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Double-hit B-cell lymphomas

Sietse M. Aukema,[1,2] Reiner Siebert,[3] Ed Schuuring,[1] Gustaaf W. van Imhoff,[2] Hanneke C. Kluin-Nelemans,[2] Evert-Jan Boerma,[1] and Philip M. Kluin[1]

[1]Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, The Netherlands; [2]Department of Hematology, University Medical Center Groningen, University of Groningen, The Netherlands; and [3]Institute of Human Genetics, Christian-Albrechts University Kiel and University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany

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 a 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. (*Blood*. 2011;117(8):2319-2331)

Introduction

Approximately 40% of all B-cell lymphomas are characterized by the presence of a recurrent reciprocal chromosomal translocations.[1-5] 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 because, from the nomenclature point of view, it is neither restricted to B-cell lymphomas (eg, inv(14)-positive T-cell prolymphocytic leukemia may carry a *MYC* translocation) nor does it exclude 2 translocations activating oncogenes other than *MYC* (eg, 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, for example, *BCL2*$^+$/*MYC*$^+$, and use the term DH lymphoma for all cases with multiple recurrent breakpoints (triple/quadruple) as well.

Because cases with a *MYC*/8q24 and *BCL2*/18q21 breakpoint (*BCL2*$^+$/*MYC*$^+$ DH lymphoma) are most common, these casesreceived 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 not otherwise specified (NOS), or diffuse large B-cell lymphomas (DLBCL), appear to represent such DH lymphomas. In the updated classification for malignant lymphomas by the World Health Organization (WHO), it is proposed to classify most if not all cases as "B-cell lymphoma unclassifiable with features intermediate

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

Study	N*	*MYC*⁺ total, n (%)	*MYC*⁺ SH, n (%)	*BCL2*⁺/*MYC*⁺ DH, n (%)	*BCL6*⁺/*MYC*⁺ DH, n (%)	*BCL2*⁺/*BCL6*⁺/*MYC*⁺ TH, n (%)	All DH and TH, n (%)	All DH & TH / *MYC* cases, n/N (%)	*BCL2*⁺ SH, n (%)	*BCL6*⁺ SH, n (%)
Barrans et al[114] 2010†	245-264	35 (14)	6 (2)	19 (8)	3 (1)	7 (3)	29 (12)	29/35 (83)	55 (21)	64 (24)
Obermann et al[130] 2009‡	220	9 (4)	7 (3)	1 (0)	1 (0)	0	2 (1)§	2/9 (22)	NA	NA
Yoon et al[129] 2008‡	137-156	14 (7)	11 (7)	1 (1)	1 (1)	1 (1)	3 (3)	3/14 (21)	3 (2)	22 (16)
Tibiletti et al[131] 2009†‖	74	12 (16)	3 (4)	4 (7)	4 (7)	1 (1)	9 (12)	9/12 (75)	12 (15)	30 (39)
Copie-Bergman et al[132] 2009‡	68-71	2 (3)	2 (3)	0	0	0	0	0	14 (20)	21 (30)
van Imhoff et al[133] 2006†¶	58-59	9 (15)	5 (8)	3 (5)	1 (2)	0	4 (7)	4/9 (44)	7 (12)	14 (24)
Savage et al[128] 2009‡	135	12 (9)	9 (7)	3 (2)	NA	NA	NA	3/12 (25)	NA	NA
Klapper et al[108] 2008‡#	117	14 (8)	NA	NA	NA	NA	NA	NA	NA	NA

All cases had DLBCL as morphology.
SH indicates single hit; DH, double hit; TH, triple hit; and NA, not available.
*Number of cases on which FISH was performed; variable numbers because of some failures in individual tests.
†FISH on conventional tissue sections.
‡FISH on tissue microarray.
§Original paper included 1 *CCND1*⁺/*MYC*⁺ DH that is not shown in table.
‖DLBCL was selected for primary nodal localization.
¶Cases from clinical trials were selected for patients with poor-risk DLBCL.
#No FISH for *BCL2* and *BCL6* was performed in the study.

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.

DH 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 (GC) 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 (~ 10%) but considerably lower than in regular BL (90%-100%). In this review we 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 is briefly discussed. (3) We discuss the timing of the occurrence of the breakpoints in lymphomagenesis as well as the synergistic action of the involved oncogenes. (4) The pathologic and clinical aspects of *BCL2*⁺/(*BCL6*⁺)/*MYC*⁺ DH and triple hit (TH) lymphomas are reviewed. (5) A short section is devoted to other DH B-cell lymphomas. (6) The relationship with "B-cell lymphoma unclassifiable with features intermediate between DLBCL and BL" is discussed. (7) Finally, we 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 8 larger studies, 3 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 because of the inclusion of one series from Asia in which the incidence of t(14;18)–carrying lymphomas may be lower than in Western countries.[7]

