well as its relatively early occurrence in the B-cell development (during V(D)J recombination) suggest it is indeed important, but also an insufficient step in lymphoma development (73). World Health Organization Classification takes into account its importance not only in follicular lymphomagenesis, but also as a variable needed for assessment of "double-hit" lymphomas (3).

### BCL6

*BCL6* (B-cell CLL/lymphoma 6) is located at 3q27. Baron B W *et al.* proposed its name in 1993 and considered it as a gene that "plays a role in the pathogenesis of certain B-cell lymphomas" (74). It codes a transcription factor that can, due to its N-terminal POZ domain, self-interact or interact with many different proteins and act as a sequence-specific repressor of transcription (75-78). Its expression is especially important for formation and function of germinal centers where it protects B-cells from apoptosis induced by double strand DNA breaks (DNA breaks required for immunoglobulin class switch recombination and somatic hypermutation) (79-82). *BCL6* is involved in cell cycle regulation, DNA repair mechanisms, apoptosis, proliferation and differentiation



**Figure 2.** A) MYC gene rearrangement; B) IGH/c-MYC translocation; C) BCL2 gene rearrangement; D) BCL6 gene rearrangement. A, C, D – single red and green signals represent split loci, B-fusion (yellow) signals represent reciprocal translocation.

(81, 83-86). It directly controls many genes, in specific *BCL2* and *p53* (80, 86, 87). Genetic aberrations of *BCL6* are mainly found in diffuse large B-cell lymphoma and follicular lymphoma, in a form of translocation or point mutations (3, 88-90). *BCL6* expression by tumor cells in DLBCL is one of key variables in algorithms for distinguishing groups of patients with better or worse survival and therapy response (10, 11). *BCL6* translocation to any of its numerous partners is considered one of the few possible events that can mark double-hit lymphoma (3, 91).

### CCND1

*CCND1* (cyclin D1) or *BCL1*, gene located at 11q13, codes a protein necessary for transition from G1 to S phase of the cell cycle. It forms a complex with kinase CDK4 and through its activity, mostly by influencing pRb function, regulates the beginning of the cycle (92). It is not expressed in normal lymphoid or myeloid lineages, but its overexpression has been described in mantle cell lymphoma (93, 94). Genetic aberration that is behind its overexpression seen in B-cell neoplasms is the translocation t(11;14)(q13;q32) – *IGH/CCND1* (94-98). This translocation is characteristic of mantle cell lymphoma but, in high percentage, it can also be found in multiple myeloma, where it is a consequence of different break-points (3, 99). In mantle cell lymphoma, *IGH/CCND1* is a result of CpG double-strand break mechanism that suggests this translocation happens during the pro B-cell developmental stage (100). Translocation itself is not necessarily a pathogenetic event – it was also found in healthy individuals (101). In rare cases of B-cell "double hit" lymphomas this translocation is believed to be the first step of the pathogenesis (3, 102).

### BCL3

*BCL3* (B-cell leukemia/lymphoma 3) gene is located at 19q13.32. Translocation (14;19)(q32;q13.1), which brings together *BCL3* and switch region of the *IGH* gene, was first found in chronic lymphocytic leukemia and acute biphenotypic leukemia (103, 104). Breakpoints responsible for this translocation are believed to be the result of aberrant class switch recombination (105). On a mouse model, it was shown that *BCL3* expression varies through different stages of B-cell development and that its transcripts are present in a higher amount during mature B-cell stages (106). *BCL3* codes a protein that belongs to a I kappa B group. It specifically binds to a NF-kappa B p50 subunit, thus interfering with inhibitory role that those homodimers have on a transcription of various genes (107, 108). *BCL3* activity is mediated by phosphorylation that allows its association with different partners and subsequently controls expression of different targets genes. (109) Furthermore, *BCL3* inhibits p53-induced apoptosis (110).

### Possible mechanisms of "double hit" lymphoma development

"Double hit" lymphomas are defined as aggressive B-cell lymphoma variants that are marked by *MYC* rearrangement and at least one other recurrent translocation (3). (Figure 2) In mature B-cell lymphomas the other "hit" usually involves *BCL2*, *BCL6* and/or *CCND1* genes. Along with a second hit, so far, all reported cases show complex cytogenetics with additional genetic alterations (23). In all cases of mature B-cell "double hit" lymphomas *MYC* translocation is considered to be a secondary event based on a timing of breakpoints responsible for certain translocations during normal B-cell development (23).

In *BCL2*<sup>+</sup>/*MYC*<sup>+</sup> lymphomas, there are two possible scenarios. The first possibility is that *BCL2* translocation is already present in a B-cell clone of a healthy individual. *MYC* translocation happens as a part of complex pathogenic karyotype and forms additional change on the ground of the already present aberration. In the other possible scenario, *BCL2* translocation is indeed one part of the oncogenic pathway (as in follicular lymphoma), followed by further aberrations involving the *MYC* gene (23). In both cases, the result is a "double hit" that might cause antiapoptotic behavior of cells, with changes in gene expression control through suppression of growth arrest, and induction of constitutive proliferation and differentiation signals. The promotion of such aberrant differentiation could be the key point that affects the severity of the disease. An idea that such pathways exist has been implied incoherently during last decade. In 2005 Martin-Subero *et al.* described two cases, showing that *IGH/BCL2* accompanied by *MYC* aberration causes aggressive behavior of germinal center B-cell lymphomas (111). In the same year, another group reported a case of follicular lymphoma with *IGH/BCL2* and its subsequent transformation to atypical Burkitt lymphoma (according to the 2008 WHO classification, grey zone lymphoma), due to additional *IGH/MYC* translocation (112).

*CCND1*<sup>+</sup>/*MYC*<sup>+</sup> lymphomas could share similar oncogenic pathways: *IGH/CCND1* is either a non-oncogenic aberration present in a small population of B-cells of healthy individuals, or the first step in the pathogenesis, similar as in the pathogenesis of mantle cell lymphoma. *MYC* rearrangement brings an additional change that adds aggressive potential to the neoplastic cell (23). *IGH/CCND1* results in constitutively induced *CCND1* that forces B-cell to enter mitosis possibly without enough time required for damage checkpoints, thus allowing multiple aberrations to persist and cause more changes in the transcription control. *MYC* rearrangement, again, could additionally promote proliferation and/or activate transcription of other oncogenes causing aggressive behavior of the neoplasm.

