The clinical presentation and prognosis of diffuse large B-cell lymphoma with t(14;18) and 8q24/*c-MYC* rearrangement

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ABSTRACT

Background and Objectives

Diffuse large B-cell lymphomas (DLBCL) are common lymphomas that have been classified into three subgroups on the basis of their patterns of gene expression. The aim of this study was to characterize the clinical, biological, immunophenotypic and cytogenetic features of DLBCL with concurrent t(14;18) and *8q24/c-MYC* rearrangement.

Design and Methods

Sixteen cases of DLBCL with the dual translocation were identified between 1998 and January 2006. The clinical features of these cases were examined and morphological, immunohistochemical, flow cytometric and cytogenetic analyses were performed.

Results

All patients had aggressive features: B symptoms (81%), ECOG performance status >2 (81%), elevated lactate dehydrogenase (100%), stage IV disease (100%) with at least one extra-nodal localization (bone marrow, blood and central nervous system involvement in 93%, 50% and 50%, respectively) and age-adjusted IPI score of 3 in 81%. Despite intensive chemotherapy regimens (including allogeneic transplants), all patients died of disease progression. Progression-free and overall survival was 4 and 5 months, respectively. Immunophenotyping analysis (CD20, CD10, Bcl-6, Mum-1, Bcl-2 CD138, MIB1, CD19, CD5, CD38 and sIg) was performed and showed DLBCL with a germinal center (GC) profile. Ki-67 staining ranged from 70 to 90%. All cases assessed by cytogenetics analysis [conventional cytogenetic and/or fluorescence *in situ* hybridization (FISH)] had a complex karyotype. In one case, we identified a *8q24/c-MYC* translocation variant never reported in DLBCL before: t(8;9)(q24;p13) and t(14;18)(q32;q21). The BCL-6 rearrangement was investigated by FISH and found to rearranged in four cases.

Interpretation and Conclusions

In conclusion, DLBCL with concurrent t(14;18) and *8q24/c-MYC* rearrangement is a subgroup of GC-DLBCLwith poor outcome. It is worth searching for the coexistence of dual translocations in Bcl-2-positive DLBCL with unusual aggressive presentation.

Key words: high grade non-Hodgkin's lymphoma, bcl-2; c-myc.

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Diffuse large B-cell lymphomas (DLBCL) are classified as a distinct lymphoma entity in the World Health Organization (WHO) classification.[1] In adults, DLBCL account for 30% to 40% of all lymphomas and represent the most frequent group of non-Hodgkin's lymphomas (NHL). DLCBL include various groups of lymphoid neoplasms and have heterogeneous clinical, histological, immunophenotypic, cytogenetic, and molecular features. The International Prognostic Index (IPI) is a clinical and biological score that segregates DLBCL patients into four prognostic groups with distinct median overall survivals.[2] The IPI score takes into account factors that are linked to the patient's characteristics (age, performance status), disease extension and tumor growth (disease stage, lactate dehydrogenase [LDH] level in the serum and extranodal involvement). However, it has been established that differences in clinical features and treatment responses are also dependent on genetic and molecular features that modify disease aggressiveness. Indeed, DLBCL are classified into three subgroups (germinal-center [GC] B-cell, activated B-cell, and primary mediastinal) according to patterns of gene expression.[3] Gene rearrangements (such as *BCL-2*, *BCL-6* or *8q24/c-MYC*) and protein expression (such as Bcl-2 or Bcl-6) have also been shown to have major prognostic value.[4-7]

A tandem t(14;18) and *8q24/c-MYC* rearrangement is a situation referred to as a dual translocation. t(14;18)(q32;q21) is reported to be present in 85% to 90% of cases of follicular lymphoma (FL).[8] The translocation t(14;18) is also found in 10% to 40% of DLBCL.[4,5,9] The translocation between these two chromosomes juxtaposes the *BCL-2* locus (located at 18q21) next to the regulatory regions of the *IGH* (locus at 14q32). This modifies the regulatory region of the proto-oncogene *BCL-2* leading to Bcl-2 overexpression. Bcl-2 is an anti-apoptotic protein and its overexpression opposes mitochondrial apoptotic pathways.[10]

The *c-MYC* gene is located at 8q24. Translocation t(8;14)(q24;q32) was the first recurrent chromosomal abnormality ever reported in lymphoproliferative disorders.[11] It juxtaposes the *c-MYC* locus next to the *IGH* locus, resulting in overexpression of c-Myc protein, a key transcription factor that promotes cell cycling and tumor proliferation. Three recurrent translocations are observed in Burkitt's lymphoma (BL): t(8;14)(q24;q32); t(2;8)(p12;q24) and t(8;22)(q24;q11). However, *8q24/c-MYC* rearrangements are identified in 7% to 15% of GC-derived DLBCL and reported to be predominantly associated with extranodal localizations.[5,12-15] The present study describes the clinical and biological features of 16 cases of *DLBCL* with concurrent t(14;18) and *8q24/c-MYC* rearrangement.

Design and Methods

Case selection and treatment

From 1998 to January 2006, we identified 16 DLBCL cases with a dual translocation. Patients who had human immunodeficiency virus infection and those who had developed a NHL after a solid organ transplants were excluded. This study was approved by the local ethics commission.