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 supplemental Tables 1-2, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).[8] 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 Revised European-American Lymphoma classification to be confident about the classification of the cases.[9] We realize that this has introduced a certain bias toward DH lymphomas, because several of these publications specifically addressed this phenomenon.[10-15] We included only mature B-cell malignancies (see supplemental data for strategies and sources). Plasma cell neoplasms were excluded because 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* and *MMSET, c-MAF*, and *MAFB*) and secondary translocations involving *MYC*.[16,17] DHs involving these genes and also combinations

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Table 2. DH and TH lymphomas in the Mitelman database

DH lymphomas	N	Percentage of all 326 DH cases	*MYC-IG* fusion, %	*MYC-IGK* or *IGL* fusion (% of cases with *MYC-IG* fusion)*
BCL2$^+$/*MYC*$^+$	203	62	66	49
BCL6$^+$/*MYC*$^+$	26	8	31	13
BCL2$^+$/*BCL6*$^+$/MYC$^+$	53	16	53	50
CCND1$^+$/*MYC*$^+$	34	10	20	43
BCL3$^+$/*MYC*$^+$	5	2		
9p13$^+$/M*YC*$^+$	4	1		
BCL3$^+$/9p13$^+$/*MYC*$^+$ TH	1	0		
Total DH and TH cases	326	100		
MYC only				
Burkitt lymphoma	205		98	18
Other lymphomas	158		61	34
Total	689			

MYC$^+$ indicates 8q24 breakpoint; *BCL2*$^+$, 18q21; *BCL6*$^+$, 3q27; *CCND1*$^+$, 11q13; *BCL3*$^+$, 19q13; and 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.

thereof were frequently seen in the Mitelman database (5%-25%; data not shown).[8] Of note, a selection bias may have occurred because 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*.[18]

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, although between 1995 and 2009, 109 of 804 DLBCLs were *BCL2*$^+$/*MYC*$^+$ or *BCL6*$^+$/*MYC*$^+$ DH cases (14%), only 12 of 445 similar DH DLBCLs were reported in the period before 1995 (3%; data not shown). As stated earlier, 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 TH lymphomas that involved *MYC*, *BCL2* and *BCL6* (16%) were more frequent than *BCL6*$^+$/*MYC*$^+$ DH cases. In DLBCL, 25 of 139 cases (18%) had a *BCL6* breakpoint, whereas 84 of 139 (60%) had a *BCL2* breakpoint (see supplemental Table 1). This preference for *BCL2*$^+$/*MYC*$^+$ is at least partially because of 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 (MCLs) in the database were *CCND1*$^+$/*MYC*$^+$ DH cases. Other recurrent DH lymphomas were 5 *BCL3*$^+$/*MYC*$^+$ cases with t(14;19)(q32;q13), 4 cases with t(9;14)(p13;q32) involving a yet unidentified locus on 9p13,[19,20] and 1 case with all 3 loci involved. A *MYC* breakpoint without any other recurrent breakpoint (*MYC*$^+$ SH) was seen in 363 of 689 cases (53%), from which 205 cases (56%) were diagnosed as BL (supplemental Table 1).

Although in typical BL *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 than the 18% in BL. We explored the direct partners of *MYC* in the other DH lymphomas. Interestingly, in the group of *BCL2*$^+$/*BCL6*$^+$/*MYC*$^+$ DH lymphomas the commonest non-immunoglobulin 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 5 cases and t(2;8)(p11;q24) in 5 cases, the latter probably representing *MYC-IGK* fusion. Both in the *BCL6*$^+$/*MYC*$^+$ DH and *BCL2*$^+$/*BCL6*$^+$/*MYC*$^+$ TH groups *BCL6* itself was a *MYC* partner in 4 and 7 cases, respectively.

Oncogenes involved in DH lymphomas

In this section we 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.[21] MYC exerts its function by dimerization with MAX and subsequent binding to specific consensus DNA sequences (CACGTG) called an "E-Box."[22-24] Many genes are directly (de)regulated by *MYC*, including *LDH-A* and *TERT*.[25] In addition *MYC* is involved in the regulation of micro-RNA (miRNA) expression,[26-28] (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 GCs is, surprisingly, lower compared with naive and memory B cells.[29] This low expression could protect against *MYC*-induced genomic instability in the GC. For a detailed review about the role of MYC in lymphomagenesis, see Klapproth and Wirth.[30] 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 that involve *MYC* and the *IGH* locus are mediated by activation-induced cytidine deaminase (AICDA) and not by recombinase activating gene 1/2 (RAG1/2).[31-34] Thus, these breakpoints should have their origin from erroneous somatic hypermutation or class switch recombination.[35] On the basis of transgenic mouse models, additional factors are needed for malignant transformation.[36,37] This is supported by the finding that

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MYC-IG translocation can be detected in nonneoplastic conditions, both in humans and mice.[38,39] The mechanism responsible for translocations affecting *MYC* and non-*IG* loci, for instance 9p13 in the t(8;9)(q24;p13) translocation, is unknown.[15,40]