Third group are *BCL6*<sup>+</sup>/*MYC*<sup>+</sup> lymphomas that are mostly "triple hit" *BCL2*<sup>+</sup>/*BCL6*<sup>+</sup>/*MYC*<sup>+</sup> lymphomas (23). This entity displays synergistic action of *BCL2* and *MYC* with an additional event, which might result in protection of B-cells from apoptosis, induced by double strand DNA breaks. Rare *BCL6*<sup>+</sup>/*MYC*<sup>+</sup> lymphomas could manifest a constant antiapoptotic signal from constitutively active *BCL6* accompanied by induced proliferation due to constant activation of various oncogenes by *MYC*.

*BCL3*<sup>+</sup>/*MYC*<sup>+</sup> lymphomas are extremely rare "double hit" entities. The underlying oncogenic pathway is most probably based on a constant antiapoptotic *BCL3* activity, followed by transcriptional activation of genes required for proliferation and differentiation induced by *MYC* rearrangement.

### Clinical presentation, current treatments and future need for therapy

In 2011, Aukema SM *et al.* analyzed the Mitelman database and cases reported in published studies, in order to review clinical data and treatment protocols concerning "double hit" lymphomas. They reported that "double hit" lymphoma patients were mostly in their 5<sup>th</sup> and 6<sup>th</sup> decades, have elevated LDH levels, extranodal involvement, high International Prognostic Index (IPI) stage and that the diagnosis was established usually in the advanced stage of the disease. In different studies, they documented various treatment protocols: high-dose chemotherapy, stem cell transplantation, specific antibody treatment (rituximab), bone marrow transplantation, radiotherapy, combination of those, and even in some cases steroids as a form of palliative therapy. Overall survival was never longer than a year and a half (23).

Hence, it seems that the category "double hit" lymphoma is just a temporary title for B-cell lymphomas with highly aggressive biologic potential, evident not only in gene rearrangements behind its development, but also in different clinical aspects that accompany such disease progression. Subsequently, patients that belong to this group require a new treatment regimen, possibly a therapy based on repression of oncogenic mechanisms arising from a "double hit" concept, or a protocol that would eliminate the effect of complex cytogenetic aberrations in the neoplastic cells.

## DISCUSSION

"Double hit" lymphomas are, by definition, a highly aggressive B-cell lymphomas with *MYC* rearrangement plus additional recurrent translocation and a very complex karyotype (3). In the recent literature, it was clearly stated that this is a temporary category that should be used until enough data for better classification were collected (23).

All disease classifications were written out of a need for better understanding of disease development in order to stratify and treat patients with adequate therapies. In case of hematological neoplasms, detailed attempts of lymphoma classification go back to Rappaport classification (1). Classification was at first based on tumor cell morphology and its similarity to normal cell counterparts. Later, gradually, different classifications take into account clinical behavior, cell surface markers, different antigen expression and genetic aberrations (2, 3, 113, 114). With time, due to gathered knowledge, it was possible to divide hematological diseases in many different groups, describe them very precisely, recognize pathways responsible for their development, and therefore to treat them almost specifically aiming at certain tumor characteristics (115-118). Today, the abundant available data about tumorigenesis allow us to stratify patients precisely, although it is still impossible to decipher crucial and therapeutically applicable pathogenetic events. "Double hit" lymphomas represent a group that encounters this problem. In the latest WHO classification, "*MYC*- plus B-cell lymphomas" belong to the grey zone category called "B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma" (3). As explained so far, the clinical presentation of those lymphomas most probably stems from the secondary *MYC* rearrangement and/or complex cytogenetics observed in almost all cases. Interestingly, the addition of *MYC* aberration to an already active pathogenetic pathway and its clinical manifestation in a form of aggressiveness is not a new concept, neither is it restricted to mature B-cell lymphomas. Adams MJ *et al.* in 1983 suggested that "plasmacytoma oncogenesis appears to involve both translocation of *c-MYC* and activation of another oncogene". Although their research was based on plasmacytoma cells lines, the postulated oncogenic mechanism involved *MYC* rearrangement plus additional aberration (40). *MYC* rearrangement as a secondary event was also observed in multiple myeloma and plasma cell leukemia. Mechanism responsible for *MYC* rearrangement in those neoplasms is still unclear, but it is most certainly different from those in mature B-cell lymphomas (55-58). Shared characteristics between plasma cell neoplasms and B-cell "double hit" lymphoma involve secondary *MYC* aberration (the result of different breakpoint mechanisms), complex karyotype, aggressive biological behavior and a decrease, or even loss of CD20 expression (3, 119). Moreover, in 2010 Ouansafi I *et al.* described a case of follicular lymphoma with transformation to plasmablastic lymphoma, possibly due to *MYC* rearrangement (120).

In conclusion, all secondary *MYC* hematological neoplasms display fascinating similarities. Their morphology, phenotype, basic genetic aberrations and postulated cells of origin differ, but the clinical aspect of those aggressive neoplasms and the main underlying pathogenic mechanisms might put them in the same category. The key event – a secondary *MYC* translocation, could integrate neoplasms emerging from different cells of origin into one group, with potential novel therapy targeting a distinct, but not unique aberration (121).

