The t(14;18) Is Associated with Germinal Center-derived Diffuse Large B-Cell Lymphoma and Is a Strong Predictor of Outcome[1]

Sharon L. Barrans,[2] Paul A. S. Evans, Sheila J. M. O'Connor, S. Jane Kendall, Roger G. Owen, Andrew P. Haynes, Gareth J. Morgan, and Andrew S. Jack

HMDS, Academic Unit of Haematology and Oncology, Leeds General Infirmary, Leeds LS1 3EX [S. L. B., P. A. S. E., S. J. M. O., S. J. K., R. G. O., G. J. M., A. S. J.], and Department of Haematology, Nottingham City Hospital, Nottingham [A. P. H.], United Kingdom

ABSTRACT

The t(14;18) is present in a significant proportion of diffuse large B-cell lymphomas (DLBCLs), however, the prognostic effect of the translocation and the relationship with transformed follicular lymphoma remains controversial.

To clarify these uncertainties, interphase fluorescence *in situ* hybridization (FISH) was used to determine the incidence of the t(14;18) in nodal DLBCL, and this was correlated with BCL2 expression, germinal center (GC) immunophenotype, and patient outcome. FISH was performed on paraffin-extracted nuclei from 137 *de novo* nodal DLBCLs.

Eighteen of 137 *de novo* DLBCLs were t(14;18) positive. The t(14;18) was most commonly associated with the subset of DLBCLs that expressed a GC phenotype, defined as CD10+, BCL6+ (GC-type DLBCL): positive in 14 of 47 (30%) cases, compared with 4 of 89 (5%) in the non-GC group (Pearson's $\chi^2 = 28.4$; $P < 0.0001$). All cases with a translocation expressed BCL2 protein, however, 40 expressed BCL2 protein without a t(14;18). GC-type DLBCL patients with a t(14;18) had a significantly worse survival compared with GC-type DLBCL patients without the translocation (2-year survivals were 29 and 63%, respectively; $P = 0.006$). Of the cases without the translocation, BCL2 protein expression did not affect survival. In contrast, in the non-GC group of DLBCLs, BCL2 protein expression reduced the 2-year overall survival from 64% in the BCL2-negative group to 38%, with a median survival of 15.0 months ($P = 0.02$).

In conclusion, the t(14;18) is common in DLBCLs, particularly in GC-type DLBCLs, where the presence of the translocation has a poor prognostic effect. BCL2 protein expression defines a group of non-GC DLBCL patients with a poor prognosis.

INTRODUCTION

The t(14;18)(q32;q21) is considered to be the major pathogenetic mechanism in FL[3] because of the deregulation of *BCL2*. The translocation is also demonstrated in up to 30% of cases of DLBCL (1, 2). The significance of the t(14;18) in DLBCL is unclear and is additionally complicated because a proportion are likely to represent cases with an underlying FL, either occult disease or previously detected. Excluding known transformed FL, the translocation has been demonstrated in 18–20% of patients (2, 3). BCL2 protein is also expressed in a number of cases of DLBCL, irrespective of the presence of the t(14;18); (3–5).

The prognostic significance of the t(14;18) in DLBCL is controversial. Some studies have shown no effect (4–8), whereas others have shown an increased incidence of relapse (2), decreased responsiveness to therapy or shorter survival (9–11), or a correlation with extensive disease (5). In some studies, poor outcome (3–5, 12) or resistance to treatment (7) has been attributed to expression of the BCL2 protein, rather than the presence of the translocation.

These inconsistencies may be partly explained by variations in methodology used to detect the translocation in previous studies. Using PCR strategies for the MBR and mcr alone, ~25% of breakpoints are not detected (13). In addition, the use of paraffin-embedded tissue results in poor quality DNA. The reported incidence of the t(14;18) as detected by MBR and mcr PCR in paraffin tissue is highly variable, with a maximum of 47% of cases of FL (14) positive for the t(14;18) using this methodology.

To attempt to clarify these uncertainties, we have used the Vysis LSI *IgH/BCL2* probe set in an interphase FISH assay, which is applicable to paraffin tissue. These probes span the *IgH* and *BCL2* genes entirely, and therefore, this method has the advantage that all breakpoints can be demonstrated. We have used this paraffin FISH assay to retrospectively determine the incidence of the t(14;18) in nodal DLBCL and correlate this with BCL2 expression, a GC immunophenotype and patient outcome.

Received 9/12/02; revised 12/23/02; accepted 12/23/02.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by a grant from the United Kingdom Leukemia Research Fund.
[2] To whom requests for reprints should be addressed, at Haematological Malignancy Diagnostic Service, Academic Unit of Hematology and Oncology, Leeds General Infirmary, Leeds LS1 3EX, United Kingdom. Phone: 441133926285; Fax: 441133926286; E-mail: sharonb@hmds.org.uk.

[3] The abbreviations used are: FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; FISH, fluorescence *in situ* hybridization; OS, overall survival; GC, germinal center; MBR, major breakpoint region; MCR, minor cluster region; IPI, international prognostic index.

MATERIALS AND METHODS

Sample Selection and Clinical Information. Formalin-fixed, paraffin wax-embedded lymph nodes from 137 presentation nodal DLBCLs were investigated. Patients were selected only on the basis of availability of clinical information and histological material. Diagnoses were confirmed by pathologic review using the diagnostic criteria defined in the Revised European-American Classification of Lymphoid Neoplasms/WHO classification. All patients were newly presenting, with no previous history of FL or other hematological malignancy, were previously untreated, and received standard primary anthracycline-containing combination chemotherapy (predominantly CHOP) with curative intent.