The initial staging evaluation included the following assessments: a complete history and physical examination, blood cell counts, complete biochemical profile including LDH level, bone marrow aspiration and/or biopsy, computed tomography scan (thorax, abdomen and pelvis) and lumbar puncture. The clinical staging system was based on the Ann Arbor classification and IPI score was calculated as reported by Shipp *et al.*[2,16]

All patients but one received chemotherapy (with or without rituximab). Nine patients received CHOP, R-CHOP or high-dose CHOP-like (CEEP) regimens.[2] Because of central nervous system (CNS) involvement, seven patients received chemotherapy regimens incorporating high-dose methotrexate (COPADM) followed or not by high-dose cytarabine (CYVE).[20] We planned to perform autologous stem cell transplantation for all patients (under the age of 60 years) in complete or partial remission after initial chemotherapy. In two patients under the age of 50 years old who had HLA matched sibling donors, we performed allogeneic stem cell transplantation upfront. This decision was based on our previous results with DLBCL patients with concurrent t(14;18) and *8q24/c-MYC* rearrangement. DHAP and IVAM regimens were used as salvage chemotherapy.[21,22] Three patients actually underwent autologous stem cell transplantation and two other patients, as described above, underwent allogeneic stem cell transplantation. Because of multiple organs failures at diagnosis, one patient (case 16) received only steroids. Progression-free survival (PFS) and overall survival (OS) times were calculated from the date of identification of the dual translocation.

Morphological analysis

Morphological features were assessed on hematoxylin-eosin-safran-stained histological sections of seven lymph nodes (inguinal, cervical, retroperitoneal), three extranodal (tonsil, skin, thoracic wall) and ten bone marrow paraffin-embedded biopsies obtained at diagnosis. Lymphoid neoplasms were classified according to the WHO Classification proposals of lymphoid tissues (WHO classification) (2001) and subclassified, when possible, according to the Kiel classification as proposed by the WHO classification. Peripheral blood and/or bone marrow aspirate smears (12 cases) and pleural effusion (1 case) stained with Wright-Giemsa were also analyzed and classified according to the WHO classification.[1]

Immunohistochemistry

Immunohistochemistry on paraffin sections was performed in 11 cases. Primary antibodies against the following antigens were used CD10 (56C6) from Novocastra (Newcastle, UK) and CD20 (L26) CD3, Bcl-6 (PG-B6p), Bcl-2 (124), Mum1 (Mum1p), Ki-67 (Mib1)

and CD138 (Mi15) all from DakoCytomation (Trappes, France). Antigen retrieval was carried out by microwaving the slides for 20 minutes in either citrate buffer (pH 6.0) for most antibodies, 1 mM EDTA buffer (pH 8.0) for anti-CD10 and anti-Mum1 antibodies or TRS-H (pH 9.9)(DakoCytomation, Trappes, France) for anti-Bcl-6 antibody. Tissue sections were stained using either a three-step indirect immunoperoxidase technique (LSAB kit, DakoCytomation, Trappes, France) for extramedullary biopsies or a two-step visualization system based on a peroxidase-conjugated dextran backbone (Dako Envision^{+TM} System, DakoCytomation, Trappes, France) for bone marrow biopsies. Immunolabeling with anti-Bcl-6 and anti-Ki-67 antibodies was not assessable on bone marrow sections because of Bouin's fixation. According to Hans's method, immunohistochemical detection of CD10, Bcl-6 and Mum1 was used to classify DLBCL into GC or non-GC groups.[23] The GC subgroup included all CD10$^+$ tumors and those with a CD10$^-$/BCL-6$^+$/MUM1$^-$ immunophenotype. Other cases were assigned to the non-GC group, including MUM1$^+$ tumors, regardless of their Bcl-6 status (CD10$^-$/Bcl-6$^+$/MUM1$^+$ or CD10$^-$/Bcl-6$^-$/MUM1$^+$). Positivity of immunohistochemistry was assessed by estimation of overall positivity.

Flow cytometry analysis

Ficoll-separated mononuclear cells from blood (1 case), pleural effusion (1 case), cerebrospinal fluid (1 case) and bone marrow aspirates (9 cases) were analyzed with a four-color FACSCALIBUR flow cytometer (Becton Dickinson, USA), and antibody combinations including allophycocyanin-conjugated CD19, peridinin chlorophyll protein-conjugated CD45, fluorescein isothiocyanate-conjugated CD10, CD20 and anti-Ig-kappa and phycoerythrin-conjugated CD5, CD38, anti-Ig lambda. The antibodies were purchased from Immunotech (France), Becton Dickinson (USA), and Dakopatts (Denmark). The positivity threshold was set at 30% of CD19$^+$ cells for all markers.

Conventional cytogenetic analysis

Nodal tissues were systematically disaggregated with a scalpel blade to obtain a single-cell suspension. Unstimulated bone marrow aspirates, blood single-cell suspensions or pleural effusions were cultured at 37°C for 17 to 24 hours in RPMI 1640 medium supplemented with 20% fetal calf serum, glutamine and antibiotics. Cells were exposed to colcemid overnight followed by hypotonic treatment (KCl 0.075 M) for 35 min and then fixed with ethanol and acetic acid (3:1). Chromosome analysis was based on RHG-banded metaphases. The karyotypes are described according to the ISCN 1995 nomenclature.[24]

Fluorescence in situ hybridization (FISH)

FISH analysis was performed on cytogenetic preparations, using probes specific for BCL2, BCL6 and MYC rearrangements, as previously described.[25] The t(14;18) translocation was analyzed using the Cα2-COS-Ig10 and PAC-210C12 probes or the dual commercial ISI *IGH/BCL2* probe (Vysis, Downers Grove, IL, USA).[25] The *8q24/c-MYC* rearrangements were analyzed using the 861F11 and 932H6 YAC probes or the dual-color break apart LSI MYC probe (Vysis, Downers Grove, IL, USA).[26] *BCL6* rearrangements were analyzed using the dual-color break-apart LSI *BCL6* probe (Vysis, Downers Grove, IL, USA). Additional FISH probes used to confirm the involvement of partner genes [t(2;8), t(8;22)] were: BAC 316 G9-5'CCN, BAC 525 L16-3'tel, BAC 1021 F11-3'tel for the kappa locus; and BAC 526 G4-5' CEN, BAC 60 B5-3'tel, BAC 865 E9-3'tel for the lambda locus.[27]

Results

Patients' clinical characteristics

The clinical characteristics and treatment of the 16 cases are listed in Table 1. All patients presented with poor prognosis features: B symptoms were present in 13 cases and ECOG Performans Status was >2 in 13 cases; all cases had stage IV disease and elevated LDH level in the serum. Seven cases had an elevated leukocyte count with circulating tumor cells. Extranodal sites of disease were bone marrow (15 cases), CNS (8 cases), blood (7 cases), pleural effusion (5 cases), skin (2 cases), liver (1 case), lung (1 case), testis (1 case) and stomach (1 case). Furthermore, 14 cases had at least two extranodal localizations. The age-adjusted IPI score was 2 in 3 cases and 3 in 13 cases.