## REFERENCES

- LENNERT K 2006 History of the European Association for Haematopathology. Springer-Verlag, Berlin, Heidelberg.
- JAFFE E S, HARRIS N L, STEIN H, VARDIMAN J W (eds) 2001 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press, Lyon.
- SWERDLOW S H, CAMPO E, HARRIS N L, JAFFE E S, PILERI S A, STEIN H, THIELE J, VARDIMAN J W (eds) 2008 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC, Lyon.
- BOSCH F, LOPEZ-GUILLERMO A, CAMPO E, RIBERA J M, CONDE E, PIRIS MA, VALLESPI T, WOESSNER S, MONT-SERRAT E 1998 Mantle cell lymphoma: presenting features, response to therapy, and prognostic factors. *Cancer* 82: 567-75
- RÄTY R, FRANSSILA K, JOENSUU H, TEERENHOVI L, ELONEN E 2002 Ki-67 expression level, histological subtype, and the International Prognostic Index as outcome predictors in mantle cell lymphoma. *Eur J Haematol* 69: 11-20
- FU K, WEISENBURGER D D, GREINER T C, DAVE S, WRIGHT G, ROSENWALD A, CHIORAZZI M, IQBAL J, GESK S, SIEBERT R, DE JONG D, JAFFE E S, WILSON W H, DELABIE J, OTT G, DAVE B J, SANGER W G, SMITH L M, RIMSZA L, BRAZIEL L M, MÜLLER-HERMELINK H K, CAMPO E, GASCOYNE R D, STAUDT L M, CHAN W C 2005 Cyclin D1-negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling. *Blood* 106: 4315-21
- GESK S, KLAPPER W, MARTIN-SUBERO J I, NAGEL I, HARDER L, FU K, BERND H W, WEISENBURGER D D, PARWARESCH R, SIEBERT R 2006 A chromosomal translocation in cyclin D1-negative/cyclin D2-positive mantle cell lymphoma fuses the CCND2 gene to the IgK locus. *Blood* 108: 1109-10
- TAGAWA H, SUGURO M, TSUZUKI S, MATSUI K, KARNAN S, OHSHIMA K, OKAMOTO M, MORISHIMA Y, NAKAMURA S, SETO M 2005 Comparison of genome profiles for identification of distinct subgroups of diffuse large B-cell lymphoma. *Blood* 106: 1770-7
- LAI R, WEISS L M, CHANG K L, ARBER D A 1999 Frequency of CD43 expression in non-Hodgkin lymphoma. A survey of 742 cases and further characterization of rare CD43+ follicular lymphomas. *Am J Clin Pathol* 111: 488-94
- HANS C P, WEISENBURGER D D, GREINER T C, GASCOYNE R D, DELABIE J, OTT G, MÜLLER-HERMELINK H K, CAMPO E, BRAZIEL R M, JAFFE E S, PAN Z, FARINHA P, SMITH L M, FALINI B, BANHAM A H, ROSENWALD A, STAUDT L M, CONNORS J M, ARMITAGE J O, CHAN W C 2004 Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 103: 275-82
- CHOI W W, WEISENBURGER D D, GREINER T C, PIRIS MA, BANHAM A H, DELABIE J, BRAZIEL R M, GENG H, IQBAL J, LENZ G, VOSE J M, HANS C P, FU K, SMITH L M, LI M, LIU Z, GASCOYNE R D, ROSENWALD A, OTT G, RIMSZA L M, CAMPO E, JAFFE E S, JAYE D L, STAUDT L M, CHAN W C 2009 A new immunostain algorithm classifies diffuse large B-cell lymphoma into molecular subtypes with high accuracy. *Clin Cancer Res* 15: 5494-502.
- ALIZADEH A A, EISEN M B, DAVIS R E, MA C, LOSSOS I S, ROSENWALD A, BOLDRICK J C, SABET H, TRAN T, YU X, POWELL J I, YANG L, MARTI G E, MOORE T, HUDSON J JR, LU L, LEWIS D B, TIBSHIRANI R, SHERLOCK G, CHAN W C, GREINER T C, WEISENBURGER D D, ARMITAGE J O, WARNKE R, LEVY R, WILSON W, GREVER M R, BYRD J C, BOTSTEIN D, BROWN P O, STAUDT L M 2000 Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403: 503-511
- LOSSOS I S, CZERWINSKI D K, ALIZADEH A A, WECHSER M A, TIBSHIRANI R, BOTSTEIN D, LEVY R 2004 Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. *N Engl J Med* 350: 1828-37
- MALUMBRES R, CHEN J, TIBSHIRANI R, JOHNSON N A, SEHN L H, NATKUNAM Y, BRIONES J, ADVANI R, CONNORS J M, BYRNE G E, LEVY R, GASCOYNE R D, LOSSOS I S 2008 Paraffin-based 6-gene model predicts outcome in diffuse large B-cell lymphoma patients treated with R-CHOP. *Blood* 111: 5509-14
- BARRANS S, CROUCH S, SMITH A, TURNER K, OWEN R, PATMORE R, ROMAN E, JACK A 2010 Rearrangement of *MYC* is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. *J Clin Oncol* 28: 3360-5
- NITSU N, OKAMOTO M, MIURA I, HIRANO M 2009 Clinical significance of 8q24/c-MYC translocation in diffuse large B-cell lymphoma. *Cancer Sci* 100: 233-7.
- ARMITAGE J O, WEISENBURGER D D 1998 New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. *J Clin Oncol* 16: 2780-95
- HUMMEL M, BENTINK S, BERGER H, KLAPPER W, WESSENDORF S, BARTH T F, BERND H W, COGLIATTI S B, DIERLAMB J, FELLER A C, HANSMANN M L, HARALAMBIEVA E, HARDER L, HASENCLEVER D, KÜHN M, LENZE D, LICHTER P, MARTIN-SUBERO J I, MÖLLER P, MÜLLER-HERMELINK H K, OTT G, PARWARESCH R M, POTT C, ROSENWALD A, ROSOŁOWSKI M, SCHWAENEN C, STÜRZENHOEFCKER B, SZCZEPANOWSKI M, TRAUTMANN H, WACKER H H, SPANG R, LOEFFLER M, TRÜMPER L, STEIN H, SIEBERT R; MOLECULAR MECHANISMS IN MALIGNANT LYMPHOMAS NETWORK PROJECT OF THE DEUTSCHE KREBSHILFE 2006 A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. *N Engl J Med* 354: 2419-30
- HARALAMBIEVA E, BOEMA E J, VAN IMHOFF G W, ROSATI S, SCHUURING E, MÜLLER-HERMELINK H K, KLUIN P M, OTT G 2005 Clinical, immunophenotypic, and genetic analysis of adult lymphomas with morphologic features of Burkitt lymphoma. *Am J Surg Pathol* 29: 1086-94
- MCCLURE R F, REMSTEIN E D, MACON W R, DEWALD G W, HABERMANN T M, HOERING A, KURTIN P J 2005 Adult B-cell lymphomas with burkitt-like morphology are phenotypically and genotypically heterogeneous with aggressive clinical behavior. *Am J Surg Pathol* 29: 1652-60.
- LE GOUILL S, TALMANT P, TOUZEAU C, MOREAU A, GARAND R, JUGÉ-MORINEAU N, GAILLARD F, GASTINNE T, MILPIED N, MOREAU P, HAROUSSEAU J L, AVET-LOISEAU H 2007 The clinical presentation and prognosis of diffuse large B-cell lymphoma with t(14;18) and 8q24/c-MYC rearrangement. *Haematologica* 92: 1335-42
- VAIDYANATHAN G, NGAMPHAIBOON N, HERNANDEZ-ILIZALITURRI F J 2011 Clinical spectrum and prognosis of follicular lymphoma with blastoid transformation: case series and a review of the literature. *Ann Hematol* 90: 955-62
- AUKEMA S M, SIEBERT R, SCHUURING E, VAN IMHOFF G W, KLUIN-NELEMANS H C, BOERMA E J, KLUIN P M 2011 Double-hit B-cell lymphomas. *Blood* 117: 2319-31
- CASTILLO J, PANTANOWITZ L, DEZUBE B J 2008 HIV-associated plasmablastic lymphoma: lessons learned from 112 published cases. *Am J Hematol* 83: 804-9
- DELECLUSE H J, ANAGNOSTOPOULOS I, DALLENBACH F, HUMMEL M, MARAFIOTI T, SCHNEIDER U, HUHNS D, SCHMIDT-WESTHAUSEN A, REICHHART P A, GROSS U, STEIN H 1997 Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. *Blood* 89: 1413-20
- COLOMO L, LOONG F, RIVES S, PITTALUGA S, MARTÍNEZ A, LÓPEZ-GUILLERMO A, OJANGUREN J, ROMAGOSA V, JAFFE E S, CAMPO E 2004 Diffuse large B-cell lymphomas with plasmablastic differentiation represent a heterogeneous group of disease entities. *Am J Surg Pathol* 28: 736-47



