Role of MYC in pediatric and adult B-cell lymphoma patients
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Double hit B-cell lymphomas

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ABSTRACT

In many B-cell lymphomas, chromosomal translocations are biologic and diagnostic hallmarks of disease. An intriguing subset is formed by the so-called double-hit (DH) lymphomas that are defined by a chromosomal breakpoint affecting the MYC/8q24 locus in combination with another recurrent breakpoint, mainly the t(14;18)(q32;q21) involving BCL2. Recently, these lymphomas have received increased attention, which contributed to the introduction of a novel category of lymphomas in the 2008 WHO classification, “B cell lymphoma unclassifiable with features intermediate between DLBCL and BL.” In this review we explore the existing literature for the most recurrent types of DH B-cell lymphomas and the involved genes with their functions, as well as their pathology and clinical aspects including therapy and prognosis. The incidence of aggressive B-cell lymphomas other than Burkitt lymphoma with a MYC breakpoint and in particular a double hit is difficult to assess, because screening by methods like FISH has not been applied on large, unselected series, and the published cytogenetic data may be biased to specific categories of lymphomas. DH lymphomas have been classified heterogeneously but mostly as DLBCL, the majority having a germinal center phenotype and expression of BCL2. Patients with DH lymphomas often present with poor prognostic parameters, including elevated LDH, bone marrow and CNS involvement, and a high IPI score. All studies on larger series of patients suggest a poor prognosis, also if treated with RCHOP or high-intensity treatment modalities. Importantly, this poor outcome cannot be accounted for by the mere presence of a MYC/8q24 breakpoint. Likely, the combination of MYC and BCL2 expression and/or a related high genomic complexity are more important. Compared to these DH lymphomas, BCL6(+)/MYC(+) DH lymphomas are far less common, and in fact most of these cases represent BCL2(+)/BCL6(+)/MYC(+) triple-hit lymphomas with involvement of BCL2 as well. CCND1(+)/MYC(+) DH lymphomas with involvement of 11q13 may also be relatively frequent, the great majority being classified as aggressive variants of mantle cell lymphoma. This suggests that activation of MYC might be an important progression pathway in mantle cell lymphoma as well. Based on clinical significance and the fact that no other solid diagnostic tools are available to identify DH lymphomas, it seems advisable to test all diffuse large B-cell and related lymphomas for MYC and other breakpoints.

INTRODUCTION

Approximately 40% of all B cell lymphomas are characterized by the presence of a recurrent reciprocal chromosomal translocations.\(^1\)\(^2\)\(^3\)\(^4\)\(^5\) In most cases an oncogene is deregulated by juxtaposition to an enhancer of the immunoglobulin (IG) loci whereas promoter substitution or fusion of genes leading to fusion proteins are less frequent. Certain translocations are characteristic for a specific type of lymphoma and are often considered as cancer initiating events. For instance, irrespective of being endemic, sporadic or AIDS associated, the t(8;14)(q24;q32) or variant translocations involving the immunoglobulin light chain loci, are considered as the lymphoma initiating event in Burkitt lymphoma (BL), constitutively activating the MYC gene. However, similar MYC breakpoints may also occur as secondary events during disease progression in other lymphomas. These secondary events can occur metachronously after a clinically evident phase of indolent lymphoma or synchronously at the moment of clinical presentation.

Lymphomas with recurrent chromosomal breakpoints activating multiple oncogenes one of which being MYC, are often referred to as “Dual – Hit” or “Double – Hit” (DH) lymphomas. Rigorously, DH lymphoma is a rather imprecise term as from the nomenclature point of view it is neither restricted to B-cell lymphomas (e.g. inv(14)-positive T-cell prolymphocytic leukemia may carry a MYC translocation) nor does it exclude two translocations activating oncogenes other than MYC (e.g. a follicular lymphoma with simultaneous BCL2 and BCL6 translocation). Nevertheless, the term DH lymphoma is mostly used for mature-B-cell lymphomas with a chromosomal breakpoint affecting the MYC locus. For clarity, we will through the text apply a nomenclature including the affected oncogenes, e.g. BCL2+/MYC+ and use the term DH lymphoma for all cases with multiple recurrent breakpoints (triple / quadruple) as well.

Since cases with a MYC/8q24 and BCL2/18q21 breakpoint (BCL2+/MYC+ DH lymphoma) are most common, these cases received most attention in the literature. In particular a subset of aggressive lymphomas in elderly patients, previously often diagnosed as Burkitt-like lymphoma, aggressive B cell lymphoma NOS or diffuse large B cell lymphomas (DLBCL) appear to represent such DH lymphomas. In the recently updated WHO classification for malignant lymphomas it is proposed to classify most if not all cases as “B cell lymphoma unclassifiable with features intermediate between DLBCL and BL”.\(^6\) This novel category is meant...
to create a (temporary) container for aggressive mature B cell lymphomas that should not be diagnosed as either BL or DLBCL.

Double hit lymphomas make up an important part of this novel WHO category, the other part representing heterogeneous cases of aggressive B cell lymphoma that have features of BL such as a monomorphic proliferation of blasts and a very high proliferation rate, often in combination with a germinal center phenotype (CD10+, BCL6+, MUM1/IRF4-). The latter lymphomas contain a MYC breakpoint in 30-50%, an incidence that is higher than seen in regular DLBCL (approximately 10%) but considerably lower than in regular BL (90-100%). In this review we will focus on DH lymphomas as follows:

1. First we explore the published cytogenetic data for the presence of known and novel recurrent chromosomal abnormalities in DH lymphomas
2. The biologic function of the involved oncogenes in these lymphomas will be briefly discussed
3. We will discuss the timing of the occurrence of the breakpoints in lymphomagenesis as well as the synergistic action of the involved oncogenes
4. The pathological and clinical aspects of BCL2+/BCL6+/MYC+ double hit and triple hit lymphomas will be reviewed
5. A short section will be devoted to other DH B-cell lymphomas
6. The relationship with “B cell lymphoma unclassifiable with features intermediate between DLBCL and BL” will be discussed
7. Finally we will draw conclusions and formulate recommendations.

PUBLISHED DH LYMPHOMAS

To get an impression on the incidence of MYC breakpoint positive and DH lymphomas diagnosed as DLBCL, we analyzed the available fluorescence in situ hybridization (FISH) studies on series of unselected DLBCL for the presence of the most common chromosomal breakpoints. Table 1 shows the results from eight larger studies, three being incomplete with respect to BCL2 and BCL6. These studies show a wide range of MYC breakpoints in 3-16% of the cases and DH lymphomas in 0-12%. The wide range of BCL2 breakpoints and therefore also DH cases may be partially due to the inclusion of one series from Asia where the incidence of t(14;18) carrying lymphomas may be lower than in Western countries.