The median follow up was 28.5 months (range, 0–165 months), and median OS was 39.4 months. Fifty-seven patients had an intermediate risk IPI with a median OS of 14.3 months, with 35% OS at 5 years. Eight patients were high risk and had a median survival of 2.2 months. In the low IPI groups (53 patients), the median survival has not yet been reached, but OS at 5 years was 68%.

Immunohistochemistry. All cases with available paraffin blocks were stained for BCL2 and BCL6 proteins using microwave antigen retrieval and standard immunocytochemistry and CD10 using Tyramine Signal Amplification. Cases were classed as BCL2 expressing if the protein was detected in >50% of tumor cells, and a GC phenotype was defined by the coexpression of CD10 and BCL6, as described previously (15). This immunophenotype was used to classify cases as GC or non-GC type.

FISH Analysis. FISH for the t(14;18) was performed on all cases using the Vysis LSI *IgH* Spectrum Green/LSI *BCL2* Spectrum Orange probe set (32-191018; Vysis, Inc.).

Validation of FISH Assay. This FISH assay has been validated by the analysis of a series of typical FL cases and comparison with a gold standard multiplex PCR technique capable of detecting the majority of known breakpoints (16, 17). Twenty-eight paired frozen and fixed cases of FL and 5 reactive controls were analyzed. FISH produced an accurate result in all paraffin-embedded cases by comparison with the results obtained in fresh tissue using FISH and PCR (18, 19).[4] The t(14;18) was detected in 23 of 28 (82%) cases using PCR on frozen material and 8 of 20 (29%) of the same cases that had amplifiable DNA from paraffin tissue. Using FISH, 24 of 26 frozen and 26 of 28 paraffin cases had a demonstrable translocation. All cases that were positive by PCR were also positive by FISH. Both PCR and FISH were highly effective for t(14;18) analysis in unfixed tissue. However, when only paraffin blocks are available, FISH is the method of choice and is capable of achieving a result in 100% of cases. As an additional control, FISH was compared with conventional G-banding in a proportion of cases that had cytogenetic results, and concordant results were observed in each case (data not shown).

FISH on Whole Nuclei Extracted from Paraffin-embedded Tissue. Thirty-five-μm thick paraffin sections were dewaxed in xylene and rehydrated through graded alcohols to water. The sections were digested at 37°C in 0.1 M Tris, 0.07 M NaCl buffer (pH 7.1), containing 0.025% Protease XXIV (Sigma P8038) and 0.1% NP40. Digested nuclei were washed in PBS and resuspended in 3:1 methanol:acetic acid, and the nuclear suspension was dropped by pipette onto aminopropyltriethoxy-silane-coated microscope slides (method as described (19, 20)).

FISH was carried out according to the product datasheet using a Vysis Hybrite hybridization system. An additional 90°C predenaturation in hybridization buffer was used to optimize the technique for paraffin nuclei. Denaturation was 73°C for 5 min, and hybridization was at 37°C for 16–24 h. Posthybridization washes were 2 × 2 min in 0.4× SSC/0.3× NP40 at 73°C. 4′,6-Diamidino-2-phenylindole was used as the counterstain.

Interpretation of FISH Results. At least 100 intact, individual nuclei were assessed, using the Zeiss Axioplan II microscope. Representative images were captured via a monochrome digital camera using Metasystems ISIS software.

Cases were defined as normal if there were two green (*IgH*) and two red (*BCL2*) signals. A t(14;18) was defined when there were extra signals of both the *BCL2* and the *IgH* probes, along with at least two colocalized signals. The presence of additional signals of either or both probes was also noted.

Cases with additional *BCL2* signals that were not accounted for as part of the translocation were additionally investigated using the CEP18 (D18Z1) alpha satellite SpectrumAqua probe (32-131018; Vysis, Inc.) to control for aneuploidy of chromosome 18.

Statistical Analysis. The χ^2 test was used to examine relationships between variables. OS time was calculated from the date of diagnosis until death or date of last follow-up. Survival curves were estimated by the Kaplan-Meier method, using the log-rank test to analyze the statistical differences between the groups. Analyses were carried out using Microsoft Excel and SSCP software.

RESULTS

Incidence of the t(14;18) in DLBCL. Eighteen of 137 (13%) cases of *de novo* DLBCL were positive for the t(14;18) using FISH (Fig. 1*a*). The t(14;18) was more commonly associated with GC-type DLBCL: positive in 14 of 47 (30%) cases, compared with 4 of 89 (4.5%) in the non-GC group (Pearson's $\chi^2 = 28.4$; $P < 0.0001$). In the non-GC group with a demonstrable translocation, CD10 was positive in 3 of 4 cases, and BCL6 was positive in the case that was CD10 negative. One t(14;18)-negative case was not classified according to GC status because of the lack of available material. All cases with a translocation expressed the BCL2 protein. None of the BCL2-negative cases had a demonstrable translocation (Pearson's χ^2 value = 157.0, $P < 0.0001$). Forty cases expressed BCL2 protein in the absence of a t(14;18), and of these, 14 (35%) cases were GC-type and 26 (65%) were non-GC DLBCLs. The results are summarized in Table 1.