27. DAWSON M A, OPAT S S, TAOUK Y, DONOVAN M, ZAMMIT M, MONAGHAN K, HORVATH N, ROBERTS A W, PRINCE H M, HERTZBERG M, MCLEAN C A, SPENCER A 2009 Clinical and immunohistochemical features associated with a response to bortezomib in patients with multiple myeloma. *Clin Cancer Res* 15: 714-22
28. DEWALD G W, KYLE R A, HICKS G A, GREIPP P R 1985 The clinical significance of cytogenetic studies in 100 patients with multiple myeloma, plasma cell leukemia, or amyloidosis. *Blood* 66: 380-90
29. BERGSAGEL P L, KUEHL W M 2005 Molecular pathogenesis and a consequent classification of multiple myeloma. *J Clin Oncol* 23: 6333-8
30. FONSECA R, BARLOGIE B, BATAILLE R, BASTARD C, BERGSAGEL P L, CHESI M, DAVIES F E, DRACH J, GREIPP P R, KIRSCH I R, KUEHL W M, HERNANDEZ J M, MINVIELLE S, PILARSKI L M, SHAUGHNESSY J D JR, STEWART A K, AVET-LOISEAU H 2004 Genetics and cytogenetics of multiple myeloma: a workshop report. *Cancer Res* 64: 1546-58
31. GREIPP P R, SAN MIGUEL J, DURIE B G, CROWLEY J J, BARLOGIE B, BLADÉ J, BOCCADORO M, CHILD J A, AVET-LOISEAU H, KYLE R A, LAHUERTA J J, LUDWIG H, MORGAN G, POWLES R, SHIMIZU K, SHUSTIK C, SONNEVELD P, TOSI P, TURESSON I, WESTIN J 2005 International staging system for multiple myeloma. *J Clin Oncol* 23: 3412-20
32. TAUB R, KIRSCH I, MORTON C, LENOIR G, SWAN D, TRO-NICK S, AARONSON S, LEDER P 1982 Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc Natl Acad Sci U S A* 79: 7837-41
33. DALLA-FAVERA R, BREGNI M, ERIKSON J, PATTERSON D, GALLO R C, CROCE C M 1982 Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc Natl Acad Sci U S A* 79: 7824-7
34. MARCU K B, HARRIS L J, STANTON L W, ERIKSON J, WATT R, CROCE C M 1983 Transcriptionally active c-myc oncogene is contained within NIARD, a DNA sequence associated with chromosome translocations in B-cell neoplasia. *Proc Natl Acad Sci U S A* 80: 519-23
35. ERIKSON J, AR-RUSHDI A, DRWINGA H L, NOWELL P C, CROCE C M 1983 Transcriptional activation of the translocated c-myc oncogene in Burkitt lymphoma. *Proc Natl Acad Sci U S A* 80: 820-4
36. NISHIKURA K, AR-RUSHDI A, ERIKSON J, WATT R, ROVERA G, CROCE C M 1983 Differential expression of the normal and of the translocated human c-myc oncogenes in B cells. *Proc Natl Acad Sci U S A* 80: 4822-6
37. BATTEY J, MOULDING C, TAUB R, MURPHY W, STEWART T, POTTER H, LENOIR G, LEDER P 1983 The human c-myc oncogene: structural consequences of translocation into the IgH locus in Burkitt lymphoma. *Cell* 34: 779-87.
38. AR-RUSHDI A, NISHIKURA K, ERIKSON J, WATT R, ROVERA G, CROCE C M 1983 Differential expression of the translocated and the untranslocated c-myc oncogene in Burkitt lymphoma. *Science* 222: 390-3
39. CROCE C M, ERIKSON J, AR-RUSHDI A, ADEN D, NISHIKURA K 1984 Translocated c-myc oncogene of Burkitt lymphoma is transcribed in plasma cells and repressed in lymphoblastoid cells. *Proc Natl Acad Sci U S A* 81: 3170-4
40. ADAMS J M, GERONDAKIS S, WEBB E, CORCORAN L M, CORY S 1983 Cellular myc oncogene is altered by chromosome translocation to an immunoglobulin locus in murine plasmacytomas and is rearranged similarly in human Burkitt lymphomas. *Proc Natl Acad Sci U S A* 80: 1982-6
41. PERSSON H, LEDER P 1984 Nuclear localization and DNA binding properties of a protein expressed by human c-myc oncogene. *Science* 225: 718-21
42. WATT R A, SHATZMAN A R, ROSENBERG M 1985 Expression and characterization of the human c-myc DNA-binding protein. *Mol Cell Biol* 5: 448-56
43. STUDZINSKI G P, BRELVI Z S, FELDMAN S C, WATT R A 1986 Participation of c-myc protein in DNA synthesis of human cells. *Science* 234: 467-70
44. BLACKWOOD E M, EISENMAN R N 1991 Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. *Science* 251: 1211-7