FISH analysis only informs on the targets for which probes are used. To get a better idea about the nature of all DH lymphomas and to search for other types than BCL2+MYC+ and BCL6+MYC+ lymphomas, we explored the ‘Mitelman Database of Chromosome Aberrations in Cancer’, edition February 2010 (see Table S1 & S2). This large publicly available database contains virtually all published cytogenetic data on a wide variety of malignancies including B cell lymphomas. We selected reports with a MYC breakpoint published after the REAL classification to be confident about the classification of the cases. We realize that this has introduced a certain bias towards DH lymphomas, since several of these publications specifically addressed this phenomenon. We included only mature B cell malignancies (see “supplementary data” for strategies and sources). Plasma cell neoplasms were excluded as they represent a different disease and are characterized by genetic aberrations different from those seen in aggressive B-cell lymphomas. Translocations in myelomas involve both primary translocations (CCND1, CCND3, FGFR3 & MMSET, c-MAF and MAFB) and secondary translocations involving MYC. Double-Hits involving these genes and also combinations thereof were frequently seen in the Mitelman database (5-25%, data not shown). Of note, a selection bias may have occurred as myelomas with MYC translocations probably have a higher success rate for culturing and karyotyping. Plasmablastic lymphomas were also excluded. A recent report identified MYC rearrangements in 49% of these lymphomas but no concomitant rearrangement of BCL2, BCL6 or PAX5.

From the 689 MYC+ breakpoint positive lymphomas 326 were DH lymphomas (47%). From the 804 cases diagnosed as DLBCL, 139 cases had a MYC breakpoint (17%). This incidence was similar to that reported for the period 1980 – 1995
(16%; data not shown). However, whereas between 1995 and 2009, 109 out of 804 DLBCL were BCL2+/MYC+ or BCL6+/MYC+ DH cases (14%), only 12/445 similar DH DLBCL were reported in the period before 1995 (3%; data not shown). As stated above, this increase in the fraction of DH lymphomas after 1994 may be due to a growing interest in these lymphomas, as well as by changes in classification.

Taking these limitations into consideration, BCL2+/MYC+ DH lymphomas formed the great majority of DH lymphomas (62%; Table 2). BCL6+/MYC+ DH lymphomas were relatively rare (8% of all cases) and in fact triple hit lymphomas involving MYC, BCL2 and BCL6 (16%) were more frequent than BCL6+/MYC+ DH cases. In DLBCL, 25/139 cases (18%) had a BCL6 breakpoint, while 84/139 (60%) had a BCL2 breakpoint. This preference for BCL2+/MYC+ is at least partially due to an underrepresentation of BCL6 breakpoints in the database, because these breakpoints at the very tip of chromosome 3 may have been missed by conventional cytogenetics. However, a very strong preference for BCL2 involvement in DH lymphomas was also found by FISH (Table 1) and suggests a selective complementary role for MYC and BCL2.

CCND1+/MYC+ DH lymphomas (N=34) formed 10% of all cases. In fact, 5% of all mantle cell lymphomas in the database were CCND1+/MYC+ DH cases. Other recurrent DH lymphomas were five BCL3+/MYC+ cases with t(14;19)(q32;q13), four cases with t(9;14)(p13;q32) involving a yet unidentified locus on 9p13 and one case with all three loci involved. A MYC breakpoint without any other recurrent breakpoint (MYC+SH) was seen in 363/689 cases (53%), from which 205 cases (56%) were diagnosed as BL (Table S1).

Whereas in typical Burkitt Lymphoma MYC is almost always juxtaposed to an IG locus (in our dataset 98%), this was only found in 66% of BCL2+/MYC+ DH lymphomas (Table 2). Moreover, in 49% of the cases with juxtaposition to an IG locus one of the IG light chain genes was involved, which is far more that the 18% in BL. We explored the direct partners of MYC in the other DH lymphomas. Interestingly, in the group of BCL2+/MYC+ DH lymphomas the commonest non-Ig partner was 9p13 (N=19; 7%). This translocation was never seen in the BCL6+/MYC+ DH and CCND1+/MYC+ DH groups. Other recurrent translocations in the BCL2+/MYC+ group were t(1;8)(p36;q24) in five cases and t(2;8)(p11;q24) in five cases, the latter likely representing MYC-IGK fusion. Both in the BCL6+/MYC+ DH and BCL2+/BCL6+/MYC+ TH groups BCL6 itself was a MYC partner in four and seven cases, respectively.

### Table 1 | Incidence of chromosomal breakpoints in unselected series of diffuse large B cell lymphoma

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>MYC+ total (%)</th>
<th>MYC+ SH (%)</th>
<th>BCL2+/MYC+ DH (%)</th>
<th>BCL6+/MYC+ DH (%)</th>
<th>BCL2+/BCL6+/MYC+ TH (%)</th>
<th>ALL DH &amp; TH (%)</th>
<th>BCL2+/MYC+ cases (SH) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barragan</td>
<td>245–266</td>
<td>35 (14)</td>
<td>3 (1)</td>
<td>29 (12)</td>
<td>55 (22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oertemuller</td>
<td>2009</td>
<td>220</td>
<td>9 (4)</td>
<td>1 (0)</td>
<td>2 (1)</td>
<td>29 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yoon</td>
<td>2008</td>
<td>137–156</td>
<td>14 (7)</td>
<td>1 (1)</td>
<td>3 (3)</td>
<td>30 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibiletti</td>
<td>2009</td>
<td>74</td>
<td>12 (16)</td>
<td>4 (7)</td>
<td>1 (1)</td>
<td>14 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copie-Bergman</td>
<td>2009</td>
<td>68–71</td>
<td>2 (3)</td>
<td>0</td>
<td>0</td>
<td>14 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Savage</td>
<td>2009</td>
<td>135</td>
<td>12 (9)</td>
<td>3 (2)</td>
<td>na</td>
<td>30 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klapper</td>
<td>2008</td>
<td>117</td>
<td>14 (14)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copie-Bergman</td>
<td>2008</td>
<td>117</td>
<td>14 (14)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td></td>
<td></td>
</tr>
<tr>
<td>van Imhoff</td>
<td>2006</td>
<td>58–59</td>
<td>9 (15)</td>
<td>3 (5)</td>
<td>4 (7)</td>
<td>49 (44)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Savage</td>
<td>2006</td>
<td>135</td>
<td>12 (9)</td>
<td>3 (2)</td>
<td>na</td>
<td>30 (20)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klapper</td>
<td>2008</td>
<td>117</td>
<td>14 (14)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All cases had DLBCL as morphology. SH indicates single-hit DH; double-hit; TH, triple hit; "+" number of cases on which FISH was performed. DH and TH are defined according to the criteria described by Campo et al. [3] and Sáez et al. [4], respectively. DH groups were selected for primary nodal localization, TH for extranodal localization. Cases from clinical trials selected for poor-risk DLBCL patients. The FISH panel used in this study included BCL2 and BCL6.
## ONCOGENES INVOLVED IN DOUBLE HIT LYMPHOMAS

In this section we will briefly discuss the function of the known oncogenes involved in DH lymphomas.