Demonstration of Alternative Patterns of FISH Signals. Multiple (≥3) *BCL2/IgH* fusion signals (Fig. 1*b*) were seen in 3 of 18 (17%) t(14;18) positive *de novo* DLBCL cases. Extra signals of *BCL2* alone ($n = 10$), *IgH* alone ($n = 14$), or both ($n = 17$; Fig. 1*c*) were demonstrated in the absence of fusion signals in an additional 41 cases. Using an alpha satellite 18 probe in combination with the *BCL2/IgH* probe set, 3 of 10 cases with extra signals of *BCL2* alone were classified as trisomy 18. The remainder had additional copies of the *BCL2*

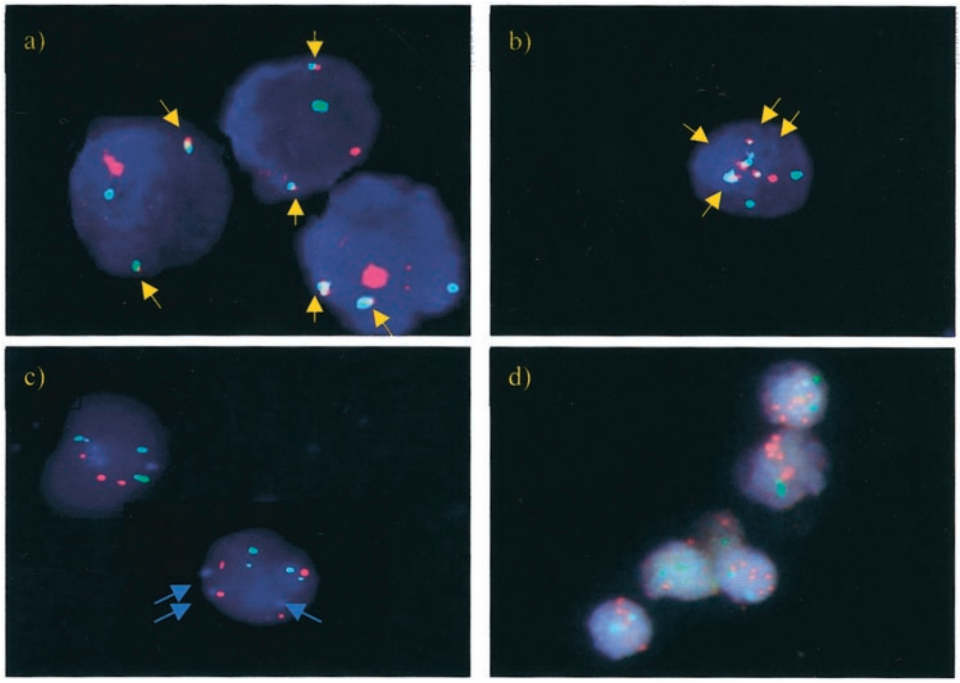

Fig. 1 Representative FISH images of the *BCL2* SO/*IgH* SG probe set on nuclei extracted from paraffin blocks of nodal DLBCL. *a*, standard t(14;18). Each cell has two fusion signals (indicated by *yellow arrows*) and a residual *red* and *green signal*. *b*, t(14;18) with multiple fusions. There are four fusion signals and a residual *red* (*BCL2*) and two residual *green* (*IgH*) *signals*. *c*, extra signals of both BCL2 and IgH. There are three to four copies of both *IgH* and *BCL2* and equivalent signals with the alpha satellite 18 probe (indicated by *blue arrows*), with no obvious fusions, suggesting that this is a case of aneuploidy. *d*, extra BCL2 signals. There are multiple clustered signals of the *red* (*BCL2*) *probe*, suggesting that this is a case of *BCL2* gene amplification. There are no fusions with *IgH*, and there are two *IgH* signals in each cell.

probe that exceeded the number of copies of the chromosome. In at least 3 of these cases, the signals were numerous and clustered (Fig. 1*d*), a pattern that is suggestive of gene amplification. BCL2 protein expression was demonstrated in all cases with additional copies of *BCL2*, and 3 of 4 expressed a GC phenotype. In all cases with extra signals of *BCL2* and *IgH*, the additional *BCL2* signals were attributable to additional copies of the chromosome.

The Presence of the t(14;18) Has an Adverse Effect on OS in *de Novo* DLBCL. The median OS of *de novo* DLBCL with a t(14;18), BCL2 expression in the absence of a translocation, and BCL2-negative cases was 12.5 and 19.8 months and median survival not yet reached, with 5-year OS of 15, 41, and 58%, respectively ($P = 0.005$; Fig. 2*a*). There was no association between the presence of a t(14;18) or BCL2 protein expression and the IPI. Bone marrow involvement was present in 14 of 89 cases investigated and was more common in 33.3% of the BCL2-positive, t(14;18)− cases and 25% of the t(14;18)+ cases, compared with 7.5% of the BCL2-negative cases (Pearson's χ^2 value = 7.0, $P = 0.03$). Ten of 14 cases were marrow infiltration with DLBCL, and 4 had evidence of low-level involvement with FL. Forty-eight patients did not have a staging bone marrow. IPI, GC phenotype, BCL2 expression, and t(14;18) all maintained a significant effect on OS in multivariate Cox regression analysis (Table 2).

Cases with extra copies of the *BCL2* gene had a similar outcome to the cases with a t(14;18) (median OS, 15.0 months compared with 12.5 months), and cases classified as aneuploid had a median OS of 28 months.

The t(14;18) Is a Strong Predictor of Outcome in GC-type DLBCL. In the *de novo* GC-type DLBCL patients, 14 of 47 (30%) had a t(14;18) and expressed BCL2 protein, 14 of 47 (30%) expressed BCL2 in the absence of a translocation, and 19 of 47 cases (40%) were negative for both the translocation and BCL2 protein. GC-type DLBCL patients with a t(14;18) had a significantly adverse median OS (12.3 months) compared with those patients without the translocation (2-year survivals were 29 and 63%, respectively; $P = 0.006$). Patients expressing BCL2 protein in the absence of a translocation had a similar outcome to those without BCL2 expression (Fig. 2*b*).