45. BLACKWOOD E M, LÜSCHER B, EISENMAN R N 1992 Myc and Max associate in vivo. *Genes Dev* 6: 71-80
46. BLACKWOOD E M, KRETZNER L, EISENMAN R N 1992 Myc and Max function as a nucleoprotein complex. *Curr Opin Genet Dev* 2: 227-35
47. GRANDORI C, MAC J, SIÉBELT F, AYER D E, EISENMAN R N 1996 Myc-Max heterodimers activate a DEAD box gene and interact with multiple E box-related sites in vivo. *EMBO J* 15: 4344-57
48. MENSSEN A, HERMEKING H 2002 Characterization of the c-MYC-regulated transcriptome by SAGE: identification and analysis of c-MYC target genes. *Proc Natl Acad Sci U S A* 99: 6274-9
49. DANG C V, O'DONNELL K A, ZELLER K I, NGUYEN T, OSTHUS R C, LI F 2006 The c-Myc target gene network. *Semin Cancer Biol* 16: 253-64
50. CHANG T C, YU D, LEE Y S, WENTZEL E A, ARKING D E, WEST K M, DANG C V, THOMAS-TIKHONENKO A, MENDEL J T 2008 Widespread microRNA repression by Myc contributes to tumorigenesis. *Nat Genet* 40: 43-50
51. ROBERTUS J L, KLUIVER J, WEGGEMANS C, HARMS G, REIJMERS R M, SWART Y, KOK K, ROSATI S, SCHUURING E, VAN IMHOFF G, PALS S T, KLUIJN P, VAN DEN BERG A 2010 MiRNA profiling in B non-Hodgkin lymphoma: a MYC-related miRNA profile characterizes Burkitt lymphoma. *Br J Haematol* 149: 896-9
52. RAMIRO A R, JANKOVIC M, EISENREICH T, DIFILIPPANTONIO S, CHEN-KIANG S, MURAMATSU M, HONJO T, NUSSENZWEIG A, NUSSENZWEIG M C 2004 AID is required for c-myc/IgH chromosome translocations in vivo. *Cell* 118: 431-8
53. DORSETT Y, ROBBIANI D F, JANKOVIC M, REINA-SAN-MARTIN B, EISENREICH T R, NUSSENZWEIG M C 2007 A role for AID in chromosome translocations between c-myc and the IgH variable region. *J Exp Med* 204: 2225-32
54. ROBBIANI D F, BOTHMER A, CALLEN E, REINA-SAN-MARTIN B, DORSETT Y, DIFILIPPANTONIO S, BOLLAND D J, CHEN HT, CORCORAN A E, NUSSENZWEIG A, NUSSENZWEIG M C 2008 AID is required for the chromosomal breaks in c-myc that lead to c-myc/IgH translocations. *Cell* 135: 1028-38
55. GABREA A, LEIF BERGSAGEL P, MICHAEL KUEHL W 2006 Distinguishing primary and secondary translocations in multiple myeloma. *DNA Repair (Amst)* 5: 1225-33
56. CHNG W J, GLEBOV O, BERGSAGEL P L, KUEHL W M 2007 Genetic events in the pathogenesis of multiple myeloma. *Best Pract Res Clin Haematol* 20: 571-96
57. TIEDEMANN R E, GONZALEZ-PAZ N, KYLE R A, SANTANA-DAVILA R, PRICE-TROSKA T, VAN WIER S A, CHNG W J, KETTERLING R P, GERTZ M A, HENDERSON K, GREIPP P R, DISPENZIERI A, LACY M Q, RAJKUMAR S V, BERGSAGEL P L, STEWART A K, FONSECA R 2008 Genetic aberrations and survival in plasma cell leukemia. *Leukemia* 22: 1044-52
58. VALERA A, BALAGUÉ O, COLOMO L, MARTÍNEZ A, DELABIE J, TADDESSE-HEATH L, JAFFE E S, CAMPO E 2010 IG/MYC rearrangements are the main cytogenetic alteration in plasmablastic lymphomas. *Am J Surg Pathol* 34: 1686-94
59. HOCKENBERY D, NUÑEZ G, MILLIMAN C, SCHREIBER R D, KORSMEYER S J 1990 Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* 348: 334-6
60. FESUS L, DAVIES P J, PIACENTINI M 1991 Apoptosis: molecular mechanisms in programmed cell death. *Eur J Cell Biol* 56: 170-7
61. GOTOW T, SHIBATA M, KANAMORI S, TOKUNO O, OHSAWA Y, SATO N, ISAHARA K, YAYOI Y, WATANABE T, LETERRIER J F, LINDEN M, KOMINAMI E, UCHIYAMA Y 2000 Selective localization of Bcl-2 to the inner mitochondrial and smooth endoplasmic reticulum membranes in mammalian cells. *Cell Death Differ* 7: 666-74
62. BRUNELLE J K, LETAI A 2009 Control of mitochondrial apoptosis by the Bcl-2 family. *J Cell Sci* 122(Pt 4): 437-41
63. PETROS A M, MEDEK A, NETTESHEIM D G, KIM D H, YOON H S, SWIFT K, MATAYOSHI E D, OLTERSDORF T, FESIK S W 2001 Solution structure of the antiapoptotic protein bcl-2. *Proc Natl Acad Sci U S A* 98: 3012-7
64. TSUJIMOTO Y, FINGER L R, YUNIS J, NOWELL P C, CROCE C M 1984 Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science* 226: 1097-9

