Pathogenetic and Clinical Implications of Non-Immunoglobulin; BCL6 Translocations in B-Cell Non-Hodgkin’s Lymphoma

Hitoshi Ohno

Chromosomal translocations affecting band 3q27, where BCL6 gene is located, are among the most common genetic abnormalities in non-Hodgkin’s lymphoma of B-cell type (B-NHL). The BCL6 gene encodes a BTB/POZ zinc finger transcription factor, which exerts repressive activity by recruiting corepressor molecules. The 3q27/BCL6 translocation is unique in that it can involve not only immunoglobulin (Ig) genes but also non-Ig chromosomal loci as a partner. To date, around 20 non-Ig partner genes have been identified. As a result of non-Ig; BCL6 translocations, many types of regulatory sequences of each partner genes substitute for the 5′ untranslated region of BCL6, and the rearranged BCL6 comes under the control of the replaced promoter. The introduction of non-Ig; BCL6 constructs into transformed cells led to high-level Bcl-6 protein expression in the nucleus, while BCL6 mRNA levels in clinical materials of diffuse large B-cell lymphoma (DLBCL) with non-Ig; BCL6 translocations were unexpectedly low. A comparative study suggested that non-Ig; BCL6 translocation and a low level of BCL6 mRNA expression are concordant indicators of a poor clinical outcome in cases of DLBCL. The coexistence of a non-Ig; BCL6 translocation with t(14; 18)(q32; q21) in a single clone did not significantly affect the clinical features of follicular lymphoma. The pathogenetic and clinical implications of non-Ig; BCL6 translocations in B-NHL subtypes may not be identical to those of Ig; BCL6. (J Clin Exp Hematopathol 46(2): 43-53, 2006)

Keywords: non-Hodgkin’s lymphoma of B-cell type, BCL6 gene, non-immunoglobulin gene, prognostic marker, concurrent translocations

INTRODUCTION

Chromosomal translocations and rearrangements of oncogenes located at breakpoints are observed in a large proportion of patients with non-Hodgkin’s lymphoma of B-cell type (B-NHL). These genetic aberrations, in general, involve immunoglobulin (Ig) gene loci as a partner. As a result of the translocation, the oncogene is transcriptionally deregulated under the influence of the juxtaposed Ig regulatory sequences. t(3; 14)(q27; q32) and its variants t(2; 3)(p11; q27) and t(3; 22)(q27; q11), involving Ig heavy chain (IgH), κ light chain (IgLκ) and λ light chain (IgLλ) genes, respectively, were first reported to be detected in 6.3% of cytogenetically analyzed B-NHLs. By analogy with previously well-characterized translocations, an oncogene was expected to be located at 3q27, and three groups independently cloned BCL6 (B-cell CLL/lymphoma 6) gene on this particular chromosomal band. A group at Columbia University headed by Dr. Dalla-Favera very recently engineered transgenic mouse strains harboring a BCL6 gene driven by the IgH Im promoter, mimicking t(3; 14)(q27; q32), using the knock-in strategy. The mice at between 15 and 20 months of age developed B-cell tumors showing features of diffuse large B-cell lymphoma (DLBCL) at a frequency of 36% to 62%, confirming clearly the oncogenic role of Ig; BCL6 translocations in the pathogenesis of B-NHL.

In contrast to other B-NHL-specific translocations, 3q27/ BCL6 translocation is unique in that it can involve not only Ig gene loci but also non-immunoglobulin (non-Ig) chromosomal loci as a partner. Molecular cloning of these translocations revealed that a gene is located at each partner locus and rearranged with BCL6 so that the normal regulatory sequences of BCL6 are replaced with those of the partner gene. Some of these translocations are not random but recurrent, indicating that non-Ig; BCL6 translocations as well as Ig; BCL6 play a pathogenetic role in B-NHL. In this review, I first describe the molecular anatomy of non-Ig; BCL6 translocations. Second, I present our experiments to show that what
extent non-Ig; BCL6 translocations affect the level of Bcl-6 protein expression. Finally, I discuss the clinical implications of this unique type of translocation in subtypes of B-NHL.