BCL2 Protein Expression Predicts Outcome of the Non-GC DLBCL Group. The median OS of the non-GC DLBCLs with BCL2 protein expression was 15.0 months but was not reached in the BCL2-negative group ($P = 0.02$; Fig. 2*c*). OS at 2 years was 64 and 38%, respectively. Only 4 cases had a t(14;18) in the non-GC series, and these patients had a median OS of 12.5.

DISCUSSION

In this study, we have examined the incidence and significance of the t(14;18) in nodal DLBCL. We consider the FISH assay described to be the most appropriate technique for this purpose because it is superior to an optimized multiplex PCR technique (16, 17) and is applicable to paraffin-embedded material (18, 19). In addition, the Vysis LSI *IgH* Spectrum Green/*BCL2* Spectrum Orange probe set used in this study consists of a 1.5-Mb locus-specific *IgH* probe spanning the entire *IgH* gene, and a 750-kb *BCL2* probe spanning the entire *BCL2* gene, ensuring that all known breakpoints can be demonstrated. Furthermore, the definition of a translocation requires both probe splitting and colocalization, minimizing the risk of false positives.

The overall incidence of the t(14;18) in *de novo* nodal DLBCL was 13% in this study using FISH analysis. The t(14;18) was almost exclusively associated with GC-type DLBCL,

Table 1 Characteristics of the cases of DLBCL investigated for the t(14;18) by FISH

A total of 137 *de novo* DLBCLs were classified according to their GC status defined by expression of BCL6 protein and CD10. BCL2 protein expression and the t(14;18), as detected by interphase FISH on paraffin-extracted nuclei, were significantly associated with a GC phenotype.

		BCL2/t(14;18) status			
	Overall n = 137	BCL2−, t(14;18)−[a] n = 79 (58%)	BCL2+, t(14;18)− n = 40 (29%)	BCL2+, t(14;18)+ n = 18 (13%)	χ^2 significance
GC status					$P < 0.001$
GC type	47	19 (40%)	14 (30%)	14 (30%)	
Non-GC	89	59 (66%)	26 (29%)	4 (5%)	
N/A[b]	1	1			
IPI					$P = 0.8$
Low (0–1)	53	32 (60%)	16 (30%)	5 (10%)	
Intermediate (2–3)	57	31 (54%)	16 (28%)	10 (18%)	
High (4–5)	8	5 (62%)	2 (25%)	1 (13%)	
N/A	19	11	6	2	
Bone marrow					$P = 0.03$
Normal	75	49 (65%)	18 (24%)	8 (11%)	
Involved[c]	14	4 (29%)	6 (42%)	4 (29%)	
Not done	48				

[a] The t(14;18) was not detected in any of the BCL2 protein negative cases.
[b] N/A, not applicable.
[c] Ten of 14 cases were marrow infiltration with DLBCL, and the remaining 4 cases had low-level involvement with FL. There was no association between the type of marrow involvement and the presence of BCL2 abnormalities (data not shown).

positive in 30% of cases, defined here by the coexpression of CD10 and BCL6 protein. An association between the t(14;18) and CD10 (21) and BCL6 expression (22) has been reported and suggested to represent a FL origin. Recent studies have demonstrated that the t(14;18) occurs exclusively in cases with a GC gene expression profile (23, 24). These cases were also shown to express BCL6 and CD10 by immunocytochemistry (23). In this study, the t(14;18) was also demonstrated in 4 non-GC DLBCL cases. CD10 was positive in the absence of BCL6 in 3 of 4, and BCL6 was positive in the case that was CD10 negative. The expression of one of the GC markers in these cases raises the possibility that an additional mutational or translocation event has resulted in loss of expression of one of the antigens, and it is possible that these cases would have been classified as GC type had gene expression analysis been used.

Taken together, these data suggest that the t(14;18) is an important event in the pathogenesis of GC-type DLBCL. High levels of mutation combined with the high proliferation rate associated with normal GC B cells renders these cells highly susceptible to translocations and favors the theory that the t(14;18) can occur during the GC phase of B-cell differentiation.

The t(14;18) was highly correlated with BCL2 protein expression. BCL2 protein is not expressed in normal GC B cells, and expression of the protein in GC-type DLBCL can therefore be explained as a direct effect of the translocation (25). Some studies have reported BCL2 negativity in significant numbers of cases with a detectable t(14;18; 3–5, 23). These differences may reflect discrepancies in antigen retrieval techniques, resulting in false negative BCL2 staining. An alternative explanation is that genetic events developing during tumor progression may abrogate the need for BCL2 (23). In this study and others (3, 4, 26), a number of cases expressed BCL2 protein in the absence of a demonstrable t(14;18), suggesting that the translocation is not the primary abnormality in all GC-derived tumors. A number of these can be accounted for by the presence of *BCL2* gene amplification (26–28), which occurs independently of the t(14;18) and also results in overexpression of the protein (26). In this study, 7 cases had extra copies of the *BCL2* gene, suggestive of gene amplification, and 3 of these were GC-DLBCL. In the remaining cases of GC-type DLBCL, expression of BCL2 in the absence of abnormalities of the gene is likely to be because of activation of other pathways such as nuclear factor κB (29).