65. HILL M E, MACLENNAN K A, CUNNINGHAM D C, VAUGHAN HUDSON B, BURKE M, CLARKE P, DI STEFANO F, ANDERSON L, VAUGHAN HUDSON G, MASON D, SELBY P, LINCH D C 1996 Prognostic significance of BCL-2 expression and bcl-2 major breakpoint region rearrangement in diffuse large cell non-Hodgkin's lymphoma: a British National Lymphoma Investigation Study. *Blood* 88: 1046-51
66. GASCOYNE R D, ADOMAT S A, KRAJEWSKI S, KRAJEWSKA M, HORSMAN D E, TOLCHERA W, O'REILLY S E, HOSKINS P, COLDMAN A J, REED J C, CONNORS J M 1997 Prognostic significance of Bcl-2 protein expression and Bcl-2 gene rearrangement in diffuse aggressive non-Hodgkin's lymphoma. *Blood* 90: 244-51
67. ROBAK E, JESIONEK-KUPNICKA D, ROBAK T, HOLUB A, WAWRZYŃIAK E, BARTKOWIAK J, BEDNAREK A, CONSTANTINU M, URBANSKA-RYS H 2007 Primary cutaneous marginal zone B-cell lymphoma in a patient with chronic lymphocytic leukaemia. *Br J Dermatol* 157: 591-5
68. PALMEDO G, HANTSCHKE M, RÜTTEN A, MENTZEL T, KEMPF W, TOMASINI D, KUTZNER H 2007 Primary cutaneous marginal zone B-cell lymphoma may exhibit both the t(14;18)(q32;q21) IGH/BCL2 and the t(14;18)(q32;q21) IGH/MALT1 translocation: an indicator for clonal transformation towards higher-grade B-cell lymphoma? *Am J Dermatopathol* 29: 231-6
69. ZHANG D, MCGUIRK J, GANGULY S, PERSONS D L 2009 Histiocytic/dendritic cell sarcoma arising from follicular lymphoma involving the bone: a case report and review of literature. *Int J Hematol* 89: 529-32
70. BASEGGIO L, GEAY M O, GAZZO S, BERGER F, TRAVERSE-GLEHEN A, FFRENCH M, HAYETTE S, CALLET-BAUCHU E, VERNEY A, MOREL D, JALLADES L, MAGAUD J P, SALLES G, FELMAN P 2012 In non-follicular lymphoproliferative disorders, IGH/BCL2-fusion is not restricted to chronic lymphocytic leukaemia. *Br J Haematol* 158: 489-98
71. LIU Y, HERNANDEZ A M, SHIBATA D, CORTOPASSI G A 1994 BCL2 translocation frequency rises with age in humans. *Proc Natl Acad Sci U S A* 91: 8910-4
72. SUMMERS K E, GOFF L K, WILSON A G, GUPTA R K, LISTER T A, FITZGIBBON J 2001 Frequency of the Bcl-2/IgH rearrangement in normal individuals: implications for the monitoring of disease in patients with follicular lymphoma. *J Clin Oncol* 19: 420-4
73. JÄGER U, BÖCSKÖR S, LE T, MITTERBAUER G, BOLZ I, CHOTT A, KNEBA M, MANNHALTER C, NADEL B 2000 Follicular lymphomas' BCL-2/IgH junctions contain templated nucleotide insertions: novel insights into the mechanism of t(14;18) translocation. *Blood* 95: 3520-9
74. BARON B W, NUCIFORA G, MCCABE N, ESPINOSA R 3RD, LE BEAU M M, MCKEITHAN T W 1993 Identification of the gene associated with the recurring chromosomal translocations t(3;14)(q27;q32) and t(3;22)(q27;q11) in B-cell lymphomas. *Proc Natl Acad Sci U S A* 90: 5262-6
75. DHORDAIN P, ALBAGLI O, ANSIEAU S, KOKEN M H, DEWEINDT C, QUIEF S, LANTOINE D, LEUTZ A, KERCKAERT J P, LEPRINCE D 1995 The BTB/POZ domain targets the LAZ3/BCL6 oncoprotein to nuclear dots and mediates homomerization in vivo. *Oncogene* 11: 2689-97
76. DEWEINDT C, ALBAGLI O, BERNARDIN F, DHORDAIN P, QUIEF S, LANTOINE D, KERCKAERT J P, LEPRINCE D 1995 The LAZ3/BCL6 oncogene encodes a sequence-specific transcriptional inhibitor: a novel function for the BTB/POZ domain as an autonomous repressing domain. *Cell Growth Differ* 6: 1495-503
77. CHANG C C, YE B H, CHAGANTI R S, DALLA-FAVERA R 1996 BCL-6, a POZ/zinc-finger protein, is a sequence-specific transcriptional repressor. *Proc Natl Acad Sci U S A* 93: 6947-52
78. PERO R, PALMIERI D, ANGRISANO T, VALENTINO T, FEDERICO A, FRANCO R, LEMBO F, KLEIN-SZANTO A J, DEL VECCHIO L, MONTANARO D, KELLER S, ARRA C, PAPA-DOPOULOU V, WAGNER S D, CROCE C M, FUSCO A, CHIARIOTTI L, FEDELE M 2012 POZ-, AT-hook-, and zinc finger-containing protein (PATZ) interacts with human oncogene B cell lymphoma 6 (BCL6) and is required for its negative autoregulation. *J Biol Chem* 287: 18308-17