After induction chemotherapy, five and seven patients reached complete remission (CR) and partial remission (PR), respectively. Four patients progressed during initial therapy. We performed autologous or allogeneic stem cell (from a matched sibling donor) transplantations in three and two cases, respectively. Status at the time of transplantation was CR in two cases and PR in three cases. The median PFS and OS were 4 and 5 months, respectively. All patients died of disease progression. Only one patient (case 2) survived more than 1 year. At the time of relapse, she received salvage chemotherapy (IVAM) and reached a second CR but relapsed a few months later. Because of a PR status after induction therapy, one patient (case 4) received three cycles of DHAP regimen before autologous stem cell transplantation. Five patients received rituximab in association with chemotherapy. Addition of rituximab did not modify either PFS or OS.

Morphological findings

Figures 1 and 2 show the morphology of tumor cells in two cases (case 5 and 2, respectively). The morphology of the tumor cells was consistent with the diagnosis of DLBCL (non-Burkitt type) according to the WHO

Table 1. Patients' clinical characteristics.

Cases	Age/Gender	IPI score	Extra-nodal sites	Prior history or concomitant low grade lymphoma	Therapy	Response	SCT	Survival (months)
1	59/M	3	BM, skin, CNS, blood	Yes, FL	CEEP, COPADM	PR	autologous (BEAM)	4
2	65/F	3	pleural effusion, CNS	no	CHOP, IVAM	CR		16
3	45/M	3	BM, lung	no	COPADM, CYVE	PR		8
4	50/F	3	BM	no	CEEP, DHAP	PR	autologous (BEAM)	7
5	62/F	2	BM, CNS, blood	no	COPADM, CYVE	PR		4
6	71/F	2	pleural effusion, BM	no	R-CHOP	PR		10
7	36/M	2	BM, liver, CNS	no	COPADM	PR	allogeneic (Bu/Cy)	4
8	69/F	3	BM, blood	no	R-CHOP	PR		5
9	56/M	3	BM, pleural effusion, CNS, stomach, blood	no	COPADM	CR	autologous (BEAM)	8
10	63/M	3	BM	Yes, FL	COPADM, CYVE	CR		4
11	48/M	3	BM, blood	no	R-CEEP	CR	allogeneic (TBI/Cy)	8
12	73/M	3	BM, blood	no	R-CHOP	CR		6
13	55/M	3	BM, CNS, blood	no	COPADM	Prog		3
14	59/M	3	BM, pleural effusion, peritoneal effusion, CNS, testis	no	R-CHOP	Prog		1
15	70/F	3	BM, CNS, pleural effusion	Yes, NOS	CHOP	Prog		1
16	72/M	3	BM, skin	Yes, NOS	steroids	Prog		1

BM,; bone marrow; CNS, central nervous system; PR, partial response, CR, complete response; SCT, stem cell transplantation; TBI, total body irradiation, Cy, cyclophosphamide; Bu: busulfan.

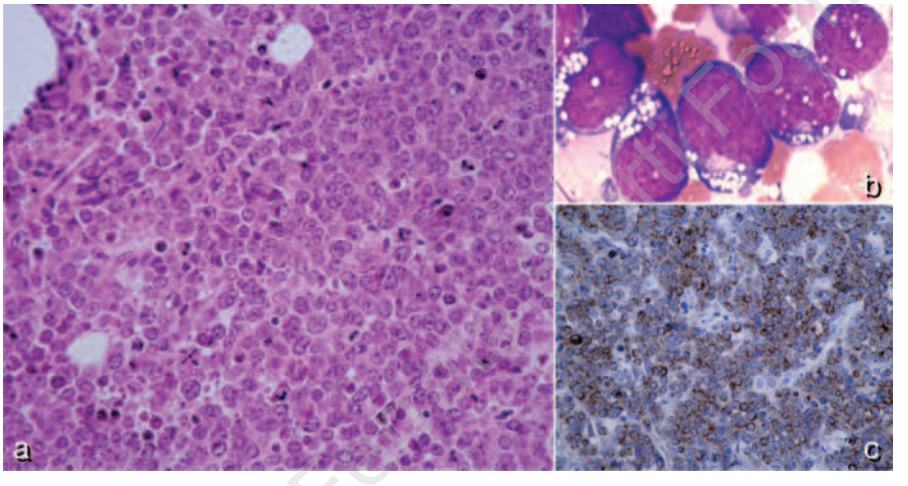

Figure 1. High-grade lymphoma with morphological features of *atypical BL*, involving bone marrow (case 5). **A.** HES x 400: diffuse infiltrate of medium-sized lymphoid cells with irregular nuclei and high mitotic index. **B.** Morphology of tumor cells. **C.** Immunohistochemistry x 200: Bcl-2 labels 100% of neoplastic cells.