A number of cases had extra copies of both *IgH* and *BCL2*, without fusion signals, suggestive of aneuploidy. Of particular interest was the demonstration of additional *BCL2/IgH* fusions in a small proportion of cases of *de novo* DLBCL. In a series of transformed FLs, over half of the cases had multiple fusions (data not shown), suggesting that duplication of the *BCL2/IgH* fusion gene may be a mechanism of disease transformation.

Of the non-GC DLBCLs, only 34% of cases expressed BCL2 protein in ≥50% of the tumor cells. Non-GC DLBCLs are thought to be post-GC derived, based on the observation that they have mutated immunoglobulin genes without ongoing somatic mutations (30). By analogy with the normal post-GC B cell, constitutive expression of BCL2 protein would be expected in the majority of these cases. A subset of monocytoid B cells has been shown to be post-GC derived but completely devoid of BCL2 expression (31). An alternative explanation is that *BCL2* may have been lost by mutation or deletion of the gene.

BCL2 protein expression was associated with a poor prognosis as previously reported (3–5, 12, 15), however, the presence of the t(14;18) had an additive adverse effect. The prognostic effect of the translocation was independent in multivariate analysis, and the survival differences cannot be explained simply by differences in the clinical features of these patients because there was no association between the presence of the translocation and the IPI. However, an association between the presence of a t(14;18) and the incidence of bone marrow involvement was noted here and in a previous study (3). Four cases had evidence of low-level bone marrow involvement

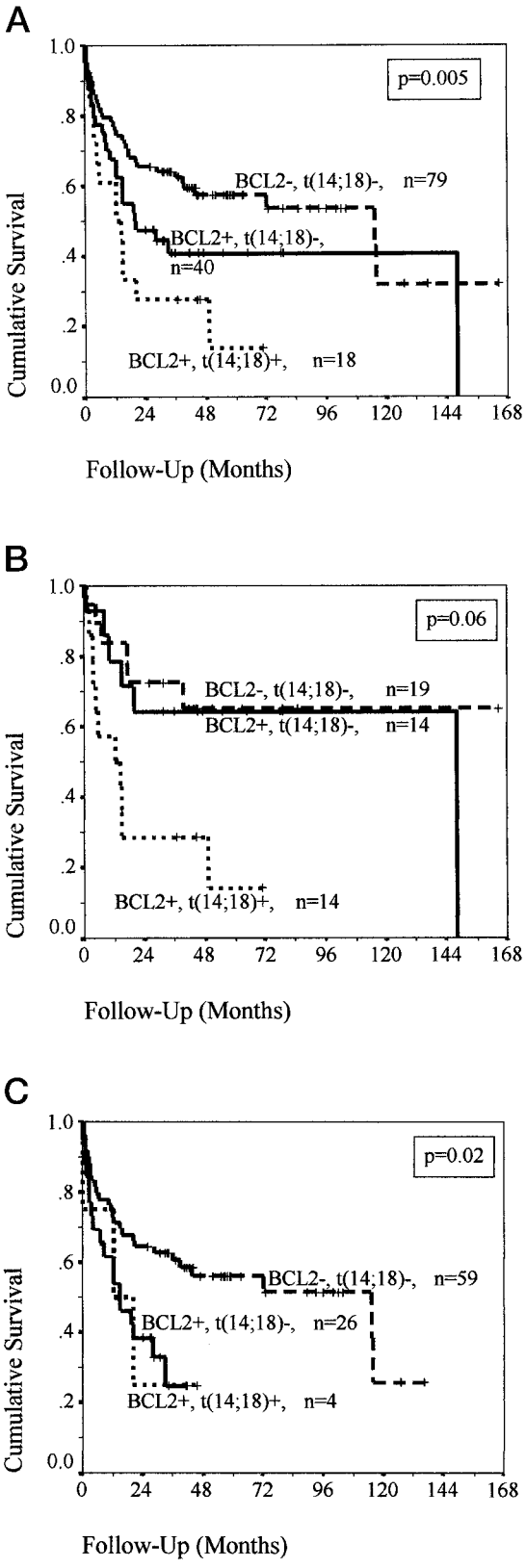

Fig. 2 *a*, OS of the *de novo* DLBCL patients classified according to their BCL2 and t(14;18) status. Kaplan-Meier analysis demonstrating that although BCL2 expression is a poor prognostic feature, the t(14;18) has an additive adverse prognostic effect and identifies a group of patients with a very poor outcome. *b*, OS of GC-type *de novo* DLBCL classified according to BCL2 and t(14;18) status. Kaplan-Meier analysis demonstrating that the t(14;18) is a strong predictor of outcome in GC-type DLBCL. Patients expressing BCL2 protein in the absence of a translocation had a similar outcome to those without BCL2 expression. *c*, OS of non-GC *de novo* DLBCL classified according to BCL2 and t(14;18) status. Kaplan-Meier analysis demonstrating that BCL2 protein expression predicts outcome of the non-GC DLBCL group. Only four cases had a t(14;18) in the non-GC *de novo* series, and these patients had a similarly poor outcome.

Table 2 Cox regression analysis of OS

IPI was stratified as low, intermediate, or high. GC phenotype was defined by CD10 and BCl6 expression. B indicates the Cox regression coefficient.