79. CATTORETTI G, CHANG C C, CECHOVA K, ZHANG J, YE BH, FALINI B, LOUIE D C, OFFIT K, CHAGANTI R S, DALLA-FAVERA R 1995 BCL-6 protein is expressed in germinal-center B cells. *Blood* 86: 45-53
80. PHAN R T, DALLA-FAVERA R 2004 The BCL6 proto-oncogene suppresses p53 expression in germinal-center B cells. *Nature* 432: 635-9
81. BASSO K, DALLA-FAVERA R 2010 BCL6: master regulator of the germinal center reaction and key oncogene in B cell lymphomagenesis. *Adv Immunol* 105: 193-210
82. BASSO K, DALLA-FAVERA R 2012 Roles of BCL6 in normal and transformed germinal center B cells. *Immunol Rev* 247: 172-83
83. SHAFFER L, YU X, HE Y, BOLDRICK J, CHAN E P, STAUDT L M 2000 BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. *Immunity* 13: 199-212
84. OHNO H 2004 Pathogenetic role of BCL6 translocation in B-cell non-Hodgkin's lymphoma. *Histol Histopathol* 19: 637-50
85. PAREKH S, POLO J M, SHAKNOVICH R, JUSZCZYNSKI P, LEV P, RANUNCOLO S M, YIN Y, KLEIN U, CATTORETTI G, DALLA FAVERA R, SHIPP M A, MELNICK A 2007 BCL6 programs lymphoma cells for survival and differentiation through distinct biochemical mechanisms. *Blood* 110: 2067-74
86. BASSO K, SAITO M, SUMAZIN P, MARGOLIN A A, WANG K, LIM W K, KITAGAWA Y, SCHNEIDER C, ALVAREZ M J, CALIFANO A, DALLA-FAVERA R 2010 Integrated biochemical and computational approach identifies BCL6 direct target genes controlling multiple pathways in normal germinal center B cells. *Blood* 115: 975-84
87. SAITO M, NOVAK U, PIOVAN E, BASSO K, SUMAZIN P, SCHNEIDER C, CRESPO M, SHEN Q, BHAGAT G, CALIFANO A, CHADBURN A, PASQUALUCCI L, DALLA-FAVERA R 2009 BCL6 suppression of BCL2 via Miz1 and its disruption in diffuse large B cell lymphoma. *Proc Natl Acad Sci U S A* 106: 11294-9
88. YE B H, LISTA F, LO COCO F, KNOWLES D M, OFFIT K, CHAGANTI R S, DALLA-FAVERA R 1993 Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large-cell lymphoma. *Science* 262: 747-50
89. KERCKAERT J P, DEWEINDT C, TILLY H, QUIEF S, LE-COCQ G, BASTARD C 1993 LAZ3, a novel zinc-finger encoding gene, is disrupted by recurring chromosome 3q27 translocations in human lymphomas. *Nat Genet* 5: 66-70
90. MIGLIAZZA A, MARTINOTTI S, CHEN W, FUSCO C, YE B H, KNOWLES D M, OFFIT K, CHAGANTI R S, DALLA-FAVERA R 1995 Frequent somatic hypermutation of the 5' non-coding region of the BCL6 gene in B-cell lymphoma. *Proc Natl Acad Sci U S A* 92: 12520-4
91. UEDA C, AKASAKA T, OHNO H 2002 Non-immunoglobulin/BCL6 gene fusion in diffuse large B-cell lymphoma: prognostic implications. *Leuk Lymphoma* 43: 1375-81
92. KATO J, MATSUSHIME H, HIEBERT S W, EWEN M E, SHERR C J 1993 Direct binding of cyclin D to the retinoblastoma gene product (pRb) and pRb phosphorylation by the cyclin D-dependent kinase CDK4. *Genes Dev* 7: 331-42
93. WITHERS D A, HARVEY R C, FAUST J B, MELNYK O, CAREY K, MEEKER T C 1991 Characterization of a candidate bcl-1 gene. *Mol Cell Biol* 11: 4846-53
94. CALLANAN M, LEROUX D, MAGAUD J P, RIMOKH R 1996 Implication of cyclin D1 in malignant lymphoma. *Crit Rev Oncog* 7: 191-203
95. LEROUX D, LE MARC'HADOUR F, GRESSIN R, JACOB M C, KEDDARI E, MONTEIL M, CAILLOT P, JALBERT P, SOTTO J J 1991 Non-Hodgkin's lymphomas with t(11;14)(q13;q32): a subset of mantle zone/intermediate lymphocytic lymphoma? *Br J Haematol* 77: 346-53
96. SETO M, YAMAMOTO K, IIDA S, AKAO Y, UTSUMI K R, KUBONISHI I, MIYOSHI I, OHTSUKI T, YAWATA Y, NAMBA M et al. 1992 Gene rearrangement and overexpression of PRAD1 in lymphoid malignancy with t(11;14)(q13;q32) translocation. *Oncogene* 7(7): 1401-6
97. RAFFELD M, SANDER C A, YANO T, JAFFE E S 1992 Mantle cell lymphoma: an update. *Leuk Lymphoma* 8: 161-6
98. AU W Y, GASCOYNE R D, VISWANATHA D S, CONNORS J M, KLASAR J, HORSMAN D E 2002 Cytogenetic analysis in mantle cell lymphoma: a review of 214 cases. *Leuk Lymphoma* 43: 783-91
99. JANSSEN J W, VAANDRAGER J W, HEUSER T, JAUCH A, KLUIN P M, GEELLEN E, BERGSAGEL P L, KUEHL W M, DREXLER H G, OTSUKI T, BARTRAM C R, SCHUURING E 2000 Concurrent activation of a novel putative transforming gene,