THE BCL6 GENE AND ITS PRODUCT

The BCL6 gene spans 24,310 bases and contains 11 exons, generating 4 types of splicing patterns (http://www.genecards.org/cgi-bin/carddisp?BCL6). The ATG signal for the initiation of protein synthesis is within exon 3 and is followed by an open reading frame. The 3q27/BCL6 translocation occurs within the major translocation cluster (MTC) spanning the non-coding exon 1 and intron 1; in a majority of cases, breakpoints are located immediately 3’ of exon 1.5,9. The translocation, therefore, does not interrupt the protein-coding region of BCL6.

The Bcl-6 protein, consisting of 706 amino acids with a calculated molecular weight of 78,846 Da, is a sequence-specific transcription factor that can repress transcription from promoters containing its DNA-binding site (Fig. 1).10 The C-terminal region of Bcl-6 contains six C2-H2 type zinc fingers, each separated by a conserved stretch of seven amino acids. The translocation, therefore, does not interrupt the protein-coding region of BCL6.

![Diagram of BCL6 protein domains and interactions](image)

**Fig. 1.** Functional domains of the Bcl-6 protein.10 The domains are involved in protein-protein interaction, transcriptional repression of target genes, post-translational modification and DNA binding. ETO/MTG8 binds to the fourth zinc finger.10,11 MTA3/NuRD interacts with the region spanning aa 141 to 507.10,11 PEST, where P = proline, E = glutamic acid, S = serine and T = threonine; KKYK, where K = lysine and Y = tyrosine.

The BTF/POZ domain (broad-complex, tramtrack, and bric-a-brac/pox virus and zinc finger) at the N-terminus is a conserved 120-amino acid motif, which is found in 5 to 10% of zinc finger proteins.11 The primary function of the BTF/POZ domain appears to be the mediation of protein-protein interactions. It has been shown that the repressive effect of Bcl-6 on the target gene is exerted via the recruitment of SMRT (silencing mediator of retinoid and thyroid receptor), NCoR (nuclear receptor corepressor) and BCoR (BCL6 corepressor) corepressors.12-15. Crystallographic analysis of the BTF/POZ domain revealed that it forms a butterfly-shaped homodimer to generate a ‘lateral groove’ motif that interfaces with a 17-residue sequence (BBD motif, Bcl-6 binding domain) of SMRT.12,15. A fusion protein that contains the HIV-TAT protein transduction domain and the BBD sequence can penetrate the cell membrane and bind to Bcl-6, thereby blocking the BTF/POZ-mediated recruitment of SMRT.16

The middle portion of Bcl-6 contains a second domain required for the repressive transcriptional activity (Fig. 1). Bereschchenko et al. showed that the KKYK motif within the PEST sequence is targeted by p300-mediated acetylation. This post-translational modification disrupts the ability of Bcl-6 to recruit histone deacetylase (HDAC), thereby hindering its capacity to repress transcription.

Targeted inactivation of BCL6 in the mouse germline leads to impaired production of secondary IgG antibody against T-cell-dependent antigens and the spleen of the BCL6−/− mouse lacks germinal center (GC) formation.18-20. A cDNA array analysis along with loss-of-function experiments revealed a set of genes that are negatively regulated by Bcl-6.21. The BLIMP1 (B-lymphocyte-induced maturation protein 1) gene, which is a representative target gene, plays a key role in the differentiation of B-cells into plasma cells by turning off the entire mature B-cell gene expression program.22,23. It is presumed that BCL6 is the master gene for the generation by B-cells of a GC, acting to modulate the transcription of genes involved in cell proliferation, differentiation and apoptosis.