	Regression coefficient (B)	Exp(B)	SE	Significance (*P*)
GC phenotype	−0.3197	0.7264	0.1110	0.0040
BCL2 status	0.7044	2.0226	0.3108	0.0234
t(14;18)	0.1861	1.2046	0.0870	0.0324
IPI	1.5576	4.7473	0.2993	0.0000

with FL, suggesting that some newly presenting DLBCL patients may have evidence of underlying occult FL. Bone marrow involvement is a common feature of FL, and the relatively high frequency of infiltration in t(14;18)-positive patients highlights similarities between FL and DLBCL. The apparent additive adverse prognostic effect of the translocation compared with cases expressing BCL2 protein in the absence of a t(14;18) may be explained by the enhanced rate of transcription (32) and level of expression of BCL2 mRNA (33, 34) and protein (25) as a result of the translocation, producing a quantitative biological effect.

A number of previous studies have failed to demonstrate any impact on survival as a result of *BCL2* gene rearrangement (3–8); however, these studies used standard MBR and mcr PCR techniques that do not detect all known translocation breakpoints. In addition, the poor quality of DNA extracted from paraffin tissue makes meaningful retrospective large-scale studies difficult.

The adverse prognostic effect of the t(14;18) was particularly significant when GC-type DLBCL was considered separately. It has been shown that DLBCL can be subdivided into GC and non-GC subtypes and that GC-type DLBCLs have a favorable prognosis (15, 24, 25). The prognostic effect of the t(14;18) has not previously been investigated within the favorable subgroup of GC-DLBCL, however, a proportion of cases with a GC-gene expression profile is associated with a poor outcome. Using genetic abnormalities, other than *BCL2* rearrangement, identified by DNA microarray analysis, 24% of patients with a GC profile were in the poor risk group, with 34 of 115 GC-DLBCL patients dead in <2 years (24). The results of this study suggest that the t(14;18) negates the beneficial prognostic effect of the presence of a GC phenotype. It is therefore possible that the poor risk GC-DLBCL patients identified by gene expression analysis (24) did have BCL2 rearrangements (detected in 23% of cases; Ref. 24), which would

also account for the poor outcome. In the absence of a detectable translocation, the OS of GC-type DLBCL patients was favorable, regardless of BCL2 protein expression. We therefore propose that the outcome of GC-type DLBCL patients should be interpreted in the context of abnormalities of the *BCL2* gene, and demonstration of the t(14;18) should be included as a routine diagnostic test in these cases. In contrast, the t(14;18) was rarely detected in non-GC DLBCLs, and BCL2 protein expression alone identified a group of patients with a very poor outcome. This suggests that the GC and non-GC types of DLBCL have distinct pathophysiology and additionally supports the rationale for the subclassification of DLBCL into GC and non-GC types.

In conclusion, this study has demonstrated the presence of the t(14;18) in a significant proportion of *de novo* DLBCL. All patients with the translocation expressed the BCL2 protein. The translocation was demonstrated almost exclusively in the GC subtype of DLBCL and was associated with an adverse prognostic effect. The outcome of patients in the non-GC group could be stratified by the expression of BCL2 protein, and these results support the subclassification of DLBCL into GC and non-GC type. The outcome of all patients with nodal DLBCL should be stratified by the analysis of their biological variables, including the presence of the t(14;18).

ACKNOWLEDGMENTS

We thank the consultants of the Yorkshire Regional Hematological Oncology Network Group for submitting cases for analysis and for clinical data.

REFERENCES

1. Weiss, L. M., Warnke, R. A., Sklar, J., and Cleary, M. L. Molecular analysis of the t(14;18) chromosomal translocation in malignant lymphomas. N. Engl. J. Med., *317:* 1185–1189, 1987.

2. Jacobson, J. O., Wilkes, B. M., Kwaiatkowski, D. J., Medeiros, L. J., Aisenberg, A. C., and Harris, N. L. bcl-2 rearrangements in *de novo* diffuse large cell lymphoma. Association with distinctive clinical features. Cancer (Phila.), *72:* 231–236, 1993.

3. Hill, M. E., MacLennan, K. A., Cunningham, D. C., Vaughan Hudson, B., Burke, M., Clarke, P., Di Stefano, F., Anderson, L., Vaughan Hudson, G., Mason, D., Selby, P., and Linch, D. C. Prognostic significance of BCL-2 expression and bcl-2 major breakpoint region rearrangement in diffuse large cell non-Hodgkin's lymphoma: a British National Lymphoma Investigation Study. Blood., *88:* 1046–1051, 1996.

4. Gascoyne, R. D., Adomat, S. A., Krajewski, S., Krajewska, M., Horsman, D. E., Tolcher, A. W., O'Reilly, S. E., Hoskins, P., Coldman, A. J., Reed, J. C., and Connors, J. M. Prognostic significance of Bcl-2 protein expression and Bcl-2 gene rearrangement in diffuse aggressive non-Hodgkin's lymphoma. Blood, *90:* 244–251, 1997.

5. Kramer, M. H., Hermans, J., Wijburg, E., Philippo, K., Geelen, E., van Krieken, J. H., de Jong, D., Maartense, E., Schuuring, E., and Kluin, P. M. Clinical relevance of BCL2, BCL6, and MYC rearrangements in diffuse large B-cell lymphoma. Blood., *92:* 3152–3162, 1998.

6. Romaguera, J. E., Pugh, W., Luthra, R., Goodacre, A., and Cabanillas, F. The clinical relevance of t(14;18)/BCL-2 rearrangement and DEL 6q in diffuse large cell lymphoma and immunoblastic lymphoma. Ann. Oncol., *4:* 51–54, 1993.

7. Martinka, M., Comeau, T., Foyle, A., Anderson, D., and Greer, W. Prognostic significance of t(14;18) and bcl-2 gene expression in follicular small cleaved cell lymphoma and diffuse large cell lymphoma. Clin. Investig. Med., *20:* 364–370, 1997.