BCL2

BCL2 was first described in the early 1980s by its involvement in the t(14;18) in follicular lymphoma.[41] As a member of a large BCL2 family of proteins, it has potent antiapoptotic functions. *BCL2* is widely expressed in immature B cells and memory B cells but is temporarily down-regulated in GC B cells, partially because of repression by *BCL6*.[29,42-44] With the occurrence of t(14;18), transcription of *BCL2* is "constitutively" deregulated with high transcription activity from the translocated *BCL2* allele.[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 nonhomologous end-joining activities of Ku proteins essential for repair of both RAG1/2- and AICDA-mediated breakpoints.[46]

With the exception of rare cases,[47] t(14;18) translocations are thought to occur early in B-cell development.[48,49] Chromosomal breaks are mediated by RAG1/2, probably in combination with low levels of AICDA.[50] Extensive mapping studies of the breakpoints as well as sequence analysis have shown that the *BCL2* breakpoints are strongly clustered at cytosine-phosphate-guanosine (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.[50] An in-depth discussion about the occurrence of the t(14;18) at later stages of B-cell development (including even the GC) and (secondary) involvement of RAG1/2 and/or other mechanisms herein[51-54] goes beyond the scope of this review.

Apparently, the t(14;18) is insufficient to cause follicular lymphoma because *IGH-BCL2* transgenic mice do not develop lymphomas, and t(14;18)-carrying mature B cells can also be found in healthy persons and probably arise by the same mechanisms.[51,55-59] 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 healthy persons, called "follicular lymphoma-like B cells," usually enter the GCs, undergo somatic hypermutations and a limited degree of immunoglobulin gene class switching, and circulate as memory B cells.[60,61] This passage across the GC 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, $BCL2^+/MYC^+$ DH lymphomas may arise in 2 ways: either they arise from a clinically overt or subclinical 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,[48,62,63] although some follicular lymphomas with *MYC* breakpoints but without evidence of morphologic transformation have been described (see "Classification").

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 it is mostly restricted to GC B cells.[64,65] *BCL6* is required for the formation of GCs because mice deficient for *BCL6* lack these structures.[66,67] Within the GC *BCL6* acts as an transcriptional repressor of many target genes involved in apoptosis, DNA-damage response, cell cycle control, proliferation, and differentiation.[68-70] Important direct targets are *BCL2*, *TP53*, *IRF4*, and *BLIMP1*, the latter being essential for maturation into plasma cells.[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 that mimicks *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 ~ 30%-40% of all DLBCLs, 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 G_1 to the S phase, by forming a complex with CDK4 and activating the RB1-E2F1 complex, allowing E2F1 to be released. Although 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, that is, translocation or copy number increase. In most MCLs and in a substantial fraction of multiple myelomas 11q13 translocations are observed.[77-80] In addition to these malignancies, also hairy cell leukemia and some cells in the proliferation centers of chronic lymphocytic leukemia 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, probably 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 persons, however at much lower frequencies than $t(14;18)^+$ cells.[83] This low frequency may be because these cells are not expanded in the GC cell reaction. This fits with the finding that MCL represents a pre-GC 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 GC B cells.[84]

BCL3

BCL3 is a distinct member of the Iκβ 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.[85] Experiments in *BCL3*-deficient mice have shown that the gene is involved in GC formation.[86] The t(14,19)(q32;q13) leads to increased *BCL3* transcription and has been described in a large variety of lymphomas and leukemias,[87] including atypical chronic lymphocytic leukemia.[88,89] Analysis of t(14;19) breakpoints indicates that this translocation is mediated by