MOLECULAR ANATOMY OF NON-Ig; BCL6 TRANSLocations

To determine the partner of the BCL6 translocation, two polymerase chain reaction (PCR)-based approaches have been applied, i.e. 5’-rapid amplification of cDNA ends (5’-RACE) and long-distance inverse (LDI-) PCR (Fig. 2).9,24,25. Both methods isolate sequences adjacent to the junction, a database search of which leads to identification of the partner gene.

Table 1 lists non-Ig partners that have been identified to date.9,24-35. These include the genes for a transcription factor, serine/threonine-protein kinase, cytokine receptor, Ras small...
GTPase, heat shock proteins and so on. In spite of this marked diversity of protein products, there are common features in the molecular anatomy of non-Ig; BCL6 translocations. First, the gene fusion occurs in the same transcriptional orientation; second, the breakpoint on the partner gene is located in close proximity to the promoter sequence; and third, the complete sequence of the promoter is fused upstream of the coding region of BCL6 on the der(3) chromosome (Fig. 3). As the result of translocation, many types of regulatory sequences of each partner gene substitute for the 5′ untranslated region of BCL6, and the rearranged BCL6 comes under the control of the replaced promoter (promoter substitution). The translocated allele generates chimeric transcripts composed of 5′ sequences of the partner followed by the open reading frame of BCL6, which is readily detectable by reverse transcriptase-mediated PCR.

Although the transcriptional control of these non-Ig genes has not been fully determined, it should be noted that some genes are transcriptionally activated by a variety of stimuli, including cell cycle control (H4), changes in the physical environment (HSP89α and HAP90β), and response to cytokines (PIM1 and CIITA). Considering that GC B-cells, in which the BCL6 translocation is presumed to occur, proliferate rapidly in response to antigen, it is likely that a BCL6 gene affected by the translocation is inappropriately expressed during B-cell proliferation.

There is evidence to suggest that the somatic hypermuta-
tion (SHM) machinery of the Ig gene is involved in the development of non-Ig: BCL6 translocations. SHM of BCL6 occurs in a large proportion of memory B-cells isolated from normal individuals, in GC-B-cells from reactive tonsils, and across the spectrum of GC/post-GC type B-cell tumors. SHM of BCL6 occurs in a large proportion of memory B-cells isolated from normal individuals, in GC-B-cells from reactive tonsils, and across the spectrum of GC/post-GC type B-cell tumors. The mutations are clustered within the first exon-intron boundary of the gene, which overlaps with the MTC, suggesting that SHM and translocations involving BCL6 are mediated by common molecular mechanisms. On the other hand, PIM1 and RHOH, both of which are non-Ig partners (Table 1), are mutated in DLBCL and the regions involved in the mutation match those in the translocation. These observations suggest that the SHM machinery, which generates double-stranded breaks of DNA, targets both the BCL6 and non-Ig partners of GC-B-cells, thereby predisposing these genes to exchanges of genetic material.

DEREGULATED EXPRESSION OF BCL-6 PROTEIN BY HISTONE H4; BCL6 TRANSLOCATION

We showed that t(3; 6)(q27: p21) results in the fusion of BCL6 with a particular histone H4 gene (HUGO nomenclature: HIST1H4I) on 6p21. The H4 gene is composed of a single exon followed by a terminal palindrome.

Transcriptional control of the histone H4 gene is mediated by two multipartite proximal promoter elements (Sites I and II), the activity of which is augmented by two distal domains (Sites III and IV). The Site II-equivalent of the H4 gene contains consensus binding sites for the transcription factors HNF-M, HNF-D and HNF-P. These Site II-binding proteins, in addition to other co-regulatory molecules, contribute to enhancing the H4 gene transcription at the G1/S phase transition. On the other hand, the terminal palindrome sequence initially contributes to cleavage of the primary H4 gene transcript and mediates mRNA destabilization at the end of S phase. The sequence approximately 16 bp downstream of the palindrome is also essential and through base-pairing interactions mediates the U7 snRNP-dependent processing of the mRNA 3′ end.