8. Vitolo, U., Gaidano, G., Botto, B., Volpe, G., Audisio, E., Bertini, M., Calvi, R., Freilone, R., Novero, D., Orsucci, L., Pastore, C., Capello, D., Parvis, G., Sacco, C., Zagonel, V., Carbone, A., Mazza, U., Palestro, G., Saglio, G., and Resegotti, L. Rearrangements of bcl-6, bcl-2, c-myc and 6q deletion in B-diffuse large-cell lymphoma: clinical relevance in 71 patients. Ann. Oncol., *9:* 55–61, 1998.

9. Offit, K., Koduru, P. R., Hollis, R., Filippa, D., Jhanwar, S. C., Clarkson, B. C., and Chaganti, R. S. 18q21 rearrangement in diffuse large cell lymphoma: incidence and clinical significance. Br. J. Haematol., *72:* 178–183, 1989.

10. Yunis, J. J., Mayer, M. G., Arnesen, M. A., Aeppli, D. P., Oken, M. M., and Frizzera, G. bcl-2 and other genomic alterations in the prognosis of large-cell lymphoma. N. Engl. J. Med., *320:* 1047–1054, 1989.

11. Tang, S. C., Visser, L., Hepperle, B., Hanson, J., and Poppema, S. Clinical significance of bcl-2-MBR gene rearrangement and protein expression in diffuse large-cell non-Hodgkin's lymphoma: an analysis of 83 cases. J. Clin. Oncol., *12:* 149–154, 1994.

12. Kramer, M. H., Hermans, J., Parker, J., Krol, A. D., Kluin-Nelemans, J. C., Haak, H. L., van Groningen, K., van Krieken, J. H., de Jong, D., and Kluin, P. M. Clinical significance of bcl2 and p53 protein expression in diffuse large B-cell lymphoma: a population-based study. J. Clin. Oncol., *14:* 2131–2138, 1996.

13. Horsman, D. E., Gascoyne, R. D., Coupland, R. W., Coldman, A. J., and Adomat, S. A. Comparison of cytogenetic analysis, southern analysis, and polymerase chain reaction for the detection of t(14;18) in follicular lymphoma. Am. J. Clin. Pathol., *103:* 472–478, 1995.

14. Shibata, D., Hu, E., Weiss, L. M., Brynes, R. K., and Nathwani, B. N. Detection of specific t(14;18) chromosomal translocations in fixed tissues. Hum. Pathol., *21:* 199–203, 1990.

15. Barrans, S. L., Carter, I., Owen, R. G., Davies, F. E., Patmore, R. D., Haynes, A. P., Morgan, G. J., and Jack, A. S. Germinal center phenotype and bcl-2 expression combined with the International Prognostic Index improves patient risk stratification in diffuse large B-cell lymphoma. Blood, *99:* 1136–1143, 2002.

16. van Dongen, J. J., Langerak, A. W., Bruggemann, M., Evans, P. A. S., Hummel, M., Lavender, L., Delabesse, E., Davi, F., Schuuring, E., Garzia Sanz, R., van Krieken, J. H., Does, J., Gondalez Diaz, D., Bastard, C., Hodges, L., Spaargaren, M., San Miguel, J. F., Parreira, A., Smith, J., Morgan, G. J., Kneba, M., and Macintyre, E. A. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene rearrangements in suspect lymphoproliferations. Report of the BIOMED-2 concerted action BMH4-CT98-3936. Leukemia (Baltimore), in press, 2003.

17. van Dongen, J. J., Langerak, A. W., San Miguel, J. F., Parreira, A., Smith, J. L., Morgan, G. J., Kneba, M., and Macintyre, E. PCR-based clonality studies for early diagnosis of lymphoproliferative disorders: report of the BIOMED-2 concerted action. Blood, *98:* 129a, 2001.

18. Barrans, S. L., Evans, P. A. S., O'Connor, S. J. M., Kendall, S. J., Owen, R. G., Morgan, G. J., and Jack, A. S. FISH is the method of choice for the detection of the t(14;18) in fixed tissue samples. Blood, *98:* 151b, 2001.

19. Barrans, S. L., Evans. P. A. S., O'Connor, S. J. M., Kendall, S. J., Owen, R. G., Morgan, G. J., and Jack, A. S. The detection of t(14;18) in archival lymph nodes: development of a FISH based method and evaluation by comparison with PCR, 2003, in press.

20. Barrans, S. L., O'Connor, S. J. M., Evans, P. A., Davies, F. E., Owen, R. G., Haynes, A. P., Morgan, G. J., and Jack, A. S. Rearrangement of the BCL6 locus at 3q27 is an independent poor prognostic factor in nodal diffuse large B-cell lymphoma. Br. J. Haematol., *117:* 322–332, 2002.

21. Fang, J. M., Finn, W. G., Hussong, J. W., Goolsby, C. L., Cubbon, A. R., and Variakojis, D. CD10 antigen expression correlates with the t(14;18)(q32;q21) major breakpoint region in diffuse large B-cell lymphoma. Mod. Pathol., *12:* 295–300, 1999.

22. King, B. E., Chen, C., Locker, J., Kant, J., Okuyama, K., Falini, B., and Swerdlow, S. H. Immunophenotypic and genotypic markers of follicular center cell neoplasia in diffuse large B-cell lymphomas. Mod. Pathol., *13:* 1219–1231, 2000.