### MYC

MYC is a transcription factor controlling the expression of a large set of target genes involved in cell cycle regulation, metabolism, DNA repair, stress response and protein synthesis.\(^2^1\) MYC exerts its function by dimerization with MAX and subsequent binding to specific consensus DNA sequences (CACGTG) called an “E-Box”.\(^2^2,2^3,2^4\) Many genes are directly (de)regulated by MYC, including LDH-A and hTERT.\(^2^5\) In addition MYC is involved in the regulation of miRNA expression,\(^2^6,2^7,2^8\) (de)regulating many target genes in an indirect way as well. Interestingly, MYC represses many miRNAs, which corroborates the idea that MYC generally is an activator of other genes. MYC expression in the germinal centers is, surprisingly, lower compared to naïve and memory B-cells.\(^2^9\) This low expression could protect against MYC induced genomic instability in the germinal center. For a detailed review about the role of MYC in lymphomagenesis see Klapproth and Wirth.\(^3^0\) Genomic alterations of the MYC gene include chromosomal translocations, mutations affecting regulatory sequences and promoter regions, as well as copy number increase. In contrast to early reports, most chromosomal breakpoints involving MYC and the IGH locus are mediated by activation induced cytidine deaminase (AICDA) and not by recombinase activating gene 1/2 (RAG1/2).\(^3^1,3^2,3^3,3^4\) Thus, these breakpoints should have their origin from erroneous somatic hypermutation or class switch recombination.\(^3^5\) Based on transgenic mouse models, additional factors are needed for malignant transformation.\(^3^6,3^7\) This is supported by the finding that MYC-IGH translocation can be detected in non neoplastic conditions, both in men and mice.\(^3^8,3^9\) The mechanism responsible for translocations affecting MYC and non-IGH loci, for instance 9p13 in the t(8;9) (q24;p13) translocation, is unknown.\(^1^5,4^0\)

### BCL2

BCL2 was first described in the early eighties by its involvement in the t(14;18) in follicular lymphoma.\(^4^1\) As a member of a large BCL2 family of proteins it has potent anti apoptotic functions. BCL2 is widely expressed in immature B-cells and memory B cells but is temporarily down regulated in germinal center B cells, partially due to repression by BCL6.\(^2^9,4^2,4^3,4^4\) With the occurrence of t(14;18), transcription of BCL2 is “constitutively” deregulated with high transcription activity from the

### Table 2 | Double and triple hit lymphomas in the Mitelman database

<table>
<thead>
<tr>
<th>DH lymphomas</th>
<th>N</th>
<th>% of all 326 DH cases</th>
<th>MYC-IG fusion (%)</th>
<th>MYC-IGK or IGL fusion (% of cases with MYC-IG fusion) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL2⁺/MYC⁺</td>
<td>203</td>
<td>62</td>
<td>66%</td>
<td>49%</td>
</tr>
<tr>
<td>BCL6⁺/MYC⁺</td>
<td>26</td>
<td>8</td>
<td>31%</td>
<td>13%</td>
</tr>
<tr>
<td>BCL2⁺/BCL6⁺/MYC⁺</td>
<td>53</td>
<td>16</td>
<td>53%</td>
<td>50%</td>
</tr>
<tr>
<td>CCND1⁺/MYC⁺</td>
<td>34</td>
<td>10</td>
<td>20%</td>
<td>43%</td>
</tr>
<tr>
<td>BCL3⁺/MYC⁺</td>
<td>5</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9p13⁺/MYC⁺</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BCL3⁺/9p13⁺/MYC⁺ TH</td>
<td>1</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TOTAL DH &amp; TH cases</td>
<td>326</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**MYC** indicates 8q24 breakpoint; BCL2⁺, 18q21; BCL6⁺, 3q27; CCND1⁺, 11q13; BCL3⁺, 19q13; 9p13⁺, yet unidentified locus; *one case had a complex t(8;14;22)(q24;q32;q11), another case both a t(8;14)(q24;q32) and t(8;22)(q24;q11). Arbitrarily, both cases were considered as having two MYC-IG events.

**MYC** only
- Burkitt lymphoma: 205 - 98% 18%
- Other lymphomas: 158 - 61% 34%
- TOTAL: 689

**MYC** and **BCL2**
- MYC⁺/BCL2⁺: 8q24 break point; 18q21 break point; 3q27 break point; 11q13 break point; 19q13 break point; 9q13 break point; yet unidentified locus; *one case had a complex t(8;14;22)(q24;q32;q11), another case both a t(8;14)(q24;q32) and t(8;22)(q24;q11). Arbitrarily, both cases were considered as having two MYC-IG events.

**BCL6**
- BCL6⁺: 18q21 break point; 3q27 break point; 11q13 break point; 19q13 break point; 9q13 break point; yet unidentified locus; *one case had a complex t(8;14;22)(q24;q32;q11), another case both a t(8;14)(q24;q32) and t(8;22)(q24;q11). Arbitrarily, both cases were considered as having two MYC-IG events.
translocated BCL2 allele.\textsuperscript{42,45} This leads to a survival advantage of the involved B cells. Recent research has shown that BCL2 overexpression in B cells also leads to impaired DNA repair by blocking non homologous end-joining activities of Ku proteins essential for repair of both RAG1/2 and AICDA mediated breakpoints.\textsuperscript{46}

With the exception of rare cases,\textsuperscript{47} t(14;18) translocations are thought to occur early in B-cell development.\textsuperscript{48,49} Chromosomal breaks are mediated by RAG1/2, likely in combination with low levels of AICDA.\textsuperscript{50} Extensive mapping studies of the breakpoints as well as sequence analysis have shown that the BCL2 breakpoints are strongly clustered at CpG islands. One hypothesis is that these CpG island are first deaminated by low levels of AICDA and that the resulting T:G mismatches are subsequently targeted by RAG1/2.\textsuperscript{50} An in depth discussion about the occurrence of the t(14;18) at later stages of B-cell development (including even the germinal center) and (secondary) involvement of RAG1/2 and/or other mechanisms herein,\textsuperscript{51,52,53,54} goes beyond the scope of this review.

Apparently, the t(14;18) is insufficient to cause follicular lymphoma since IGH – BCL2 transgenic mice do not develop lymphomas and t(14;18) carrying mature B cells can also be found in healthy individuals and probably arise by the same mechanisms.\textsuperscript{51,55,56,57,58} Hence the translocation may rather facilitate than directly cause malignant transformation. The secondary genetic changes responsible for development into follicular lymphoma are not known. Importantly, these t(14;18) carrying B cells in normal individuals, called “follicular lymphoma-like B cells” usually enter the germinal centers, undergo somatic hypermutations and a limited degree of immunoglobulin gene class switching, and circulate as memory B cells.\textsuperscript{60,61} This passage across the germinal center cell reaction with exposure to high levels of AICDA may make these cells susceptible for additional genomic alterations such as a MYC or BCL6 mutations and breakpoints. Thus BCL2\textsuperscript{+}/MYC\textsuperscript{+} DH lymphomas may arise in two ways: either they arise from a clinically overt or subclincial follicular lymphoma or they directly arise from the much more prevalent B cells with a t(14;18) that otherwise had not attained any malignant potential (Figure 1). Under both circumstances MYC translocation may function as a progression event,\textsuperscript{48,62,63} although some follicular lymphomas with MYC breakpoints but without evidence of morphologic transformation have been described (see section below).