- mycov, and cyclin D1 in a subset of multiple myeloma cell lines with t(11;14)(q13;q32). *Blood* 95: 2691-8
100. TSAI A G, LU H, RAGHAVAN S C, MUSCHEN M, HSIEH C L, LIEBER M R 2008 Human chromosomal translocations at CpG sites and a theoretical basis for their lineage and stage specificity. *Cell* 135: 1130-42
  101. LECLUSE Y, LEBAILLY P, ROULLAND S, GAC A C, NADEL B, GAUDUCHON P 2009 t(11;14)-positive clones can persist over a long period of time in the peripheral blood of healthy individuals. *Leukemia* 23: 1190-3
  102. DUROT E, PATEY M, LUQUET I, GAILLARD B, KOLB B, DELMER A 2012 An aggressive B-cell lymphoma with rearrangements of MYC and CCND1 genes: a rare subtype of double-hit lymphoma. *Leuk Lymphoma*: doi:10.3109/10428194.2012.710329.
  103. CARTER R, DUBÉ I, MCKEITHAN T, CARSTAIRS K, DEHARVEN E, BAILEY D, SCOTT J G 1991 Translocation (14;19) in acute biphenotypic leukemia. *Cancer Genet Cytogenet* 53: 67-73
  104. OHNO H, DOI S, YABUMOTO K, FUKUHARA S, MCKEITHAN T W 1993 Molecular characterization of the t(14;19)(q32;q13) translocation in chronic lymphocytic leukemia. *Leukemia* 7: 2057-63
  105. MCKEITHAN T W, TAKIMOTO G S, OHNO H, BJORLING V S, MORGAN R, HECHT B K, DUBÉ I, SANDBERG AA, ROWLEY J D 1997 BCL3 rearrangements and t(14;19) in chronic lymphocytic leukemia and other B-cell malignancies: a molecular and cytogenetic study. *Genes Chromosomes Cancer* 20: 64-72
  106. BHATIA K, HUPPI K, MCKEITHAN T, SIWARSKI D, MUSHINSKI J F, MAGRATH I 1991 Mouse bcl-3: cDNA structure, mapping and stage-dependent expression in B lymphocytes. *Oncogene* 6: 1569-73
  107. WULCZYN FG, NAUMANN M, SCHEIDEREIT C 1992 Candidate proto-oncogene bcl-3 encodes a subunit-specific inhibitor of transcription factor NF-kappa B. *Nature* 358: 597-9.
  108. FRANZOSO G, BOURS V, PARK S, TOMITA-YAMAGUCHI M, KELLY K, SIEBENLIST U 1992 The candidate oncoprotein Bcl-3 is an antagonist of p50/NF-kappa B-mediated inhibition. *Nature* 359: 339-42
  109. VIATOUR P, DEJARDIN E, WARNIER M, LAIR F, CLAUDIO E, BUREAU F, MARINE J C, MERVILLE M P, MAURER U, GREEN D, PIETTE J, SIEBENLIST U, BOURS V, CHARIOT A 2004 GSK3-mediated BCL-3 phosphorylation modulates its degradation and its oncogenicity. *Mol Cell* 16: 35-45.
  110. KASHATUS D, COGSWELL P, BALDWIN A S 2006 Expression of the Bcl-3 proto-oncogene suppresses p53 activation. *Genes Dev* 20: 225-35
  111. MARTÍN-SUBERO J I, ODERO M D, HERNANDEZ R, CIGUDOSA J C, AGIRRE X, SAEZ B, SANZ-GARCÍA E, ARDANAZ M T, NOVO F J, GASCOYNE R D, CALASANZ M J, SIEBERT R 2005 Amplification of IGH/MYC fusion in clinically aggressive IGH/BCL2-positive germinal center B-cell lymphomas. *Genes Chromosomes Cancer* 43: 414-23
  112. TOMITA N, NAKAMURA N, KANAMORI H, FUJIMAKI K, FUJISAWA S, ISHIGATSUBO Y, NOMURA K 2005 Atypical Burkitt lymphoma arising from follicular lymphoma: demonstration by polymerase chain reaction following laser capture microdissection and by fluorescence in situ hybridization on paraffin-embedded tissue sections. *Am J Surg Pathol* 29: 121-4
  113. LENNERT K 1975 Morphology and classification of malignant lymphomas and so-called reticulosos. *Acta Neuropathol Suppl* 6: 1-16
  114. ISAACSON PG 1995 The revised European- American lymphoma (REAL) classification. *Clin Oncol (R Coll Radiol)* 7: 347- 8.
  115. SIKORA K 1982 Monoclonal antibodies in oncology. *J Clin Pathol* 35: 369-75.
  116. MARWICK C 1997 Monoclonal antibody to treat lymphoma. *JAMA* 278: 616-18
  117. SAUSVILLE E A 1999 A Bcr/Abl kinase antagonist for chronic myelogenous leukemia: a promising path for progress emerges. *J Natl Cancer Inst* 91: 102-3.
  118. GREENWOOD J, GORMAN S D, ROUTLEDGE E G, LLOYD I S, WALDMANN H 1994 Engineering multiple-domain forms of the therapeutic antibody CAMPATH-1H: effects on complement lysis. *Ther Immunol* 1: 247-55.
  119. WU D, WOOD B L, DORER R, FROMM J R 2010 „Double-Hit“ mature B-cell lymphomas show a common immunophenotype by flow cytometry that includes decreased CD20 expression. *Am J Clin Pathol* 134: 258-65
  120. OUANSAFI I, HE B, FRASER C, NIE K, MATHEW S, BHANJI R, HODA R, ARABADJIEF M, KNOWLES D, CERUTTI A, ORAZI A, TAM W 2010 Transformation of follicular lymphoma to plasmablastic lymphoma with c-myc gene rearrangement. *Am J Clin Pathol* 134: 972-81
  121. SOUCEK L, WHITFIELD J, MARTINS C P, FINCH A J, MURPHY D J, SODIR N M, KARNEZIS A N, SWIGART L B, NASI S, EVAN GI 2008 Modelling Myc inhibition as a cancer therapy. *Nature* 455: 679-83