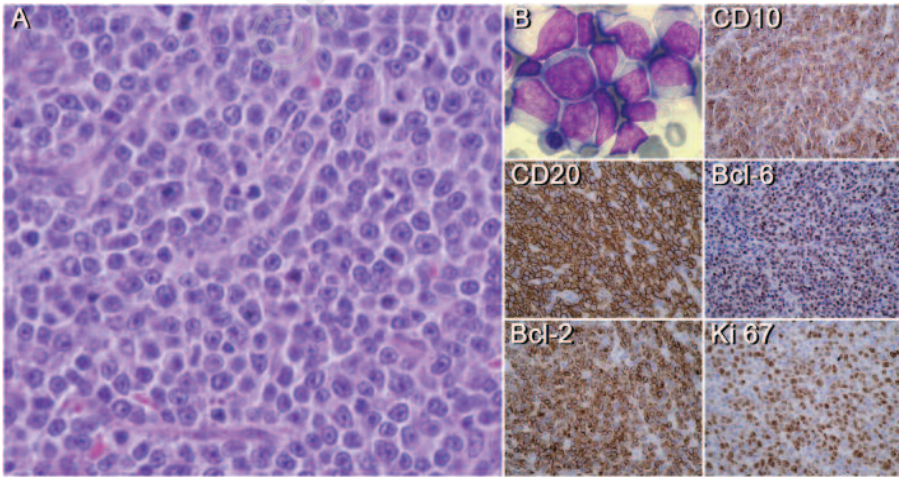

Figure 2. Morphological and immunohistochemical features of DLBCL of an immunoblastic subtype involving a lymph node (case 2). **A.** (HES x 400) sheets of immunoblastic cells with high mitotic index. Immunohistochemistry: tumor cells exhibited a GC profile: CD20 positive, CD10 positive, Bcl-6 positive, Bcl-2 positive and 60-70% of cells are immunostained with Ki67. **B.** Morphology of tumor cells.

2001 classification. In the majority of cases, peripheral blood and bone marrow aspirate smears showed medium-sized to large lymphoid cells with round to ovoid nuclei, fine chromatin and centrally located or few or no vacuoles. In most cases apoptosis was moderate to absent with a high mitotic index. Histologic sections of

nodal and extranodal biopsies showed a diffuse infiltrate of medium- to large-sized non-cleaved lymphoid cells with vesicular, sometimes irregular nuclei. Two cases of nodal lymphomas presented with a nodular and diffuse architecture (cases 4 and 6). Seven cases were subclassified as centroblastic (including the two cases with prior or co-existing FL) and one as immunoblastic. Among the cases with involved bone marrow biopsy (n=8), six showed an interstitial infiltrate of large lymphomatous cells (including a massive, diffuse infiltrate in four cases). All these cases had a high mitotic index. The two other cases showed focal involvement with paratrabecular nodules of small lymphoid cells. In one case (case 5), tumor cells had the appearance of *atypical BL*. In this case, bone marrow biopsy revealed a diffuse infiltrate of medium-sized, non-cleaved lymphoid cells, with irregular nuclei and basophilic cytoplasm.

Immunophenotypic findings

Immunophenotypic findings are summarized in Table 2. Immunohistochemistry analysis was performed in 11 cases. All tumor cells were positive for CD20 and negative for CD3. We demonstrated a GC profile ($CD10^+$ [11/11] $Bcl6^+$ [6/7] and $Mum1^-$ [11/11]) in all cases (Hans' system). Bcl-2 protein was consistently expressed with strong immunostaining (100%) in ten out of 11 cases, including the case of so-called *atypical BL*. The Ki-67 staining ranged from 70% to 90%.

Flow cytometry analysis was performed on peripheral blood and/or bone marrow aspirates in 13 cases. Lymphoma cells demonstrated a B-cell lineage. All cases were positive for CD19, CD20 and CD38. Only one case (case 8) was CD5 positive. In 11 cases, lymphoma cells were CD10 positive. A monotypic surface immunoglobulin light chain was observed in 12 cases (κ in 6 cases and λ in 6 cases).

Conventional cytogenetic (CC) and FISH findings

CC and FISH findings are presented in Table 3 and Figure 2 shows the karyotype and FISH analysis in one case (case 9). CC was performed in ten cases. All cases had a complex karyotype with several abnormalities. CC showed t(14;18) in eight cases (patients 3-6, 8-10). In two cases (cases 2 and 7), CC suggested a 14q32 rearrangement that was confirmed by FISH. CC showed t(8;22)(q24;q11) in three cases (cases 5, 8 and 10), t(8;14)(q24;q32) in two cases (cases 3 and 7) and t(2;8)(p12;q24) in one case (case 9). Interestingly, CC identified a t(8;9)(q24;p13) in one case (case 4).

IGH/BCL2, *BCL6* and *8q24/c-MYC* rearrangements were assessed in all cases using FISH. FISH was performed from BM aspirates in 14 cases and pleural effusion in two cases. A total of 200 interphases cells was systematically analyzed. All cases were positive for both *IGH/BCL2* and *8q24/c-MYC* rearrangements. Four of 16 cases also had a *BCL6* rearrangement.

Discussion

We described a series of 16 cases of DLBCL with concurrent t(14;18) and *8q24/c-MYC* rearrangement. Mufti *et al*. were the first to report this dual translocation in a patient with acute lymphoblastic leukemia.[28] In our series, the morphological and immunophenotypic findings were consistent with the diagnosis of DLBCL with a GC profile in all cases. In contrast to Kunango and colleagues, but as recommended by the WHO classification, we classified the present lymphoid neoplasms as DLBCL according not only to morphological criteria but also to immunophenotypic and cytogenetic findings.[29]

All patients had very aggressive clinical and biological features: stage IV disease, multiple extranodal sites of disease, elevated LDH level, intermediate-high (n=3) or

Table 2. Immunophenotypic findings.