**BCL6**

BCL6 is a zinc finger transcription factor with an N-terminal POZ domain. The gene is localized at 3q27, a position at the very end of the chromosome. BCL6 is widely expressed in many tissues, but in B cells mostly restricted to germinal center B-cells.\textsuperscript{64,65} BCL6 is required for the formation of germinal centers as mice deficient for BCL6 lack these structures.\textsuperscript{66,67} Within the germinal center BCL6 acts as an transcriptional repressor of many target genes involved in apoptosis, DNA-damage response, cell cycle control, proliferation and differentiation.\textsuperscript{68,69,70} Important direct targets are BCL2, TP53, IRF4 and BLIMP1, the latter being essential for maturation into plasma cells.\textsuperscript{71,72} Interestingly, BLIMP1 is a repressor of
both BCL6 and MYC in plasma cells. As a result of the BCL6-mediated repression of TP53,73 somatic hypermutation and class switch recombination are facilitated. Interestingly, the AICDA mediated somatic hypermutation machinery can target many non-IG genes including BCL6 itself. By facilitating activating mutations or chromosomal translocations, BCL6 activation may therefore indirectly lead to its own mutation and constitutive activation.74,75 Deregulated expression of BCL6 in a mouse-model mimicking BCL6 translocations resulted in lymphoproliferative disease and ultimately in a disorder resembling DLBCL.76

Only half of the translocations involving BCL6 affect an IG locus, in other cases the translocation partner is very diverse. Translocations involving BCL6 can be found in approximately 30-40% of all DLBCL, some follicular lymphomas and even some marginal zone B cell lymphomas.

CCND1
The gene encoding cyclin D1 (CCND1) is located on 11q13 and is involved in cell cycle progression from the G1 to the S phase, by forming a complex with CDK4, and activating the RB1-E2F1 complex, allowing E2F1 to be released. Whereas CCND2 and CCND3 are expressed in normal B cells, CCND1 is not. In consequence, CCND1 is almost exclusively expressed in neoplastic B cells with genetic alterations of 11q13, i.e. translocation or copy number increase. In the vast majority of mantle cell lymphomas and in a substantial fraction of multiple myelomas 11q13 translocations are observed.77,78,79,80 In addition to these malignancies also hairy cell leukemia and some cells in the proliferation centers of CLL as well as extremely rare DLBCL cases may express CCND1, the mechanism being unknown.81 Like the t(14;18), the t(11;14) in MCL is mediated by RAG1/2, likely in combination with low levels of AICDA as well as other mechanisms.50,82 Similar to the t(14;18), also CCND1 breakpoints are strongly centered at CpG islands indicating a concerted action of AICDA and RAG1/2 in precursor B cells.50 As seen for the t(14;18), also occasional t(11;14) cells can be found in the blood of healthy individuals, however at much lower frequencies than t(14;18)+ cells.83 This low frequency may be due to the fact that these cells are not expanded in the germinal center cell reaction. This fits with the finding that MCL represents a pre-germinal center B cell lymphoma. Of note, the t(11;14) in myeloma has a different configuration of the breakpoint with strong indications of an AICDA mediated breakpoint initiated in germinal center B cells.84

BCL3
BCL3 is a distinct member of the IκB protein family and resides on 19q13. Its expression is dependent on the stage of B-cell differentiation with higher expression in mature than immature B-cells.50 Experiments in BCL3 deficient mice have shown that the gene is involved in germinal center formation.86 The (14;19) (q32;q13) leads to increased BCL3 transcription and has been described in a large variety of lymphomas and leukemias,87 including atypical CLL.88,89 Analysis of t(14;19) breakpoints indicates that this translocation is mediated by illegitimate class switch recombination.90,91,92 Eμ-BCL3 transgenic mice overexpressing BCL3 show lymphoid hyperplasia but do not develop lymphomas.90
TIMING AND SYNERGY OF TRANSLOCATIONS IN DH LYMPHOMA

DH mature B-cell lymphomas are by definition characterized by a MYC breakpoint in combination with another recurrent chromosomal breakpoint. Most MYC breakpoints are likely mediated by AICDA in mature B cells. In contrast, and with the exception of CCND1 in myeloma, BCL2 and CCND1 breakpoints are most likely mediated by RAG1/2 in precursor B cells. This strongly suggests that the MYC/8q24 breakpoint is a secondary event in the cases with a BCL2/18q21 or CCND1/11q13 breakpoint (Figure 1). This sequence of events is also supported by the fact that approximately 5% of all follicular lymphoma with a BCL2/18q21 breakpoint will acquire a MYC/8q24 breakpoint during the course of the disease, and that at the cytogenetic level incidental cases show two or more subclones, one with only a t(14;18) and the other with both translocations.46 Another argument for the secondary nature may be that - in comparison to the “primary” MYC breakpoints in BL where 82% affect the IGH locus at 14q32 (Table 2), many more of these “secondary” breakpoints affect the light chain loci. Probably, tumor cells still require a functional heavy chain protein for signaling and cell survival, as cells with disruption of both IGH alleles are deleted.94

For BCL6/MYC and BCL3/MYC DH lymphomas the timing of events is less clear since most breakpoints affecting MYC, BCL6 and BCL3 are likely mediated by the same mechanism.

As shown in Table 2 there are interesting differences between BCL2/MYC DH and CCND1/MYC DH lymphomas with respect to the partner of the MYC gene. These differences might shed light on the mechanisms causing the MYC breakpoint. In the majority of the BCL2/MYC DH lymphomas (66%) the MYC partner is an IG locus, which might reflect a high activity of AICDA that can induce mutations and breakpoints in both the IG and MYC loci. This may be due to the fact that the t(14;18) translocation forces tumor cells to accumulate as germinal center B cells in which high AICDA levels are present. Exposure to high levels of AICDA may then lead to a MYC-IG breakpoint. In contrast, in CCND1/MYC DH lymphomas MYC is in only 20% of the cases joined to an IG locus (see also Table 2 and Table S2). Indeed, the t(11;14) translocation does not result in accumulation of germinal center B cells since the tumor cells are already blocked in an earlier stage of development. Likely, the occasional MYC breakpoints without an IG partner in these cases are not caused by AICDA or by AICDA expression in extrafollicular B cells.

What could be the biological synergy of acquiring two or three breakpoints, and thus activating both oncogenes? This is again most evident for BCL2 and MYC where BCL2 is anti-apoptotic without mediating proliferative signals. Instead, MYC drives the cells in an active proliferative and metabolic state, for instance by allowing anaerobic glycolysis in an anaerobic state by upregulating LDH-A.25 Moreover, in normal cells MYC induces DNA stress and activates the TP53 checkpoint leading to apoptosis; in consequence tumor cells with constitutive MYC activation frequently have acquired inactivating TP53 mutations or other mechanisms to protect them from apoptosis. In that view a pre-existent BCL2 activation may also protect the cells from apoptosis. This synergy may be further enhanced by the fact that BCL2 can also repress important proteins involved in repair of non-homologous end joining mediated DNA double strand breaks.46 When cells carrying a t(14;18)(q32;q21) enter the germinal center this might facilitate an increased accumulation of chromosomal abnormalities, including MYC translocations, as a result of the processes of somatic hypermutation and class switch recombination. In that respect it would be interesting to study whether a combination of MYC and BCL2 translocation favors a molecular signature of genomic instability and therefore could explain the high genomic complexity that is so frequent in this type of lymphoma.96

As discussed, BCL6 is a strong repressor of many genes, including BCL2 and MYC.44,68,70 Therefore, both constitutive activation of MYC and BCL2 by chromosomal translocation might be of advantage for BCL6 positive tumor cells. In reverse, since DNA damage induced by MYC can repress BCL6 expression, constitutive activation of BCL6 by a translocation might be of selective advantage for MYC overexpressing tumor cells.