We cloned 5 H4; BCL6 fusion genes and found that the breakpoints on H4 were distributed within the 3′ half of the H4 protein-coding region or in the vicinity of the palindrome. Therefore, the mechanism of 3′ end formation of H4 is perturbed and the resulting fusion mRNAs with BCL6 are predicted to be processed like normal polyadenylated mRNAs. It appears then that the deregulation of Bcl-6 protein expression is facilitated by ‘capturing’ sequences that support cell cycle control of H4 gene transcription during the G1/S phase transition.

Table 1. Non-Ig partner genes of BCL6 translocation

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene product</th>
<th>Chromosomal locus</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBNL1 (KIAA0428)</td>
<td>Muscleblind-like protein (Triplet-expansion RNA-binding protein)</td>
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<tr>
<td>TFRC</td>
<td>Transferrin receptor (p90, CD71)</td>
<td>3q26.2- qter/3q29</td>
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<tr>
<td>ST6GAL1 (CD75)</td>
<td>Sialyltransferase 1 (beta-galactoside alpha-2, 6-sialytransferase)</td>
<td>3q27-q28/3q27.3</td>
<td>34</td>
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<td>EIF4A2</td>
<td>Eukaryotic translation initiation factor 4A, isoform 2</td>
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<td>28</td>
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<td>RHOH (RhoH, TTF)</td>
<td>Rho-related GTP-binding protein RhoH (GTP-binding protein TTF)</td>
<td>4p13/4p14</td>
<td>24, 43</td>
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<td>HSPCB (HSP90b)</td>
<td>Heat shock 90kDa protein 1, beta</td>
<td>6p12/6p21.1</td>
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</tr>
<tr>
<td>PIM1</td>
<td>Pim-1 oncogene product</td>
<td>6p21.2</td>
<td>9, 28, 43</td>
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<tr>
<td>SFRS3 (SpR20)</td>
<td>Splicing factor, arginine/serine-rich 3 (Pre-mRNA splicing factor SRP20)</td>
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<td>32</td>
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<tr>
<td>HIST1H4I (H4/m)</td>
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<td>U50HG</td>
<td>Small nucleolar RNA</td>
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<td>30</td>
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<td>ZNFN1A1 (IKAROS)</td>
<td>Ikaros (zinc finger protein)</td>
<td>7p13-p11.1</td>
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<td>GRHPR (GLXR)</td>
<td>Glyoxylate reductase/hydroxybutyrate reductase</td>
<td>9q12/9p13.2</td>
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<td>POU domain class 2, associating factor 1 (B-cell-specific coactivator OB-1) (OCT binding factor 1) (BOB-1) (OCT-binding factor 1) (BOB-1) (OCA-B)</td>
<td>11q23.1</td>
<td>25</td>
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<tr>
<td>LRMP (JAW1)</td>
<td>Lymphoid-restricted membrane protein</td>
<td>12p12.1</td>
<td>34</td>
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<td>GAPDH</td>
<td>Glyceroldehyde-3-phosphate dehydrogenase</td>
<td>12p13.31</td>
<td>35</td>
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<tr>
<td>NACA</td>
<td>Nascent-polypeptide-associated complex alpha polypeptide</td>
<td>12q23-q24.1/12q13.3</td>
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<td>LPC1</td>
<td>L-plastin (Lymphocyte cytotoxic protein 1) (LCP-1) (LC64P)</td>
<td>13q14.3/13q14.13</td>
<td>26</td>
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<tr>
<td>HSPCA (HSP90a)</td>
<td>Heat shock 90kDa protein 1, alpha</td>
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<td>9, 31</td>
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<td>IL21R</td>
<td>Interleukin-21 receptor</td>
<td>16p11/16p12.1</td>
<td>33</td>
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<tr>
<td>CIITA</td>
<td>MHC class II transactivator</td>
<td>16p13/16p13.13</td>
<td>9, 28</td>
</tr>
</tbody>
</table>

1 LocusLink and/or Ensembl cytogenetic band
2 not included in the HUGO gene nomenclature database
cell cycle, while simultaneously inactivating the regulatory sequences required for post-transcriptional control of H4 gene expression.