			Immunohistochemistry						Flow cytometry					sIgκ/λ
Cases	*Morphology*	*Materials*	*CD20*	*CD10*	*Bcl-6*	*Mum1*	*Bcl-2*	*CD138MIB1*	*CD19*	*CD5*	*CD20*	*CD10*	*CD38*	
1	DLBCL	BM, blood							95%	Neg	Pos	Pos	Pos	κ
2	DLBCL, IB	LN	100%	100%	100%	Neg	100%	Neg60-70%						
3	DLBCL, CB	BM	100%	100%	NE	Neg	100%	Neg NE	70%	Neg	Pos	Neg	Pos	κ
4	DLBCL CB	LN, BM	100%	100%	>60%	Neg	100%	Neg 80%						
5	DLBCL	BM	20%	100%	NE	Neg	100%	ND NE	98%	Neg	Pos	Pos	Pos	κ
6	DLBCL CB	LN, BM, pleural	100%	70%	>50%	Neg	100%	Neg70-80%	99%	Neg	Pos	Pos	Pos	λ
7	DLBCL	BM, blood							94%	Neg	Pos	Pos	Pos	Neg
8	DLBCL	BM, blood							82%	Pos	Pos	Pos	Pos	κ
9	DLBCL	LN, BM, pleural							96%	Neg	Pos	Pos	Pos	λ
10	DLBCL, CB	LN, BM	100%	100%	NE	Neg	70%	ND 70%	95%	Neg	Pos	Pos	Pos	λ
11	DLBCL, CB	tonsil, LN, BM, blood	100%	100%	>60%	Neg	100%	Neg 90%	90%	Neg	Pos	Pos	Pos	κ
12	DLBCL	BM, blood	NE	100%	NE	Neg	100%	ND NE	91%	Neg	Pos	Pos	Pos	κ
13	DLBCL	BM, blood							99%	Neg	Pos	Pos	Pos	λ
14	DLBCL, CB	LN, BM	100%	100%	10-20%	Neg	100%	ND 70%	99%	Neg	Pos	Neg	Pos	λ
15	DLBCL	pleural, thoracic wall	100%	100%	Neg	Neg	100%	ND 80%	ND	ND	ND	ND	ND	ND
16	DLBCL,CB	LN, skin	100%	100%	> 50%	Neg	100%	ND 80%	97%	Neg	Pos	Pos	Pos	λ

IB, immunoblastic; CB, centroblastic; LN, lymph node; BM, bone marrow; NE, not evaluable; ND, not done; Neg, negative; Pos, positive; FL, follicular lymphoma; NOS not otherwise specified.

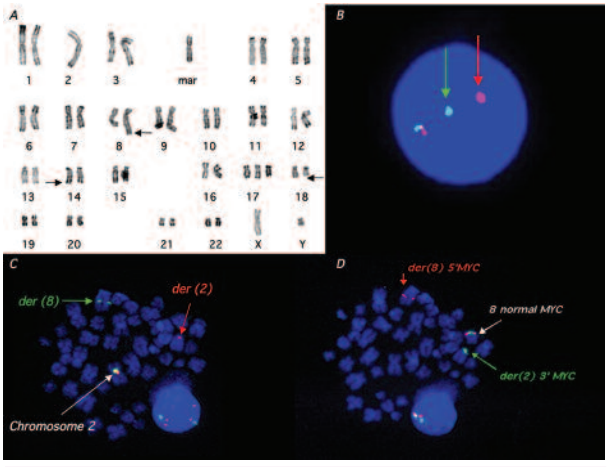

Figure 3. Case 9. A. The karyotype shows complex cytogenetic abnormalities including t(14;18) and t(2;8); arrows indicate abnormal chromosomes. B. Interphase FISH in case 9: *IGK* FISH assay; one fusion signal from the intact *IGK* locus and a dissociation of one red and one green signal (arrows) indicating a breakpoint in the *IGK* locus. C. Metaphase in case 9: *IGK* FISH assay showing a fusion of the dual color probe on the chromosome 2 with an intact *IGκ* locus, an isolated green signal from the telomeric probe on derivative chromosome 8, and a single red signal from the centromeric probe on a marker chromosome [der (2), probably]. D. The same metaphase with the dual color *c-MYC* FISH assay showing one fusion from the normal chromosome 8, one red signal from the derivative chromosome 8 (5' centromeric *c-MYC* probe) and one green signal from the marker chromosome (3' telomeric *c-MYC* probe).

high (n=13) IPI score and high Ki-67 staining (70% to 90%). Extranodal sites of disease were bone marrow (93%), CNS (50%), blood (44%) and pleural effusion (31%). The median PFS and OS were 4 and 5 months, respectively. These results are clearly worse than those reported in standard intermediate-high or high IPI DLBCL and consistent with the findings of both. Kanungo and Macpherson.[2,29,30] In the same period of time, we identified 22 DLBCL patients with complex karyotypes without t(14;18) and *8q24/c-MYC* rearrangements. In this cohort, six patients died of disease progression. The other patients are still alive with a median follow-up of 17.5 months (range, 9 to 94 months). This suggests that the poor prognosis of patients with t(14;18) and *8q24/c-MYC* rearrangement is due to this specific dual translocation and not to karyotype complexity. The duration of response and outcomes of the patients with the dual translocation were worse than those of patients with BL or transformed lymphoma.[31-33] This may be related to Bcl-2 protein that opposes mitochondrial apoptotic pathways and confers resistance to chemotherapy-induced apoptosis whereas c-MYC protein promotes cell cycling and tumor proliferation.