For CCND1 and MYC, the synergy may be based on the fact that cyclin D mediates G1-S phase transition. Activation of MYC may bring the cells in an advantageous metabolic state allowing cells to progress further. This synergy has also been shown in CCND1-MYC transgenic mice.96,97 Indeed acquisition of a MYC translocation is associated with a dramatic morphologic change in mantle cell lymphoma (blastic98 or even mimicking Burkitt lymphoma99).
CLINICOPATHOLOGIC ASPECTS OF MATURE DH B-CELL LYMPHOMAS INVOLVING MYC, BCL2 AND BCL6

Classification

As can be concluded from the analysis of the Mitelman database and the original publications therein, DH lymphomas show heterogeneous morphologies, the majority of the BCL2+/MYC+ and BCL6+/MYC+ DH cases being classified as DLBCL or mature B cell lymphoma NOS (Figure 2). Except for the BCL6+/MYC+ DH cases, less than 20% were classified as BL. Of note, the category of “mature B cell neoplasm NOS”, was frequently called “Burkitt-like lymphoma” in the past and therefore possibly lumped with BL, also in the Mitelman database. Based on this literature data and our own experience, there are no unique unifying morphological features of DH lymphomas. Rare cases were classified as lymphoblastic lymphoma / leukemia. These cases express CD10 and terminal deoxynucleotidyl transferase (TdT) and lack expression of immunoglobulins.48,100,101 Intriguingly, at least some of these cases do not really represent a neoplasm of precursor B cells but B cells that may have re-expressed TdT since they have accumulated somatic hypermutations, a hallmark of germinal center B cells.102,103 Whatever the nature of these exceptional cases may be, many DH lymphoblastic cases in the Mitelman database are incompletely documented without data on expression of TdT or CD34.

Based on the difficulties to classify many DH cases and on the fact that they represent a subset of highly aggressive lymphomas (see below), it was considered that these lymphomas, in particular the BCL2+/MYC+ DH cases, should be separated from other lymphomas. In the 2008 WHO classification, these cases are therefore called “B cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL”. Discussion is ongoing whether otherwise morphologically regular DLBCL with a BCL2+/MYC+ DH should be placed in this category as well. As discussed below, both molecular and clinical data indeed suggest this should be done.

Certainly not all DH lymphomas represent morphological aggressive lymphomas. In the BCL2+/MYC+ group rare cases represented morphological untransformed follicular lymphoma while other cases had blastoid features or were classified as follicular lymphoma grade 3a or 3b.14 In three studies that systematically addressed MYC-rearrangements in follicular lymphoma, the frequency was 2-8%.104,105,106 However all studies showed deficits in histology (grading, Ki67 proliferation index) or clinical follow up. One interesting observation was that MYC translocation may be associated with a blastic/blastoid morphology of the tumor cells (four of seven cases being DH follicular lymphoma),106 which is usually associated with progressive disease. The implications of the presence of MYC rearrangement at initial diagnosis in follicular lymphoma thus deserves further study.

Immunophenotype

We collected immunophenotypical data from larger studies on DH lymphoma.10,11,12,13,14,15 Most lymphomas had a germinal center phenotype with expression of CD10 (107/122 cases; 88%) and BCL6 (45/60; 75%) and lack of MUM1/IRF4 (12/69; 17%). This corroborates the observation that BCL2 translocations are mainly found in GC type of DLBCL,107 and that also MYC translocations are associated with a GC molecular profile in DLBCL.108 Most importantly, BCL2 protein was detected in 101/106 cases (95%). The Ki67/MIB1 proliferation rate varied between 50 and 100% with a median of 90% in the 58 cases for which accurate data were given. Thus, although not very specific, co-expression of CD10, BCL6, BCL2 and a high Ki67 proliferation index might be used to select potential DH lymphomas in tumors morphologically diagnosed as DLBCL.

Figure 2 | Distribution of morphologies according to breakpoints

For readability of the figure BCL3+/MYC+ and 9p13+/MYC+ DHs (n=10) are omitted.
Gene expression profile

So far only two studies on BL and grey zone lymphomas between BL and DLBCL addressed gene expression specifically for BCL2/MYC+ DH lymphomas.108,110 In a collaborative study of the German network MMML project on 220 aggressive B cell lymphomas,106 16 cases represented DH lymphomas with a BCL2/MYC+, BCL6+/MYC+ DH or TH configuration. All cases except one with a borderline profile had a gene expression profile that was “intermediate between Burkitt lymphoma and DLBCL” or “non-BL”. Using a molecular algorithm in which the molecular profiles were constructed in a different way, the LLMPP consortium investigated the gene expression profile in three BCL2+/MYC+ DH lymphomas.110 These lymphomas had not been diagnosed as Burkitt lymphomas but had a molecular BL score of 98 or 99% and thus were classified as discrepant lymphomas. These discrepant cases had much higher genomic imbalances than true BL (6.9±4.4 versus 1.5±1.8 in BL), suggesting that they are nevertheless different from real BL.111 Importantly, six “regular” DLBCL cases with a MYC+DH score of 98 or 99% and thus were classified as discrepant lymphomas. These cases were used to construct a gene expression profile in three BL-like DH lymphomas with different regulatory properties.40

An additional interesting finding by the MMML was that in 14 of the 35 MYC breakdowm patients, the profile had shifted towards molecular Burkitt lymphoma after acquiring a MYC translocation.40

Clinical aspects

In this section we focus on DH lymphomas involving MYC, BCL2 and BCL6. We collected clinical features of DH cases from eight studies with ≥ 10 DH cases and with sufficient clinical data (Table 3).10,11,12,13,14,15,16,17 Median age for the DH lymphomas ranged from 51 – 65 years. DH lymphomas are extremely rare in children below the age of 18 years.113

A prior history of indolent lymphoma was documented only in a minority of cases. In the majority of cases elevated LDH and an advanced stage of disease was re-