To determine the level of Bcl-6 protein expression directed by the H4; BCL6 fusion gene as compared with the germ-line BCL6, we constructed expression plasmids that mimicked the structure of t(3; 6)(q27; p21) (Fig. 4A)37. Transient transfection of the plasmids into COS-7 cells resulted in transcription of H4; BCL6 fusion mRNA that had the same structure as mRNA from clinical materials with t(3; 6)(q27; p21). Comparison of the levels of Bcl-6 expression revealed that H4; BCL6-transfected cells produced markedly more Bcl-6 than cells transfected with a plasmid carrying BCL6 driven by its normal promoter (Fig. 4B). We next subjected the COS-7 cells to indirect immunofluorescence microscopy using a polyclonal antibody against Bcl-6 and found that H4; BCL6-transfected cells displayed bright nuclear staining with a characteristic granular pattern (Fig. 4C); the granules have been shown to contain SMRT and N-CoR co-repressors13. The introduction of a series of deletion mutants that lacked the Site II sequences led to a reduction in the expression of Bcl-6 to the basal level (Fig. 4C). These findings indicate that H4; BCL6 gene fusion leads to enhanced Bcl-6 protein expression, which is promoted by the H4 regul

![Diagram of Bcl-6 expression plasmids](image)

**Fig. 4.** Construction of H4; BCL6 fusion gene mimicking t(3; 6)(q27; p21) and the effect of transfection into COS-7 cells37. (A) Diagram of the Bcl-6-expression plasmids driven by the SV40 promoter, normal BCL6 promoter, and H4; BCL6 fusion gene of case no. 457. Transcription initiated from the H4; BCL6 fusion gene was contiguous with the BCL6 exon2 at the cryptic 5' splice-donor site (▲). A series of deletion mutants of the last fragments was generated by digestion with exonuclease III and mung bean nuclease. IRF2, interferon regulatory factor 2; B, BamHI; X, XbaI; H, HindIII; G, BglII; and S, SacI. (B) Western blot analysis of COS-7 transfectants for Bcl-6 expression. FL-218 is a follicular lymphoma cell line. (C) Effect of the promoters of H4 upon the level of Bcl-6 expression. The COS-7 transfectants were subjected to Western blot analysis and indirect immunofluorescence microscopy. (D) Scatter plot analysis comparing the expression profile of H4; BCL6-transfected cells (Y axis) to that of normal BCL6 promoter-transfected cells (X axis). Alteration of the levels of expression was determined by the Atlas Human Array (Clontech) and ArrayGauge software (Fuji Photo Film). Up- or down-regulation by > 1.5-fold, indicated by two diagonal lines, was considered significant.
primary target genes negatively regulated by Bcl-6. These included latter reference cells (Fig. 4D). The down-regulated genes fold under-expressed in the former cells as compared with the latter reference cells (Fig. 4D). The down-regulated genes included BLIMP1 and cyclin D2 (CCND2), both of which are primary target genes negatively regulated by Bcl-651. These experiments provided clear evidence that the primary target genes negatively regulated by Bcl-6.

PROGNOSTIC SIGNIFICANCE OF NON-Ig; BCL6 TRANSLOCATIONS IN DLBCL

The correlation between BCL6 translocations and clinical features of DLBCL has been a subject of controversy. An earlier study showed that BCL6 rearrangements determined by Southern blotting occurred more frequently in extranodal DLBCL than in node-based diseases and correlated with a favorable clinical outcome46. However, later studies failed to find a statistically significant impact of BCL6 translocations on the clinical outcome of DLBCL47-50.