Translocation t(14;18) is a cytogenetic abnormality associated with FL. We postulated that the *8q24/c-MYC* rearrangement is an additional oncogenic event arising in FL cells and linked to aggressive transformation.[34] Transformation from indolent lymphoma with t(14;18) to aggressive lymphoma with *8q24/c-MYC* rearrangement was described by McDonnell and Korsmeyer.[35] These authors demonstrated that mice with overexpression of Bcl-2 had an indolent follicular hyperplasia that progressed to DLBCL with *8q24/c-MYC* rearrangement in half of the cases. In our series, one patient had a prior history of documented FL (case 1) and one (case 10) had coexisting FL and DLBCL cells at diagnosis. We isolated low grade lymphoma within the bone marrow in two cases (case 15 and 16), although there was not sufficient material to classify the low grade NHL. Thus, four cases out of 16 were considered as aggressive transformation of prior indolent lymphoma. However, it remains possible that the 12 remaining cases were transformed FL or low grade NHL in which the underlying FL or low grade NHL was not detected. In Kanungo's report, three patients were diagnosed as having DLBCL and nine patients as having BL or *atypical BL*. One case had a low-grade B-cell lymphoma neoplasm but no case had a prior history of FL.[29] Yano and colleagues reported on a series of 36 patients with transformed FL with *BCL2* rearrangement and identified three patients with a *8q24/c-MYC* rearrangement.[36] Macpherson *et al*. reported on a series of 13 DLBCL with this dual translocation including six patients with transformed FL.[30] In Thangavelu's report, two patients out of six had a prior history of FL.[37] Indeed, most cases do not have t(14;18) prior to *8q24/c-MYC* rearrangement. The *8q24/c-MYC* rearrangement is observed in BL, *atypical BL* and DLBCL. In the present series, t(8;14)(q24;q32) and t(8;22)(q24;q11) translocations were the most frequent translocations. This observation is consistent with Kunango's findings. Interestingly, we also identified a *8q24/c-MYC* translocation variant associated with t(14;18)(q32;q21). The t(8;9)(q24;p13) translocation has never previously been reported in DLBCL. We identified *PAX-5* as the partner gene. To the best of our knowledge, only three such cases have been reported in the literature in patients with B-cell acute lymphocytic leukemia.[38,39]

The Bcl-6 protein is a transcriptional repressor expressed within GC B cells and required for GC formation in response to antigen stimulation.[40,41] Cattoretti *et al*. demonstrated the oncogenic role of Bcl-6 in DLBCL.[42] Compared to activated B-like DLBCL, GC-DLBCL have been shown to have a better prognosis.[43] Our findings and other studies do not confirm this result in the subgroup of DLBCL with t(14;18) and *8q24/c-MYC* rearrangement. However, only four patients in our series had the Bcl-6 rearrangement and conclusions are not, therefore, possible. Based on transcriptional and genomic profiling analysis, two reports highlighted the molecular differences that distinguish BL and *atypical BL* from DLBCL.[14,15] Hummel and colleagues distinguished three subgroups of DLBCL without a molecular BL-signature.[14] According to their classification, our series of patients would be classified in the so-called *c-MYC-complex* subgroup. In Hummel's study, 46% of the cases of DLBCL

Table 3. Cytogenetic findings.

Cases	Convential cytogenetics	FISH IGH/BCL-2	FISH c-MYC	FISH BCL-6
1	ND	pos	pos	neg
2	48~50,XX,add(3)(q27),+3,iso(6)(p10),-8,add(9)(p12-13),-14,add(18)(q21), +mar1,+mar2,+min1,+min2,inc[cp10]/46,XX[5]pos	pos	pos	
3	46,XY,t(8;14)(q24;q32),t(14;18)(q32;q21),i(17q)[8]/46,XY[5]	pos	pos	neg
4	46,X,-X,der(?)t(?;2)(?;q12),add(3)(p21),del(6)(q21qter),+7,t(8;9)(q24;p13), +11,t(14;18)(q32;q21),-15,add(17)(p11)[7]/47,idem,+mar[4]47-52,idem,+mars[9]	pos	pos	neg
5	46,XX,add(1)(p36),add(3)(q27),t(8;22)(q24;q11),add(9)(p?),t(14;18)(q32;q21)[20]	pos	pos	neg
6	47,XX,add(2)(p1?6),-4,i(6)(p10),+7,del(10)(q22qter),t(14;18)(q32;q21),+mar[20] nt(8;14)	pos	pos	neg
7	52,XY,+X,+Y,+7,del(9)(p13p24),t(8;14)(q24;q32),add(14)(q32),add(18)(q21), +20,+der(21)t(1;21)(q11;p11)[18]/46,XY[2]	pos	pos	neg
8	74-75,XX,+X,add(X)(q2?5),+iso(1q),+2,+3,+3,der(3)(3pter->3q27::8q13 ->8q24::22q11->22qter),+4,+4,t(4;7)(q24-25;q35),+5,+6,+7,+7,t(8;22)(q24;q11), +9,+9,+11,+12,+12,+13,t(14;18)(q32;q21),+15,+16,+16,+17,add(17)(p12), +18,+19,+20,+21,+21,+mar1,mar2,+mar3[20] pos	pos	pos	pos
9	47,XY,-2,add(3)(q27),der(8)t(2;8)(p12;q24),add(9)(q34),t(14;18)(q32;q21), +17,+mar[10]/46,XY[10]	pos	pos	neg
10	50,XY,+X,+?Y,t(8;22)(q24;q11),+12,add(14)(q32),t(14;18)(q32;q21), +mar[10]/46,XY[3]	pos	pos	neg
11	ND	pos	pos	pos
12	ND	pos	pos	neg
13	ND	pos	pos	neg
14	ND	pos	pos	neg
15	47,XY,add(4)(p13-15),+7,t(14;18)(q32;q21)[18]/48,idem,+mar[2]	pos	pos	neg
16	ND	pos	pos	pos

with *8q24/c-MYC* breakpoints had concurrent *BCL2* and *BCL6* translocations. The authors demonstrated that the presence of *8q24/c-MYC* breakpoints is associated with a poor 5-year survival rate. They hypothesized that the *c-MYC* breakpoint is a secondary event which appears during clonal evolution. Dave and colleagues showed that the molecular signature of DLBCL with *c-MYC* t(8;14) differs from that of BL.[15] These two reports confirm the molecular differences between BL and DLBCL and show that DLBCL with *8q24/c-MYC* constitute a heterogeneous subgroup of DLBCLs.