23. Huang, J. Z., Sanger, W. G., Greiner, T. C., Staudt, L. M., Weisenburger, D. D., Pickering, D. L., Lynch, J. C., Armitage, J. O., Warnke, R. A., Alizadeh, A. A., Lossos, I. S., Levy, R., and Chan, W. C. The t(14;18) defines a unique subset of diffuse large B-cell lymphoma with a germinal center B-cell gene expression profile. Blood, *99:* 2285–2290, 2002.

24. Rosenwald, A., Wright, G., Chan, W. C., Connors, J. M., Campo, E., Fisher, R. I., Gascoyne, R. D., Muller-Hermelink, H. K., Smeland, E. B., Giltnane, J. M., Hurt, E. M., Zhao, H., Averett, L., Yang, L., Wilson, W. H., Jaffe, E. S., Simon, R., Klausner, R. D., Powell, J., Duffey, P. L., Longo, D. L., Greiner, T. C., Weisenburger, D. D., Sanger, W. G., Dave, B. J., Lynch, J. C., Vose, J., Armitage, J. O., Montserrat, E., Lopez-Guillermo, A., Grogan, T. M., Miller, T. P., LeBlanc, M., Ott, G., Kvaloy, S., Delabie, J., Holte, H., Krajci, P., Stokke, T., and Staudt, L. M. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N. Engl. J. Med., *346:* 1937–1947, 2002.

25. Chen-Levy, Z., Nourse, J., and Cleary, M. L. The bcl-2 candidate proto-oncogene product is a 24-kilodalton integral-membrane protein highly expressed in lymphoid cell lines and lymphomas carrying the t(14;18) translocation. Mol. Cell. Biol., *9:* 701–710, 1989.

26. Monni, O., Joensuu, H., Franssila, K., Klefstrom, J., Alitalo, K., and Knuutila, S. BCL2 overexpression associated with chromosomal amplification in diffuse large B-cell lymphoma. Blood, *90:* 1168–1174, 1997.

27. Monni, O., Joensuu, H., Franssila, K., and Knuutila, S. DNA copy number changes in diffuse large B-cell lymphoma: comparative genomic hybridization study. Blood, *87:* 5269–5278, 1996.

28. Rao, P. H., Houldsworth, J., Dyomina, K., Parsa, N. Z., Cigudosa, J. C., Louie, D. C., Popplewell, L., Offit, K., Jhanwar, S. C., and Chaganti, R. S. Chromosomal and gene amplification in diffuse large B-cell lymphoma. Blood, *92:* 234–240, 1998.

29. Bureau, F., Vanderplasschen, A., Jaspar, F., Minner, F., Pastoret, P. P., Merville, M. P., Bours, V., and Lekeux, P. Constitutive nuclear factor-κB activity preserves homeostasis of quiescent mature lymphocytes and granulocytes by controlling the expression of distinct Bcl-2 family proteins. Blood, *99:* 3683–3691, 2002.

30. Lossos, I. S., Alizadeh, A. A., Eisen, M. B., Chan, W. C., Brown, P. O., Botstein, D., Staudt, L. M., and Levy, R. Ongoing immunoglobulin somatic mutation in germinal center B cell-like but not in activated B cell-like diffuse large cell lymphomas. Proc. Natl. Acad. Sci. USA, *97:* 10209–10213, 2000.

31. Stein, K., Hummel, M., Korbjuhn, P., Foss, H. D., Anagnostopoulos, I., Marafioti, T., and Stein, H. Monocytoid B cells are distinct from splenic marginal zone cells and commonly derive from unmutated naïve B cells and less frequently from postgerminal center B cells with polyclonal transformation. Blood, *94:* 2800–2808, 1999.

32. Seto, M., Jaeger, U., Hockett, R. D., Graninger, W., Bennett, S., Goldman, P., and Korsmeyer, S. J. Alternative promoters and exons, somatic mutation and deregulation of the Bcl-2-Ig fusion gene in lymphoma. EMBO J., *7:* 123–131, 1988.

33. Graninger, W. B., Seto, M., Boutain, B., Goldman, P., and Korsmeyer, S. J. Expression of Bcl-2 and Bcl-2-Ig fusion transcripts in normal and neoplastic cells. J. Clin. Investig., *80:* 1512–1515, 1987.

34. Quattrone, A., Papucci, L., Santini, V., Schiavone, N., Noferini, D., Calastretti, A., Copreni, E., Morelli, S., Rossi Ferrini, P. L., and Nicolin, A. Quantitation of bcl-2 oncogene in cultured lymphoma/leukemia cell lines and in primary leukemia B-cells by a highly sensitive RT-PCR method. Haematologica, *80:* 495–504, 1995.

35. Alizadeh, A. A., Eisen, M. B., Davis, R. E., Ma, C., Lossos, I. S., Rosenwald, A., Boldrick, J. C., Sabet, H., Tran, T., Yu, X., Powell, J. I., Yang, L., Marti, G. E., Moore, T., Hudson, J., Jr., Lu, L., Lewis, D. B., Tibshirani, R., Sherlock, G., Chan, W. C., Greiner, T. C., Weisenburger, D. D., Armitage, J. O., Warnke, R., and Staudt, L. M. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature (Lond.), *403:* 503–511, 2000.

Clinical Cancer Research

The t(14;18) Is Associated with Germinal Center-derived Diffuse Large B-Cell Lymphoma and Is a Strong Predictor of Outcome

Sharon L. Barrans, Paul A. S. Evans, Sheila J. M. O'Connor, et al.

Clin Cancer Res 2003;9:2133-2139.