We studied whether the partner in BCL6 translocations influences the clinical behavior and/or treatment outcome of DLBCL. Of 43 DLBCL patients having BCL6 abnormalities, 26 had Ig; BCL6 translocation, while 15 had non-Ig; BCL6. The remaining 2 had a deletion of a > 1 kb segment encompassing exon 1 of BCL6. Although there were no significant differences in pretreatment clinical features between the 26 patients with Ig; BCL6 translocation and the 17 patients with non-Ig; BCL6, including the 2 with a deletion, overall survival (OS) of the non-Ig; BCL6 group was inferior to that of the Ig; BCL6 group (Fig. 5A). Fourteen patients of the non-Ig; BCL6 group died within 2 years, while 5 patients of the Ig; BCL6 group have been disease-free for some time. The estimated 2-year OS of the Ig; BCL6 and non-Ig; BCL6 groups was 58.1% and 17.6% (P = 0.003), respectively. Although the total number of patients analyzed was quite small, our study suggested that non-Ig; BCL6 translocation is an indicator of poor prognosis in DLBCL, and additional studies of larger patient populations are warranted.

A cDNA microarray analysis revealed that DLBCL patients with the GC B-cell-like (GCB) pattern of gene expression have a significantly better OS than those with the activated B-cell-like (ABC) expression profile52. BCL6 is a representative gene of the GCB-type signature and high-level expression of BCL6 at both the mRNA and protein levels has been shown to be a predictor of a favorable treatment outcome in cases of DLBCL. To address the relationship between our finding that DLBCL with non-Ig; BCL6 translocation has a worse prognosis than DLBCL with Ig; BCL6 and the fact that the level of BCL6 expression is related to clinical outcome, we compared the levels of BCL6 mRNA between the two groups. The amount of mRNA measured by real-time quantitative PCR was divided by that of the GAPDH. The levels of the last group were significantly higher than those of the former two DLBCL groups. Ramos, a Burkitt lymphoma cell line; FL-18 and FL-218, follicular lymphoma cell lines; YM, HBL-2 and KIS-1, DLBCL cell lines.

Fig. 5. Prognostic impact of non-Ig; BCL6 translocation on DLBCL, and BCL6 mRNA levels in DLBCL subgroups stratified by BCL6 translocation*. (A) Overall survival curves of DLBCL patients with Ig; BCL6 or non-Ig; BCL6 translocation. The latter group included two patients having a deletion within the MTC region. (B) BCL6 mRNA levels of B-NHL cell lines, clinical materials of DLBCL patients with Ig; BCL6 or non-Ig; BCL6 translocation, and those lacking BCL6 translocation. The levels of the last group were significantly higher than those of the former two DLBCL groups. Ramos, a Burkitt lymphoma cell line; FL-18 and FL-218, follicular lymphoma cell lines; YM, HBL-2 and KIS-1, DLBCL cell lines.
non-Ig; BCL6 group (n = 8; range, 0.4-1.9; mean, 1.0)\textsuperscript{51}, and the level of the latter group was below the threshold (= 1.3) for a poor prognosis determined by Lossos et al\textsuperscript{23} (Fig. 5B). This observation suggests that high versus low-level BCL6 mRNA expression and an Ig versus non-Ig partner of BCL6 translocation are concordant prognostic indicators of DLBCL. Further study is required to determine whether BCL6 translocation are concordant prognostic indicators of non-Ig DLBCL. The level of the latter group was below the threshold (\textsuperscript{5B}). This observation suggests that high versus low-level BCL6 expression and/or BCL6 translocation were concordant prognostic indicators of development of B-NHL. However, 3q27/BCL6 translocations involved other translocations in a single clone\textsuperscript{55,56}, including t(8;14)(q24;q32) and t(14;18)(q32;q21) involving c-MYC and BCL2, respectively, making the role of the 3q27/BCL6 translocation in the pathogenesis of B-NHL unclear.