<table>
<thead>
<tr>
<th>Study</th>
<th>DH (N=9)</th>
<th>Age (range)</th>
<th>Stage</th>
<th>Prior history of indolent lymphoma</th>
<th>IPI</th>
<th>PI</th>
<th>BM+</th>
<th>CNS+</th>
<th>&gt;1E site</th>
</tr>
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<tbody>
<tr>
<td>Bertrand et al. 2007</td>
<td>10/17 (59%)</td>
<td>1/10 (9%)</td>
<td>19/30 (63%)</td>
<td>1/9 (9%)</td>
<td>9/9 (100%)</td>
<td>1/9 (11%)</td>
<td>1/9 (11%)</td>
<td>2/9 (22%)</td>
<td>1/9 (11%)</td>
</tr>
<tr>
<td>Johnson et al. 2009</td>
<td>5/46 (11%)</td>
<td>1/10 (9%)</td>
<td>16/27 (59%)</td>
<td>1/10 (9%)</td>
<td>8/8 (100%)</td>
<td>1/8 (12%)</td>
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<td>Kanungo et al. 2006</td>
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<td>2/13 (15%)</td>
<td>22/27 (81%)</td>
<td>1/10 (9%)</td>
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<td>1/20 (5%)</td>
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<td>1/10 (9%)</td>
<td>19/19 (100%)</td>
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<td>Nitti et al. 2006</td>
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<td>1/10 (9%)</td>
<td>20/21 (95%)</td>
<td>1/10 (9%)</td>
<td>19/19 (100%)</td>
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<td>Suárez et al. 2010</td>
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<td>19/19 (100%)</td>
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<td>Tomita et al. 2009</td>
<td>15/51 (29%)</td>
<td>1/10 (9%)</td>
<td>20/21 (95%)</td>
<td>1/10 (9%)</td>
<td>19/19 (100%)</td>
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<td>1/19 (5%)</td>
<td>0/19 (0%)</td>
<td>0/19 (0%)</td>
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</tbody>
</table>

LDH indicates lactate dehydrogenase; ULN, upper limit of normal; BM+, bone marrow involvement; CNS+, central nervous system involvement; >1E, involvement of >1 extranodal site; Stage, I/II/III/IV; PI, International Prognostic Index; HI, high-intermediate (IPI score 3); H, high (IPI score 4 or 5); NA, no specific information available; +/-, are available but no ULN provided; IPI not available in 1 case; not available in 9 cases; IPI score at least Low-Intermediate (IPI score 2 or higher).
ported. In addition patients often had extranodal involvement. The bone marrow and central nervous system were most frequently involved, though the frequency of CNS involvement varied widely between studies (9-50%). In addition, pleural effusions were commonly reported. Most patients had a high-intermediate or high International Prognostic Index (IPI) risk profile (IPI score 3 or 4/5).

**Therapy & outcome**

In Table 4, a summary is given for the most relevant published clinical studies in which DH lymphoma patients could be identified. Patients were treated with a variety of regimens (including doxorubicin based chemotherapy regimens as well as high dose chemotherapy regimens with stem cell transplantation). In some instances only palliative therapy was given. Taken these limitations into account, DH lymphomas generally tended to have a very poor survival with a median overall survival of only 0.2 – 1.5 years.

In a recent study on 303 DLBCL patients treated with RCHOP, 35 had a MYC rearrangement of which 27 (77%) were DH cases. All MYC rearranged cases had an inferior outcome, also when compared for the individual IPI categories. Additional breakpoints of BCL2 or BCL6 had no significant additional impact on survival, but this may have been caused by the fact that only eight of the 27 patients did not have a DH lymphoma. Very few data are available for other treatment modalities. All four DLBCL patients with a DH who received high intensity chemotherapy with CODOX-M/IVAC, died within 5 months after start of treatment.

**How can the dismal outcome in DH lymphomas be explained?**

Several biological mechanisms may arise to explain the dismal outcome in DH lymphoma patients:

First, it could be that activation of MYC is directly responsible. However, the observation that adult BL patients have a much more favorable outcome is against this hypothesis and suggest that additional factors are essential.

Secondly, the synergistic action of MYC and BCL2 might be responsible for this behavior. Observations in transgenic mice (see previous sections) as well as some clinical observations support this hypothesis. For instance in one report with relatively large numbers of cases and relatively homogeneous therapy the 19 BCL2+MYC+ cases had a worse survival than the 24 patients with a single MYC+ or the 18 patients with a single BCL2+ translocation, as well as all 333 other DLBCL patients.

Thirdly, it could be that other molecular features play an important role as well. DH lymphomas often have a very complex karyotype with many additional genetic alterations, and the poor outcome may reflect many of these alterations. The role of genomic complexity as such is suggested by the studies of Hummel et al and Seegmiller et al, both indicating that the genomic complexity correlates with survival in lymphomas with a MYC rearrangement. Interestingly, based on their biological functions (see above), it might be speculated that MYC and BCL2 themselves play a role in the generation of this genomic complexity.

Most clinical studies concerned BCL2+MYC+ DH lymphomas and only little can be concluded for BCL6+MYC+ DH lymphomas, also since most of these lymphomas in fact might represent BCL2+/BCL6+/MYC+ TH lymphomas (see above).
<table>
<thead>
<tr>
<th>Study</th>
<th>DH (N=) / total study size (N=)</th>
<th>Treatment regimen*</th>
<th>Overall Response Rate†</th>
<th>Median Survival (yr)</th>
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<tbody>
<tr>
<td>Bertrand40</td>
<td>10/17 (59%)</td>
<td>NA</td>
<td>(5/10) 50%</td>
<td>&lt; 11</td>
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<tr>
<td>Johnson15</td>
<td>54/54 (100%)</td>
<td>RCHOP (11/54); HD+/-SCT (6/54); CHOP (23/54); P (14/54)</td>
<td>NA</td>
<td>HD 0.26; RCHOP 1.40; CHOP-like 0.42; P 0.07</td>
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<tr>
<td>Kanungo13</td>
<td>14/14 (100%)</td>
<td>CT-NOS (11); R+/- (1); CT &amp; BMT (1); CT, BMT &amp; RT (1)</td>
<td>NA</td>
<td>&lt; 11</td>
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<tr>
<td>Le Gouill10</td>
<td>16/16 (100%)</td>
<td>CEEP/COPADM + Auto-SCT/BEAM (1); CHOP/IVAM (1); COPADM/ CYVE (3); COPADM (1); COPADM + Auto-SCT/BEAM (1); COPADM + Allo-SCT/Bur/Cy (1) CEEP/DHAP + Auto-SCT/BEAM (1); RCHOP (4); CHOP (1); Steroids (1); R-CEEP Allo-SCT/TBVCy (1)</td>
<td>12/16 (75%)</td>
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<td>Macpherson12</td>
<td>15/39 (38%)</td>
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<td>Niitsu12</td>
<td>19/19 (100%)</td>
<td>CycOBEAP (6); CHOP+ HD MTX (3); CHOP (4); RCHOP (3); CycOBEAP+R (3)</td>
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<td>Snuderl14</td>
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<td>R-ICE/MTX/ASCT (1); CHOP (1); RCHOP (3); RCHOP+MTX (6); RCHOP+MTX+ASCT (1); R-EPOCH+MTX (3); CODOX+MTX-= R-IVAC (3); P (1); NK (1)</td>
<td>10/20 (50%)</td>
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<td>Tomita11</td>
<td>27/27 (100%)</td>
<td>CHOP or CODOX-M/IVAC or HyperCVAD (+ R N=14; -R N=8)</td>
<td>6/23 (26%)</td>
<td>0.501</td>
</tr>
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</table>

NA indicates not available; RCHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; HDC, high-dose chemotherapy; SCT, stem cell transplantation; P, palliative; R, rituximab; CT-NOS, intensive combination chemotherapy, not otherwise specified; BMT, bone marrow transplantation; RT, radiotherapy; CEEP, cyclophosphamide, etoposide, epidoxorubicin, and cisplatin; COPADM, cyclophosphamide, vincristine, prednisone, doxorubicin, and high-dose methotrexate; BEAM, carmustine, etoposide, cytarabine, and melphalan; IVAM, ifosfamide, etoposide, and high-dose methotrexate; CYVE, cyclophosphamide, vincristine, and doxorubicin; ASCT, autologous stem cell transplantation; EPOCH, rituximab plus etoposide, doxorubicin, vincristine, and high-dose methotrexate; CODOX, cyclophosphamide, vincristine, and doxorubicin; IVAC, ifosfamide, etoposide, and high-dose cytarabine; NK, not known; and CVAD, cyclophosphamide, vincristine, doxorubicin, and dexamethasone.