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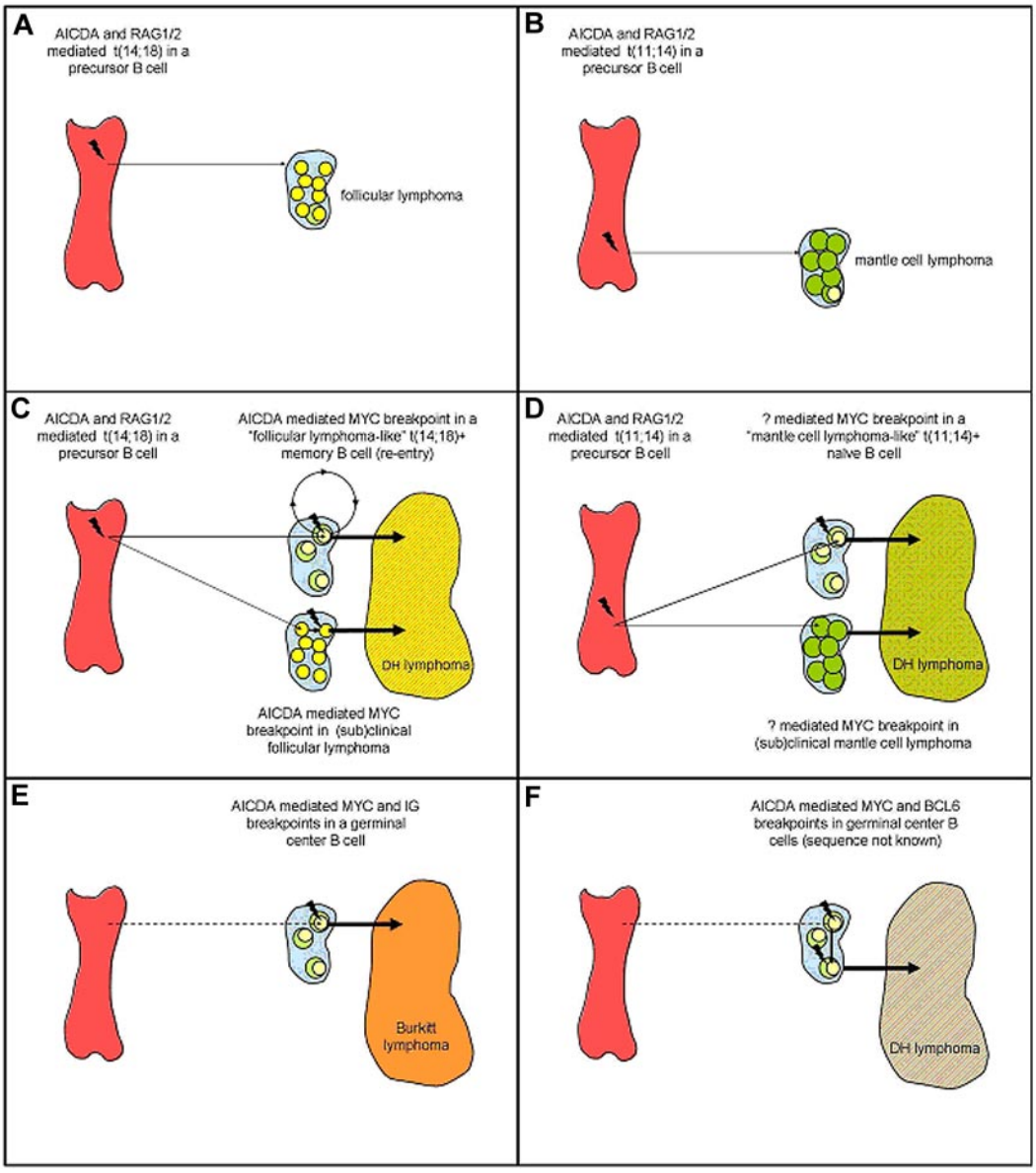

Figure 1. Schematic scenarios for the origin of follicular lymphoma, MCL, BL (*MYC-IG* single hit lymphomas), and DH lymphomas. (A) Follicular lymphoma; (B) MCL; (C) *BCL2*$^+$/*MYC*$^+$ DH lymphoma with 2 scenarios, one with an origin from relatively common benign "follicular lymphoma–like B cells" that can be detected in most healthy persons, and the other from a preexistent follicular lymphoma. (D) *CCND1*$^+$/*MYC*$^+$ DH lymphoma with 2 scenarios, one with an origin from rare benign "MCL-like B cells" that can be detected in few healthy persons, and the other from a preexistent MCL. (E) BL; (F) *BCL6*$^+$/*MYC*$^+$ DH lymphoma with unknown order of events. For readability possible occurrence of the t(14;18) at later stages of B-cell development (including the GC) and (secondary) involvement of RAG1/2 and/or other mechanisms herein are not displayed in the figure (see "Oncogenes involved in DH lymphomas"). AICDA indicates activation-induced cytidine deaminase; RAG1/2, recombinase activating gene 1/2. Not drawn to scale.

illegitimate class switch recombination.[90-92] *Eμ-BCL3* transgenic mice overexpressing *BCL3* show lymphoid hyperplasia but do not develop lymphomas.[93]

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 probably mediated by AICDA in mature B cells. In contrast, and with the exception of *CCND1* in myeloma, *BCL2* and *CCND1* breakpoints are most probably 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 ~ 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 ≥ 2 subclones, one with only a t(14;18) and the other with both translocations.[48] Another argument for the secondary nature may be that, in comparison to the "primary" *MYC* breakpoints in BL whereby 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, because cells with disruption of both *IGH* alleles are deleted.[94]