We conducted a series of LD/LDI-PCR assays, which readily detect rearrangements involving c-MYC, BCL2 and BCL6\textsuperscript{57,58}, using clinical materials of B-NHL, and found that a total of 14 patients carried a rearrangement of BCL6 concurrently with rearrangements of c-MYC and/or BCL2; 10 patients had both BCL2 and BCL6 rearrangements, 3 had both c-MYC and BCL6 rearrangements, and the remaining patient had rearrangements of all 3 genes (Table 2). The constant genes affected by c-MYC; Igf, which is the molecular equivalent of t(8;14)(q24;q32), were Cy. The BCL2 genes in 10 patients were rearranged at the 3\textsuperscript{rd}, i.e. from the major breakpoint cluster region (MBR) through to the minor cluster region (mcr), while 1 patient had a 5\textsuperscript{th} breakpoint\textsuperscript{59}. BCL6 translocations involved Ig genes as partners in 5 patients, and non-Ig genes as partners in 8 patients. The remaining patient had a deletion of a 1.2-kb segment within the MTC region of BCL6\textsuperscript{60}.

Seven of the 10 patients with both BCL2 and BCL6 rearrangements had follicular lymphoma (FL), while all of the

### Table 2. Clinical and molecular features of B-NHL patients concurrently carrying rearrangements of BCL6 and of c-MYC and/or BCL2