*For details about the treatment regimens we refer to the original papers.
†Overall response rate (ORR), complete remission (unconfirmed) partial response.
‡Dead before treatment, n=1.
§Calculation of median survival is based on data presented in the original paper.
Auto-SCT, n=3; Allo-SCT, n=1.
¶Given for low-grade B-cell lymphoma, NOS.
#Steroids as palliative therapy.
**Therapy ongoing, n=1.
††Lymphoma-type DH only, n=23.
‡‡Twenty-three DH lymphomas and 4 DH leukemias.
CLINICOPATHOLOGIC ASPECTS OF OTHER DH LYMPHOMAS

In the Mitelman database as analyzed by us, 34 DH lymphoma cases had a \textit{CCND1+/MYC+} combination (Table 2, Figure 2). CCND1+/MYC+ DH cases accounted for 5% of all mantle cell lymphomas. Many of these cases were leukemic and had a blastoid, pleomorphic or even a BL like morphology. Perhaps because of the retained and easily detectable cyclinD1 protein expression in tissue sections, the great majority of these cases were readily diagnosed as mantle cell lymphoma. An overrepresentation of such cases in the database may have also occurred since leukemic MCL lymphomas are possibly more frequently karyotyped than non-leukemic MCL, and most of these cases presented with overtly leukemic disease. Intriguingly, some \textit{CCND1+/MYC+} DH cases had aberrant expression of CD10 and BCL6, which parallels the observation that most MYC rearranged DLBCL also express CD10. Since most cases were studied on leukemic cells, only few cases have been documented for the Ki67 proliferation index.69,118,119 Six out of eight documented cases had a Ki67 index of >75%, suggesting that MYC might confer an important additional proliferative boost to the tumor cells in which proliferation is a very strong driving force.120 Interestingly, a recent gene expression study of 65 MCL cases showed that high MYC expression is the most important factor for outcome but only marginally associated with the Ki67 proliferation index, suggesting a role of MYC in the (TP53) DNA damage pathway rather than in the proliferation pathway.121 As far as can be concluded from the reports, mantle cell lymphoma with involvement of 8q24 tend to have an aggressive clinical course, the average survival of \textit{CCND1+/MYC+} MCL being only 8 months.119

The reported numbers of the rare other DH lymphomas involving BCL3 or other loci are too few to draw any conclusions. One interesting feature that needs more attention is a subset of lymphomas in which 9p13 is involved, either as a 9p13'/MYC+ DH lymphoma were MYC is directly fused to this locus.

DOUBLE HIT LYMPHOMAS AND “B CELL LYMPHOMAS, UNCLASSIFIABLE WITH FEATURES INTERMEDIATE BETWEEN DIFFUSE LARGE B CELL LYMPHOMA AND BURKITT LYMPHOMA”

According the 2008 WHO classification, the category “B cell lymphomas, unclassifiable with features intermediate between diffuse large B cell lymphoma and Burkitt lymphoma”6 is a heterogeneous category of lymphomas that for biological and clinical reasons should not be classified as Burkitt lymphoma or DLBCL. It is meant as a temporary category, necessary until better discriminating criteria and more distinct categories of lymphomas are available. Apart from the DH lymphomas there are three other problematic issues with respect to the diagnosis of Burkitt lymphoma versus DLBCL that justified this category:

1. The most problematic area is formed by non DH lymphomas that are diagnosed as DLBCL but nonetheless share several morphological and immunophenotypical features with Burkitt lymphoma, in particular a cohesive growth pattern, a very high Ki-67 proliferation index and expression of germinal center associated proteins like CD10. The dimension of this diagnostic problem is difficult to assess but certainly is different between pediatric and adult patients. Many of such cases in children contain an \textit{MYC-IG} breakpoint, do not have any other breakpoint and have a gene expression profile similar to Burkitt lymphoma and, therefore, likely should be better diagnosed as Burkitt lymphoma.122 In contrast, for adult patients no data are available for large series of DLBCL collected in population based studies where all ancillary tests to exclude Burkitt or DH lymphomas have been applied. According to Nordic lymphoma group,123 which more or less reflects a population based registry, 10% of all 185 DLBCL had a Ki-67 proliferation index of 90% or more. This should mean that after exclusion of DH lymphomas and DLBCL with a phenotype not compatible with Burkitt lymphoma, far less than 10% of all DLBCL are problematic in this respect.124,109

2. Burkitt lymphoma has a characteristic expression of CD20, CD10, BCL6 and absence of BCL2 protein, while MUM1/IRF4 may be expressed at low levels. However, in up to 20% of all otherwise classical Burkitt lym-
Double hit B-cell lymphomas some immunophenotypic abnormalities have been reported, for instance weak expression of BCL2 protein in 0-20%\textsuperscript{125,129} or aberrant expression of T cell markers such as CD4 or CD5.\textsuperscript{126} As already discussed in this review, such cases should only be accepted as Burkitt lymphoma after vigorous exclusion of a DH lymphoma.

3. Finally, in approximately 10% of all lymphomas that are otherwise indistinguishable from Burkitt lymphoma, including endemic and pediatric Burkitt lymphomas, a MYC breakpoint is not detectable by current FISH methods. It might be considered to restrict the diagnosis Burkitt lymphoma to cases with a proven MYC-iG breakpoint and to consign all other cases to the “intermediate” group. Likely it is too early to do so since certain MYC breakpoints are missed with the current (FISH) methods and since in rare cases of Burkitt lymphoma a similar high MYC expression can be induced by downregulation of micro RNA 34B (miRNA-34B).\textsuperscript{127}

These dilemmas and the fact that many recent publications suggest that the presence of a MYC breakpoint in DLBCL, either or not involved in a double hit, has an important prognostic value,\textsuperscript{109,108,128,114,117,129} suggest that all aggressive mature B lymphomas should be systematically studied with ancillary methods, in particular FISH analysis, to provide the best possible diagnosis and therapeutic prospects.