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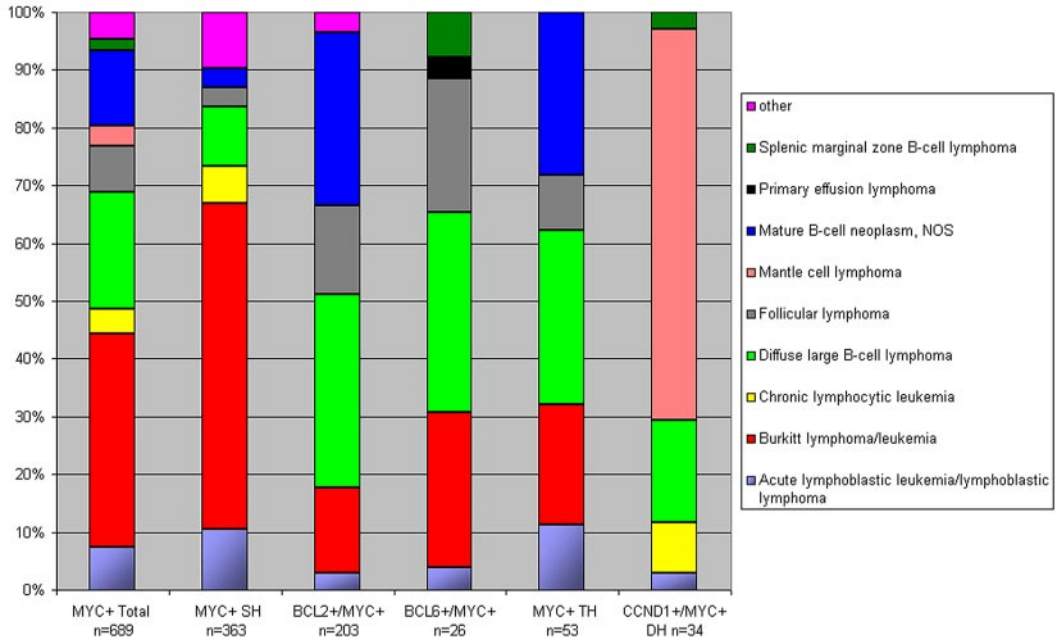

Figure 2. Distribution of morphologies according to breakpoints. For readability of the figure *BCL3*$^+$/*MYC*$^+$ and 9p13$^+$/*MYC*$^+$ DHs (n = 10) are omitted. SH indicates single hit.

For *BCL6*$^+$/*MYC*$^+$ and *BCL3*$^+$/*MYC*$^+$ DH lymphomas the timing of events is less clear because most breakpoints affecting *MYC*, *BCL6*, and *BCL3* are probably 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 because the t(14;18) translocation forces tumor cells to accumulate as GC 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 supplemental Table 2). Indeed, the t(11;14) translocation does not result in accumulation of GC B cells because 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 biologic synergy of acquiring 2 or 3 breakpoints, and thus activating both oncogenes? This is again most evident for *BCL2* and *MYC* whereby *BCL2* is antiapoptotic 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 up-regulating lactate dehydrogenase 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 preexistent *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 GC, 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.[95]

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*$^+$ tumor cells. In reverse, because 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 G_1-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 MCL (blastic[98] or even mimicking BL[99]).

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[8] and the original publications therein, DH lymphomas show heterogeneous morphologies, most 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, $< 20\%$ 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 was lumped with

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BL, also in the Mitelman database.[8] On the basis of these literature data and our own experience, there are no unique unifying morphologic 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 reexpressed TdT, because they have accumulated somatic hypermutations, a hallmark of GC B cells.[102,103] Whatever the nature of these exceptional cases may be, many DH lymphoblastic cases in the Mitelman database[8] are incompletely documented without data on expression of TdT or CD34.

On the basis of the difficulties to classify many DH cases and because they represent a subset of highly aggressive lymphomas (see this and following sections), it was considered that these lymphomas, in particular the *BCL2*$^+$/*MYC*$^+$ DH cases, should be separated from other lymphomas. In the 2008 WHO classification,[6] 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 morphologic aggressive lymphomas. In the *BCL2*$^+$/*MYC*$^+$ group rare cases represented morphologic untransformed follicular lymphoma, whereas other cases had blastoid features or were classified as follicular lymphoma grade 3a or 3b.[14] In 3 studies that systematically addressed *MYC* rearrangements in follicular lymphoma, the frequency was 2%-8%.[104-106] 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 (4 of 7 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.[10-15] Most lymphomas had a GC phenotype with expression of CD10 (107 of 122 cases; 88%) and BCL6 (45 of 60; 75%) and lack of MUM1/IRF4 (12 of 69; 17%). This corroborates the observation that *BCL2* translocations are mainly found in GC type of DLBCL, and that also *MYC* translocations are associated with a GC molecular profile in DLBCL.[107,108] Most importantly, the BCL2 protein was detected in 101 of 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, coexpression of CD10, BCL6, BCL2, and a high Ki67 proliferation index might be used to select potential DH lymphomas in tumors morphologically diagnosed as DLBCL.