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/ Sex</th>
<th>WHO\textsuperscript{1} Stage/ PS</th>
<th>Involved organs</th>
<th>LDH (IU)</th>
<th>CD10</th>
<th>sIg</th>
<th>Immuno-phenotype</th>
<th>Treatment\textsuperscript{1}</th>
<th>Outcome\textsuperscript{4}</th>
<th>Survival (days)\textsuperscript{5}</th>
<th>Rearrangement</th>
<th>BCL6 Breakpoint\textsuperscript{6}</th>
<th>Constant gene \textsuperscript{7}</th>
<th>Partner gene \textsuperscript{7}</th>
<th>c-MYC \textsuperscript{7}</th>
<th>BCL2 \textsuperscript{7}</th>
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<tr>
<td>644</td>
<td>74/F</td>
<td>FL, IV/1</td>
<td>LN, BM, spleen</td>
<td>265</td>
<td>−</td>
<td>μλ</td>
<td>BCL6, BCL2</td>
<td>CHOP, other</td>
<td>PR</td>
<td>2822+</td>
<td>−</td>
<td>150 bp-MBR</td>
<td>RHDL TTF</td>
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<td>514</td>
<td>43/F</td>
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<td>LN, BM</td>
<td>208</td>
<td>−</td>
<td>μλ</td>
<td>BCL6, BCL2</td>
<td>CHOP, radiation</td>
<td>CR-R-CR-R-CR</td>
<td>5146+</td>
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<td>652</td>
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<td>BCL6, BCL2</td>
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<td>CHOP, radiation, other</td>
<td>PR</td>
<td>1860+</td>
<td>−</td>
<td>150 bp-MBR</td>
<td>CD34</td>
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<tr>
<td>1053</td>
<td>70/M</td>
<td>FL, IV/0</td>
<td>LN, BM, stomach</td>
<td>302</td>
<td>+</td>
<td>μλ</td>
<td>BCL6, BCL2</td>
<td>CHOP, radiation, other</td>
<td>PR</td>
<td>1458+</td>
<td>−</td>
<td>150 bp-MBR</td>
<td>3q25-27</td>
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<tr>
<td>422</td>
<td>69/F</td>
<td>DLBCL, IV/2</td>
<td>Bone</td>
<td>412</td>
<td>+</td>
<td>μλ</td>
<td>CHOP</td>
<td>CHOP</td>
<td>CR</td>
<td>4054+</td>
<td>C\textsubscript{y}</td>
<td>−</td>
<td>Igf</td>
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<td>742</td>
<td>61/M</td>
<td>DLBCL, IV/1</td>
<td>LN, spleen, tonsils</td>
<td>1825</td>
<td>+</td>
<td>μλ</td>
<td>CHOP</td>
<td>CHOP, other</td>
<td>PR-B-PD</td>
<td>397</td>
<td>−</td>
<td>5\textsuperscript{th} - BCL2</td>
<td>IL21R</td>
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<td>854</td>
<td>86/M</td>
<td>DLBCL, IV/1</td>
<td>LN</td>
<td>549</td>
<td>−</td>
<td>ND</td>
<td>Radiation, VEPA</td>
<td>PR-B-PD</td>
<td>229</td>
<td>C\textsubscript{y}</td>
<td>−</td>
<td>HSRPC</td>
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<td>893</td>
<td>66/M</td>
<td>DLBCL, IV/2</td>
<td>LN, stomach, pleural effusion</td>
<td>1530</td>
<td>+</td>
<td>μλ</td>
<td>CHOP</td>
<td>CHOP, other</td>
<td>PR-B-PD</td>
<td>164</td>
<td>C\textsubscript{y}</td>
<td>−</td>
<td>IgH</td>
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<td>1230</td>
<td>49/M</td>
<td>DLBCL, III/1</td>
<td>LN</td>
<td>195</td>
<td>+</td>
<td>−</td>
<td>CHOP, radiation, other</td>
<td>PR</td>
<td>1045+</td>
<td>C\textsubscript{y}</td>
<td>−</td>
<td>mcr</td>
<td>Igf3,</td>
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<td>1262</td>
<td>53/M</td>
<td>DLBCL, II/0</td>
<td>LN, tonsil</td>
<td>180</td>
<td>+</td>
<td>αυ/ε</td>
<td>CHOP, radiation, other</td>
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<td>451</td>
<td>−</td>
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<td>IgH</td>
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<td>1263</td>
<td>54/F</td>
<td>DLBCL, IV/2</td>
<td>LN, BM, skin, bones</td>
<td>310</td>
<td>+</td>
<td>μλ</td>
<td>BCL6, BCL2</td>
<td>CHOP</td>
<td>PR</td>
<td>187+</td>
<td>−</td>
<td>mcr</td>
<td>MEF2C</td>
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1: FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; 2: LN, lymph node; BM, bone marrow; 3: VEPA and CHOP, vincristine, cyclophosphamide, doxorubicin, prednisolone; LSG9, multi-agent combination chemotherapy designed by the Japan Lymphoma Study Group, 4: CR, complete response; PR, partial response; R, relapse; PD, progressive disease. 5: Vital status was followed through January 2004. 6: The BCL2 breakpoints were as defined previously58. 7: HNPRC, heterogeneous nuclear ribonucleoprotein C (C1/C2) on 14q11.1; MEF2C, myocyte enhancer factor 2C, MADS box transcription enhancer factor 2, polypeptide C on 5q14
4 patients with c-MYC rearrangement had DLBCL. In particular, of 7 FL patients, 5 had a non-Ig; BCL6 translocation; they had advanced-stage disease at presentation and 3 were positive for CD10 expression. All but one patient with FL showed indolent clinical behavior, although initial treatment with a doxorubicin-containing regimen failed to achieve a complete clinical response. Thus, in contrast to the general consensus that multiple translocations can occur at the time of transformation from low- to high-grade disease, the coexistence of a non-Ig; BCL6 translocation with a BCL2 rearrangement did not significantly affect the clinical features of FL defined by t(14; q32; q21). In contrast, the pretreatment parameters as well as treatment outcome of DLBCL patients varied. Thus, a dual/triple rearrangement does not necessarily result in synergic effects on the malignant phenotype of B-NHL subtypes.

CONCLUSION

We showed that the introduction of expression plasmids mimicking the non-Ig; BCL6 fusion genes into transformed cells led to high levels in GC B-cells. When a GC B-cell acquires a non-Ig; BCL6 translocation, transcription of BCL6 mRNA from the translocated allele is further enhanced under the GC micro-environment. Once the neoplastic B-cells gain growth advantage over normal cells, the over-expression of Bcl-6 may be down-regulated by an unknown mechanism.

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