**CONCLUSIONS**

Double and triple hit lymphomas are B cell lymphomas characterized by a recurrent chromosomal translocation in combination with a MYC/8q24 breakpoint, the latter mostly as a secondary event involved in transformation. The compiled cytogenetic and FISH data strongly suggest that many lymphomas other than Burkitt lymphoma with a MYC breakpoint represent DH lymphomas. This implies that the studies that only focused on the impact of MYC breakpoints in DLBCL have to be reconsidered. Most DH have a \textit{BCL2}/MYC combination and most \textit{BCL6}/MYC DH lymphomas represent \textit{BCL2}/\textit{BCL6}/MYC TH lymphomas. \textit{CCND1}/MYC DH lymphomas may be more frequent than anticipated and should receive more attention. In view of the frequency of these aberrations and their clinical impact, it seems timely to test all aggressive B cell lymphomas, including the mantle cell lymphomas with a high proliferation index or blastic morphology, for MYC (and \textit{MYC-IG}) breakpoints by FISH. In selected cases \textit{BCL2} and \textit{BCL6} FISH tests should be performed as well, for instance in those cases with a MYC breakpoint and concomitant BCL2 protein expression. A \textit{BCL2}/MYC DH lymphoma should be considered in all aggressive and highly proliferating B-cell lymphomas with a distinct GCB phenotype in combination with BCL2 expression, in particular when the patient presents with extensive disease including bone marrow and/or CNS involvement. However these parameters are insufficient to identify all cases. Moreover, individual morphological and immunophenotypical parameters may suffer from a low reproducibility. Although we realize that detection of \textit{MYC}, \textit{BCL2} and \textit{BCL6} breakpoints does not reflect all biological aspects of these complex tumors, we suggest to perform these assays until more biological data and better tests become available.

DH lymphoma patients generally have rapidly progressive disease and a dismal outcome, even with high intensity chemotherapy. The course of disease might reflect not only the synergistic actions of the two or more oncogenes involved, but also the high genomic complexity in most of these tumors.
Acknowledgements:
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Authorship statement:
S.M.A. performed literature and database searches, wrote the manuscript
R.S. contributed to the design and supervised the cytogenetic / molecular investigations
E.S. contributed to the scientific cytogenetic / molecular part of the manuscript
G.W.v.I. contributed to the design of the manuscript and wrote parts of the manuscript
H.K.N. contributed to the design of the manuscript
E.J.B. contributed the design of the manuscript
P.M.K. contributed to the design of the manuscript and wrote parts of it.

Conflict of interest statement:
The authors declare no competing clinical or financial interest.

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Chapter 6 Double hit B-cell lymphomas


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SUPPLEMENTARY DATA

To identify candidate oncogenes involved in double hits we explored the ‘Mitelman Database of Chromosome Aberrations in Cancer’,(1) edition February 2010. We selected cases with a 8q24 breakpoint published after the publication of the REAL classification to be confident about the pathologic classification of the cases.(2) Although the database relies on karyotypic features only and no full proof is given that the indicated genes are really involved in all cases, we used the names of genes and not the loci, for the sake of readability. Our cytogenetic search included 8q24, 18q21, 3q27, 11q13, 19q13, 14q32, 2p12 and 22q11 as queries for breakpoints involving MYC, BCL2, BCL6, CCND1, BCL3, IGH, IGK and IGL. Due to a variable cytogenetic assignment of some loci, potentially some breakpoints mapped to neighbouring gene loci might have remained undetected by our algorithm. Only mature malignancies of the B-cell lineage were included and all plasma cell dyscrasias including myeloma were excluded. DH lymphomas were identified by searching cases with an 8q24 breakpoint and an other recurrent breakpoint with IG (IGH, IGK or IGL) as a partner that were found three or more times in the database. Karyotypes from the study of Snuderl et al(3) which were not available in the February edition of the Mitelman database as well as some cases that due to technical errors like typing errors were not included in the Mitelman database, were manually added to our dataset. Our dataset included in total 689 cases. To identify DH or TH lymphomas with a novel combination (not BCL2+/MYC+ or BCL6+/MYC+ DH lymphomas) we explored our dataset of 689 cases on all loci that may be juxtaposed to the IG-partners as published in the in the "Atlas of Genetics and Cytogenetics in Oncology and Haematology" (URL: http://AtlasGeneticsOncology.org ). Loci with a frequency ≥3 were selected and included MYC (8q24), BCL2, (18q21), BCL6 (3q27), CCND1 (11q13), BCL3 (19q13) and a yet unidentified locus on 9p13. With respect to breakpoints involving 11q13, cases with interstitial deletions involving a11q13 as the sole breakpoint involving 11q13, e.g. del(11)(q13q23 ) and variants, were not considered as a CCND1+/ MYC+ DH as these events as the sole abnormality are not likely to cause increased CCND1 expression.

We included cases of follicular lymphoma and cases with transformation from follicular lymphoma. All cases with a clinical history of follicular lym-
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phoma but in whom the karyotypic abnormalities were detected at the time of transformation were classified according to the aggressive (trans- formed) morphology. We included such cases since the distinction between DH lymphoma may be arbitrary and it may be difficult to proof that a patient with a de novo DH lymphoma may have an underlying follicular

lymphoma. Few cases showed a DH phenomenon already at the presentation of follicular lymphoma. To avoid any selection bias, we also included these cases in our study. With some exceptions (see above for some transformed follicular lymphoma cases that had to be corrected on basis of the original manuscript), the histological classification assigned to the cases in the Mitelman database was used. The classification of all cases is shown in Supplementary Table 1. In Supplementary Table 2 all data as retrieved from the Mitelman database with addition of few individual other publications are listed.

Supplementary table 1 | classification of recurrent DH en TH lymphomas in the Mitelman database

<table>
<thead>
<tr>
<th>Lymphoma</th>
<th>MYC+</th>
<th>MYC+</th>
<th>BCL2+/MYC+</th>
<th>BCL6+/MYC+</th>
<th>BCL2+/BCL6+/MYC+</th>
<th>CCND1+/MYC+</th>
<th>BCL3+and/or 9p13+/MYC+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>689</td>
<td>100</td>
<td>363</td>
<td>100</td>
<td>203</td>
<td>100</td>
<td>53</td>
</tr>
<tr>
<td>ALL/LL</td>
<td>51</td>
<td>7</td>
<td>38</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>ALL/L</td>
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<td>37</td>
<td>205</td>
<td>56</td>
<td>30</td>
<td>15</td>
<td>7</td>
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<tr>
<td>DLBCL</td>
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<td>38</td>
<td>10</td>
<td>68</td>
<td>33</td>
<td>9</td>
</tr>
<tr>
<td>CLL</td>
<td>30</td>
<td>4</td>
<td>23</td>
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<tr>
<td>MM</td>
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<td>12</td>
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<td>31</td>
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<tr>
<td>MBCL-NOS</td>
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<td>4</td>
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<tr>
<td>SMZL</td>
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<td>7</td>
<td>2</td>
<td>1</td>
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</tr>
</tbody>
</table>

ALL/LL indicates acute lymphoblastic leukemia/lymphoblastic lymphoma; BL/L, Burkitt lymphoma/leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MBCL-NOS, mature B-cell lymphoma, not otherwise specified; NMZL = nodal marginal zone lymphoma; PEL, primary effusion lymphoma; SMZL, splenic marginal zone lymphoma.
REFERENCE LIST