Gene expression profile

So far only 2 studies on BL and gray zone lymphomas between BL and DLBCL addressed gene expression specifically for *BCL2*$^+$/*MYC*$^+$ DH lymphomas.[109,110] In a collaborative study of the German network Molecular Mechanisms in Malignant Lymphoma (MMML) project on 220 aggressive B-cell lymphomas,[109] 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." With the use of a molecular algorithm in which the molecular profiles were constructed in a different way, the Lymphoma/Leukemia Molecular Profiling Project consortium investigated the gene expression profile in 3 *BCL2*$^+$/*MYC*$^+$ DH lymphomas.[110] Morphologically, these lymphomas had not been diagnosed as BLs 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 $\pm$ 4.4 versus 1.5 $\pm$ 1.8 in BL), suggesting that they are nevertheless different from real BL.[111] Importantly, 6 "regular" DLBCL cases with a *MYC* breakpoint (no DH) did not have a BL type of gene expression in the Lymphoma/Leukemia Molecular Profiling Project study, indicating that some MYC translocations might be insufficient to enforce a full-blown MYC-driven gene expression program, probably because the partner is a non-IG gene locus with different regulatory properties than IG loci or because the genetic or cellular background interacts with the possibility to fully express a MYC program.[40]

An additional interesting finding by the MMML consortium was that in 14 of the 35 MYC breakpoint-positive cases that lacked the molecular BL signature, a non-IG partner was involved in the *MYC* breakpoint. Thus, in particular the MMML study suggests that DH lymphomas are biologically and clinically different from both classical BL and DLBCL. In fact, they cannot be classified easily, probably because the profile has shifted toward molecular BL after acquiring a MYC translocation.

Clinical aspects

In this section we focus on DH lymphomas involving *MYC*, *BCL2*, and *BCL6*. We collected clinical features of DH cases from 8 studies with $\geq$ 10 DH cases and with sufficient clinical data (Table 3).[10-15,40,112] Median age for the DH lymphomas ranged from 51 to 65 years. DH lymphomas are extremely rare in children younger than 18 years of age.[113]

A prior history of indolent lymphoma was documented only in a minority of cases. In the majority of cases elevated lactate dehydrogenase and an advanced stage of disease was reported. In addition patients often had extranodal involvement. The bone marrow and central nervous system (CNS) were most frequently involved, although the frequency of CNS involvement varied widely between studies (9%-50%). In addition, pleural effusions were commonly reported.[10-12] Most patients had a high-intermediate or high International Prognostic Index (IPI) risk profile (IPI score 3 or 4/5).

Therapy and outcome

In Table 4, a summary is given for the most relevant published clinical studies in which patients with DH lymphoma 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. With these limitations taken into account, DH lymphomas generally tended to have a poor survival, with a median overall survival of only 0.2-1.5 years.[10-15,40,112]

In a recent study on 303 patients with DLBCL treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone, 35 had a *MYC* rearrangement of which 27 (77%) were

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Table 3. Clinical features of DH lymphomas

Study	No. of DH/total study size (%)	DHs with prior history of indolent lymphoma, n/N (%)*	Age, median (range)	Stage III/IV, n/N (%)	LDH > ULN, n/N (%)	BM+, n/N (%)	CNS+, n/N (%)	>1E site, n/N (%)	IPI HI-H, n/N (%)
Bertrand et al[40] 2007	10/17 (59)	1/10 (10)	58 (45-81)	7/10 (70)	NA†	NA	NA	NA	5/9 (56)‡
Johnson et al[15] 2009	54/54 (100)	20/54 (46)	62 (24-93)	41/54 (76)	27/54 (50)	32/45 (71)§	NA	19/54 (35)	38/54 (70)‖
Kanungo et al[13] 2006	14/14 (100)	None	55 (29-72)	NA	13/14 (93)	11/14 (79)	3/14(21)	8/14 (57)¶	NA
Le Gouill et al[10] 2007	16/16 (100)	4/16 (25)	61 (36-73)	16/16 (100)	16/16 (100)	15/16 (94)	8/16(50)	14/16 (88)	13/16 (81)
Macpherson et al[112] 1999#	15/39 (38)	6/13 (46)	65**	12/13 (92)	8/10†† (80)	9/13 (69)	NA	8/13 (62)	9/10 (90)††
Niitsu et al[12] 2009	19/19 (100)	None	61 (29-79)	19/19 (100)	19/19 (100)	16/19 (84)	4/19(21)	12/19 (63)	17/19 (89)
Snuderl et al[14] 2010‡‡	20/20 (100)	3/20§§ (15)	64 (32-91)	18/19 (95)	18/18 (100)	10/17 (59)	5/11(45)	6/20 (30)¶	17/20 (85)
Tomita et al[11] 2009‖‖	27/27 (100)	4/23 (17)	51 (36-79)	22/23 (96)	25/27 (93)	15/23 (65)	2/23 (9)	15/23 (65)	20/23 (87)

LDH indicates lactate dehydrogenase; ULN, upper limit of normal; BM+, bone marrow involvement; CNS+, central nervous system involvement; >1E, involvement of >1 extranodal site; IPI, International Prognostic Index; HI, high-intermediate (IPI score 3); H, high (IPI score 4 or 5); and NA, no specific information available.

*Follicular lymphoma, n = 29; chronic lymphocytic leukemia, n = 1; and low-grade lymphoma (not otherwise specified), n = 8.

†LDH values 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). No specific information about distribution between patients in low-intermediate and HI groups available.

¶Involvement of > 1 extranodal site calculation was based on data presented in the original paper.