The present report describes a subgroup of DLBCL defined by a concurrent t(14;18) and *8q24/c-MYC* rearrangement. To the best of our knowledge, our series is the most important reported in the literature so far. DLBCL with t(14;18) and *8q24/c-MYC* rearrangement are characterized by an aggressive clinical presentation, morphological and immunophenotypic features of GC-DLBCL, complex karyotype and very poor prognosis. It is worth searching for concurrent t(14;18) and *8q24/c-MYC* rearrangement in Bcl-2-positive DLBCL with an unusually aggressive presentation because, given their dismal prognosis, these patients need to be identified as soon as possible and should be considered eligible for innovative upfront, treatments.

Authors' Contributions

SLG conducted the study and analyzed data; PT performed cytogenetic analyses; CT collected and analyzed data; AM performed immunohistochemistry and morphological analyses; RG performed morphological and flow cytometry analyses; J-M collected clinical data; FG performed immunohistochemistry and morphologic; TG collected clinical data; NM collected clinical data and treated patients; PM collected clinical data and treated patients, J-LH initiated this study; HA-L co-ordinated and supervized this study.

Conflict of Interest

The authors reported no potential conflcits of interest.

References

1. Diebold J, Jaffe ES, Raphael M, Warnke RA. Burkitt lymphoma. In: Jaffe ES, Harris NL, Stein H, Wardiman JW, eds. World Health Organisation Classification of tumours. Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press 2001. p. 171-74.
2. Shipp MA, Harrington DP, Anderson JR, Armitage JO, Bonadonna G, Brittinger G, et al. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. N Engl J Med 1993; 329:987-94.
3. Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, et al. Lymphoma/Leukemia Molecular Profiling Project. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 2002; 346: 1937-47.
4. Gascoyne RD, Adomat SA, Krajewski S, Krajewski M, Connors JM, Reed JC, et al. Prognostic significance of bcl-2 protein expression and bcl-2 gene rearrangement in diffuse aggressive non-Hodgkin's lymphoma. Blood 1997;90:244-51.
5. Kramer MH, Hermans J, Wijburg E, Philippo K, Geelen E, Van Krieken JH et al. Clinical relevance of BCL-2, BCL-6 and MYC rearrangements in diffuse large B-cell lymphoma. Blood 1998;92:3152-62.
6. Lossos IS, Jones CD, Warnke R, Natkunam Y, Kaiser H, Zehnder JL, et al. Expression of a single gene, BCL-6, strongly predicts survival in patients with diffuse large B-cell lymphoma. Blood 2001;98:945-51.
7. Winter JN, Weller EA, Horning SJ, Krajewska M, Variakojis D, Habermann TM, et al. Prognostic significance of Bcl-6 protein expression in DLBCL treated with CHOP or R-CHOP: a prospective correlative study. Blood 2006;107:4207-13.
8. Offit K, Wong G, Filippa DA, Tao Y, Chaganti RSK. Cytogenetic analysis of 434 consecutive ascertained specimens of non-Hodgkin's lymphoma:

clinical correlations. Blood 1991;77: 1508-15.
9. Hill ME, MacLennan KA, Cunningham DC, Vaughan-Hudson B, Burke M, Clarke P, et al. Prognostic significance of bcl-2 expression and bcl-2 major breakpoint region rearrangement in diffuse large cell non-Hodgkin's lymphoma: a British National Lymphoma Investigation study. Blood 1996;88:1046-51.
10. Hockenberry D, Nunez G, Miliman C, Schreiber R, Korsmeyer S.J. BCL2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 1990;348:334-6.
11. Zech L, Haglund U, Nilsson K, Klein G. Characteristic chromosomal abnormalities in biopsies and lymphoid-cell lines from patients with Burkitt and non-Burkitt's lymphomas. Int J Cancer 1976;17:47-56.
12. Ladanyi M, Offit K, Jhanwar SC, Filippa DA, Chaganti RSK. Myc rearrangements and translocations involving band 8q24 in diffuse large-cell lymphomas. Blood 1991;77: 1057-63.
13. Scarpa A, Borgato L, Chilosi M, Capelli P, Menestrina F, Bonetti F, et al. Evidence of MYC gene abnormalities in mediastinal large B-cell lymphoma of young adult age. Blood 1991;78:780-8.
14. Hummel M, Bentink S, Berger H, Klapper W, Wessendorf S, Barth T, et al. Molecular mechanisms in malignant lymphomas Network Project of the Deutsche Krebshilfe. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med 2006; 354:2419-30.
15. Dave SS, Fu K, Wright GW, Lam LT, Kluin P, Boerma EJ, et al. Lymphoma/leukemia molecular profiling project. Molecular diagnosis of Burkitt's lymphoma. N Engl J Med 2006;354:2431-42.
16. Carbone PP, Kaplan SS, Musshoff K, Smithers DW, Tubiana M. Report of the Committee on Hodgkin's Disease Staging. Cancer Res 1971; 31: 1860-1.
17. Elias L, Portlock CS, Rosenberg SA. Combination chemotherapy of diffuse histiocytic lymphoma with cyclophosphamide, adriamycin, vincristine, and prednisone (CHOP). Cancer 1978;42:1705-10.
18. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. N Engl J Med 2002;346:235-42.
19. Milpied N, Deconinck E, Gaillard F, Delwail V, Foussard C, Berthou C, et al. Groupe Ouest-Est des Leucemies et des Autres Maladies du Sang. Initial treatment of aggressive lymphoma with high-dose chemotherapy and autologous stem-cell support. N Engl J Med 2004;350:1287-95.
20. Patte C, Auperin A, Michon J, Behrendt H, Leverger G, Frappaz D, et al. Societe Francaise d'Oncologie Pediatrique. The Societe Francaise d'Oncologie Pediatrique LMB89 protocol: highly effective multiagent chemotherapy tailored to the tumor burden and initial response in 561 unselected children with B-cell lymphomas and L3 leukemia. Blood 2001;97:3370-9.
21. Velasquez WS, Cabanillas F, Salvador P, Mc Laughlin P, Fridirik M, Tucher S, et al. Effective salvage therapy for lymphoma with cisplatin in combination with high-dose Ara-C and dexamethasone (DHAP). Blood 1988;71:117-22.
22. Stamatoullas A, Fruchart C, Bastit D, Boulet D, Monconduit M, Piguet H, et al. Ifosfamide, etoposide, cytarabine, and methotrexate as salvage chemotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma. Cancer 1996;77:2302-7.
23. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. Blood 2004;103:275-82.
24. Mitelman F. An international system for human cytogenetic nomenclature. Report of the Standing Committee on Human Cytogenetic Nomenclature. Karger; ISCN: Basel, Switzerland. 1985. p. 1-117.
25. Godon A, Avet-Loiseau H. Is t(14;18)(q32;q21) a constant finding in follicular lymphoma? An interphase FISH study on 63 patients. Leukemia 2003;17:255-9.
26. Avet-Loiseau H. Rearrangements of the myc-oncogene are present in 15% of primary human multiple myeloma tumors. Blood 2001;98: 3082-6.
27. Martín-Subero JI, Harder L, Gesk S, Schlegelberger B, Grote W, Martinez-Climent JA, et al. Interphase FISH assays for the detection of translocations with breakpoints in immunoglobulin light chain loci. Int J Cancer 2002; 98:470-4.
28. Mufti GJ, Hamblin TJ, Oscier DG, Johnson S. Common ALL with pre-B-cell features showing (8;14) and (14;18) chromosome translocations. Blood 1983;62:1142-6.
29. Kanungo A, Medeiros LJ, Abruzzo LV, Lin P. Lymphoid neoplasms associated with concurrent t(14;18) and 8q24/c-MYC translocation generally have a poor prognosis. Mod Pathol 2006;19:25-33.
30. Macpherson N, Lesack D, Klasa R, Horsman D, Connors JM, Barnett M et al. Small noncleaved, non-Burkitt's (Burkitt-like) lymphoma: cytogenetics predict outcome and reflect clinical presentation. J Clin Oncol 1999;17:1558-67.
31. Blum KA, Lozanski G, Byrd JC. Adult Burkitt leukemia and lymphoma. Blood 2004;14:3009-20.
32. Williams CD, Harrison CN, Lister TA, Norton AJ, Blystad AK, Coiffier B et al for the European bone marrow transplant lymphoma working party. High-dose therapy and autologous stem-cell support for chemosensitive transformed low-grade follicular non hodgkin's lymphoma: a case-matched study from the European bone marrow transplant registry. J Clin Oncol 2001; 19:727-35.
33 Le Gouill S, Moreau P, Morineau N, Harousseau JL, Milpied N. Tandem high-dose therapy followed by autologous stem-cell transplantation for refractory or relapsed high grade non Hodgkin's lymphoma with poor prognosis factors: a prospective pilot study. Haematologica 2002;87:333-4.
34. De Jong D, Voetdijk BM, Beverstock GC, van Ommen GJ, Willemze R, Kluin PM. Activation of the c-myc oncogene in a precursor-B-cell blast crisis of follicular lymphoma, presenting as composite lymphoma. N Engl J Med 1988;318:1373-8.
35 McDonnell TJ, Korsmeyer SJ. Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14;18). Nature 1991;349:254-6.
36 Yano T, Jaffe ES, Longo DL, Raffeld M. MYC rearrangements in histologically progressed follicular lymphomas. Blood 1992;80:758-67.
37 Thangavelu M, Olopade O, Beckman E, Vardiman JW, Larson RA, Mc Keithan TW, et al. Clinical, morphologic, and cytogenetic characteristics of patients with lymphoid malignancies characterized by both t(14;18)(q32;q21) and t(8;14)(q24;q32) or t(8;22)(q24;q11). Genes Chromosomes Cancer 1990;2:147-58.
38. Dunphy CH, van Deventer HW, Carder KJ, Rao KW, Dent GA. Mature B-cell acute lyphoblastic leukemia with associated translocations (14;18)(q32;q21) and (8;9) (q24;p13). A Burkitt variant. Arch Pathol Lab Med 2003;127:610-3.
39. Nacheva E, Dyer MJ, Fisher P, Stranks G, Heward JM, Marcus RE, et al. C-MYC translocations in de novo B-cell lineage acute leukemias with t(14;18)(cell lines Karpas 231 and 353).Blood 1993;82:231-40.
40. Ye BH, Lista F, Lo Coco F, Knowles DM, Offit K, Chaganti RS, et al. Alterations of a zinc finger-encoding gene, BCL-6, in diffuse large-cell lymphoma. Science 1993;262:747-50.
41. Ye BH, Cattoretti G, Shen Q, Zhang G, Hawe N, de Waard R, et al. The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. Nat Genet 1997; 16:161-70.
42. Cattoretti G, Pasqualucci L, Ballon G, Tam W, Nandula SV, Shen Q, et al. Deregulated BCL6 expression recapitulates the pathogenesis of human diffuse large B cell lymphomas in mice. Cancer Cell 2005; 7:445-55.
43. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000; 403:503-11.