#For the clinical parameters only information for *BCL2*$^+$/*MYC*$^+$DH cases (n = 13) was available; for *BCL6*$^+$/*MYC*$^+$ DH (n = 2) cases no specific information was available.

**No age-range for DHs available.

††LDH and IPI not available in all cases.

‡‡For some cases not all clinical parameters were available.

§§Three cases with confirmed preexisting follicular lymphoma.

‖‖Lymphoma type DH (n = 23), leukemia type DH (n = 4).

DH cases.[114] All *MYC*-rearranged cases had an inferior outcome, also compared with the individual IPI categories. Additional breakpoints of *BCL2* or *BCL6* had no significant additional effect on survival, but this may have been because only 8 of the 27 patients did not have a DH lymphoma. Few data are available for other treatment modalities. All 4 patients with DLBCL with a DH who received high-intensity chemotherapy with cyclophosphamide, vincristine, doxorubicin, high-dose methotrexate/ifosfamide, etoposide, and high-dose cytarabine died within 5 months after the start of treatment.[115]

Table 4. Treatment and survival of DH lymphomas

Study	No. of DH/total study size (%)	Treatment regimen*	Overall response rate, n/N (%)†	Median survival, y
Bertrand et al[40] 2007	10/17 (59)	NA	5/10‡ (50)	< 1§
Johnson et al[15] 2009	54/54 (100)	RCHOP (11/54);HDC+/− SCT‖ (6/54); CHOP (23/54); P (14/54)	NA	HD, 0.26; RCHOP, 1.40; CHOP-like, 0.42; P 0.07
Kanungo et al[13] 2006	14/14 (100)	CT-NOS (11); R¶ (1); CT and BMT (1); CT, BMT, and RT (1)	NA	< 1§
Le Gouill et al[10] 2007	16/16 (100)	CEEP/COPADM + Auto-SCT/BEAM (1); CHOP/IVAM (1); COPADM/CYVE (3); COPADM (1); COPADM + Auto-SCT/BEAM (1); COPADM + Allo-SCT/Bu/Cy (1) CEEP/DHAP + Auto-SCT/BEAM (1); RCHOP (4); CHOP (1); Steroids# (1); R-CEEP Allo-SCT/TBI/Cy (1)	12/16 (75)	0.42
Macpherson et al[112] 1999	15/39 (38)	CHOP-variant or cyclophosphamide + MTX (6); HDC +/− SCT (3); P (4)	NA	0.21
Niitsu et al[12] 2009	19/19 (100)	CyclOBEAP (6); CHOP + HD MTX (3); CHOP (4); RCHOP (3), CyclOBEAP + R (3)	17/19 (89)	1.50
Snuderl et al[14] 2010	20/20 (100)	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)	10/20** (50)	0.38
Tomita et al[11] 2009	27/27 (100)	CHOP or CODOX-M/IVAC or HyperCVAD (+ R, n = 14; -R, n = 8)††	6/23 (26)††	0.50‡‡

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, cytarabine, and methotrexate; CYVE, cytarabine and etoposide; Bu, busulfan; Cy, cyclophosphamide; DHAP, dexamethason, high-dose cytarabine, and cisplatin, TBI, total body irradiation; MTX, methotrexate; CyclOBEAP, cyclophosphamide, vincristine, bleomycin, etoposide, doxorubicin, prednisone; HD, high dosage; R-ICE, rutuximab plus ifosfamide, carboplatin, and etoposide plus rituximab; ASCT, autologous stem cell transplantation; EPOCH, rituximab plus etoposide, doxorubicin, vincristine, prednisone, and cyclophosphamide; 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.

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How can the dismal outcome in DH lymphomas be explained?

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

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

Second, the synergistic action of *MYC* and *BCL2* might be responsible for this behavior. Observations in transgenic mice (see "Oncogenes involved in DH lymphomas" and "Timing and synergy of translocations in DH lymphomas") 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 patients with other DLBCL.[12]

Third, it could be that other molecular features play an important role as well. DH lymphomas often have a 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[109] and Seegmiller et al,[116] both indicating that the genomic complexity correlates with survival in lymphomas with a *MYC* rearrangement. Interestingly, on the basis of their biologic functions, 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 because most of these lymphomas in fact might represent *BCL2*$^+$/*BCL6*$^+$/*MYC*$^+$ TH lymphomas.[11,117]

Clinicopathologic aspects of other DH lymphomas

In the Mitelman database[8] as analyzed by us, 34 DH lymphoma cases had a *CCND1*$^+$/*MYC*$^+$ combination (Table 2; Figure 2). *CCND1*$^+$/*MYC*$^+$ DH cases accounted for 5% of all MCLs. 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 cyclin D1 protein expression in tissue sections, most of these cases were readily diagnosed as MCL. An overrepresentation of such cases in the database may have also occurred because leukemic MCLs are possibly more frequently karyotyped than nonleukemic MCLs, and most of these cases presented with overtly leukemic disease. Intriguingly, some *CCND1*$^+$/*MYC*$^+$ DH cases had aberrant expression of CD10 and BCL6, which parallels the observation that most *MYC*-rearranged DLBCL also express CD10. Because most cases were studied on leukemic cells, only few cases have been documented for the Ki67 proliferation index.[99,118,119] Six of 8 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 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 was 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, MCLs with involvement of 8q24 tend to have an aggressive clinical course, the average survival of *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, (see "Published DH lymphomas" and supplemental Table 2).

DH lymphomas and "B-cell lymphomas, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma"

According to 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 biologic and clinical reasons should not be classified as BL 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 3 other problematic issues with respect to the diagnosis of BL versus DLBCL that justified this category:

First, the most problematic area is formed by non-DH lymphomas that are diagnosed as DLBCL but nonetheless share several morphologic and immunophenotypical features with BL, in particular a cohesive growth pattern, a very high Ki-67 proliferation index, and expression of GC associated proteins such as 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 *MYC-IG* breakpoint, do not have any other breakpoint, and have a gene expression profile similar to BL and, therefore, probably should be better diagnosed as BL.[122] In contrast, for adult patients no data are available for large series of DLBCLs collected in population-based studies in which all ancillary tests to exclude BL or DH lymphomas have been applied. According to the Nordic Lymphoma Group,[123] which more or less reflects a population-based registry, 10% of all 185 DLBCL cases had a Ki-67 proliferation index of $\geq 90\%$. This should mean that after exclusion of DH lymphomas and DLBCL with a phenotype not compatible with BL, far $< 10\%$ of all DLBCL are problematic in this respect.[109,124]

Second, BL has a characteristic expression of CD20, CD10, BCL6 and the absence of BCL2 protein, whereas MUM1/IRF4 may be expressed at low levels. However, in $\leq 20\%$ of all otherwise classical BLs some immunophenotypic abnormalities have been reported, for instance weak expression of BCL2 protein in 0%-20%[109,125] or aberrant expression of T-cell markers such as CD4 or CD5.[126] As already discussed in this review, such cases should only be accepted as BL after vigorous exclusion of a DH lymphoma.

Finally, in $\sim 10\%$ of all lymphomas that are otherwise indistinguishable from BL, including endemic and pediatric BLs, a *MYC* breakpoint is not detectable by current FISH methods. It might be considered to restrict the diagnosis of BL 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 because certain *MYC* breakpoints are missed with the current (FISH) methods and because in rare cases of BL a similar high MYC expression can be induced by down-regulation of miRNA-34B.[127]

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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 DH, has an important prognostic value,[109,108,114,117,128,129] 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

DH and TH 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 BL with a *MYC* breakpoint represent DH lymphomas. This implies that the studies that only focused on the effect of *MYC* breakpoints in DLBCL have to be reconsidered. Most DHs have a $BCL2^+/MYC^+$ combination, and most $BCL6^+/MYC^+$ DH lymphomas represent $BCL2^+/BCL6^+/MYC^+$ TH lymphomas. $CCND1^+/MYC^+$ DH lymphomas may be more frequent than anticipated and should receive more attention.

In view of the frequency of these aberrations and their clinical effect, it seems timely to test all aggressive B-cell lymphomas, including the MCLs with a high-proliferation index or blastic morphology, for *MYC* (and *MYC-IG*) breakpoints by FISH. In selected cases *BCL2* and *BCL6* FISH tests should be performed as well, for instance in those cases with a *MYC* breakpoint and concomitant BCL2 protein expression. A $BCL2^+/MYC^+$ DH lymphoma should be considered in all aggressive and highly proliferating B-cell lymphomas with a distinct GC B phenotype in combination with BCL2 expression, in particular when the patient presents with extensive disease, including bone marrow or CNS involvement or both. However. these parameters are insufficient to identify all cases. Moreover, individual morphologic and immunophenotypical parameters may have a low reproducibility. Although we realize that detection of *MYC*, *BCL2*, and *BCL6* breakpoints does not reflect all biologic aspects of these complex tumors, we suggest to perform these assays until more biologic data and better tests become available.

Patients with DH lymphoma 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 ≥ 2 oncogenes involved but also the high genomic complexity in most of these tumors.

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Authorship

Contribution: S.M.A. performed literature and database searches and 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.C.K.-N. and E.-J.B. contributed to the design of the manuscript; P.M.K. contributed to the design of the manuscript and wrote parts of it.

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The current affiliation for E.-J.B. is Department of Surgery, Maasstad Hospital, Rotterdam, The Netherlands.

Correspondence: Sieste M. Aukema, Department of Pathology and Medical Biology, University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands; e-mail: s.m.aukema@student.rug.nl.

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Double-hit B-cell lymphomas

Sietse M. Aukema, Reiner Siebert, Ed Schuuring, Gustaaf W. van Imhoff, Hanneke C. Kluin-Nelemans, Evert-Jan Boerma and Philip M. Kluin

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