ORIGINAL ARTICLE

Expression of the polycomb-group gene *BMI1* is related to an unfavourable prognosis in primary nodal DLBCL

Joost C van Galen, Jettie J F Muris, Joost J Oudejans, Wim Vos, Cindy P E Giroth, Gert J Ossenkoppele, Arie P Otte, Frank M Raaphorst, Chris J L M Meijer

J Clin Pathol 2007;**60**:167–172. doi: 10.1136/jcp.2006.038752

See end of article for authors' affiliations

Correspondence to: Dr J J Oudejans, Department of Clinical Pathology, VU Medical Center, De Boelelaan 1117, 1007 MB Amsterdam, The Netherlands; jj.oudejans@vumc.nl

Accepted 22 June 2006
Published Online First 12 July 2006

Background : Clinical outcome in patients with diffuse large B cell lymphomas (DLBCL) is highly variable and poorly predictable. Microarray studies showed that patients with DLBCL with a germinal centre B cell-like (GCB) phenotype have a better prognosis than those with an activated B cell-like (ABC) phenotype. The *BMI1* proto-oncogene was identified as one of the genes present in the signature of the ABC type of DLBCL, associated with a poor prognosis.
Objectives : (1) To investigate, in primary nodal DLBCL, the expression of *BMI1* and its association with clinical outcome and DLBCL signature; (2) to look for an association between *BMI1* expression and the expression of its putative downstream targets *p14ARF* and *p16INK4a*.
Results : *BMI1* expression was found to be associated with poor clinical outcome, but not clearly with an ABC-like phenotype of DLBCL. Expression of *BMI1* was frequently, but not always, related to low levels of expression of *p14ARF* and *p16INK4a*.
Conclusion : Expression of *BMI1* is associated with an unfavourable clinical outcome of primary nodal DLBCL.

Diffuse large B cell lymphomas (DLBCL) are heterogeneous in terms of clinical behaviour, histological features and differences in response to treatment. Currently, the clinical parameters comprised in the International Prognostic Index are used as a prognostic indicator in DLBCL.[1] Also, various cellular and molecular factors that have prognostic significance in DLBCL have been identified.[2–9]

Neoplastic cells in DLBCL cases originate from germinal centre B (GCB) cells or their descendents.[10] Recent studies based on microarray analysis showed that part of DLBCL phenotypically resemble non-neoplastic GCB cells, but that part of DLBCL show an expression profile more consistent with an activated B cell (ABC)-like phenotype.[11 12] Furthermore, DLBCL with a GCB-like phenotype have a considerably better prognosis than DLBCL with an ABC-like phenotype.[4] *BMI1* was identified as one of the genes that distinguish the GCB-like from ABC-like DLBCL (supplement of studies by Alizadeh *et al*[11] and de Boer *et al*[13]), with high expression levels in ABC-like DLBCL.

BMI1 belongs to the Polycomb group of genes that are important regulators of mammalian lymphopoiesis (reviewed in Raaphorst *et al*[14]) Furthermore, *BMI1* has been shown to be essential for self-renewal of haematopoietic and neural stem cells, in part through inhibition of genes regulating senescence.[15] Initially, *BMI1* was identified as a proto-oncogene in the development of lymphomas.[16] Further studies showed that overexpression of *BMI1* in transgenic mice results in down regulation of the cell cycle inhibitors *p19ARF* and *p16INK4a*, and that *BMI1* cooperates with *c-Myc* in tumorigenesis by inhibiting *c-Myc*-induced apoptosis through *p14ARF*.[15 17 18]

p14ARF and *p16INK4a* are alternatively spliced products from the *INK4a/ARF* (CDKN2A) locus, with no structural homology. *p14ARF* is involved in the induction of apoptosis via p53, whereas *p16INK4a* is involved in the inhibition of cell cycle progression through cyclin D1 and CDK4/6.[19] Both these processes can be controlled by *BMI1* via the INK4a/ARF locus.[20 21]

In non-neoplastic lymphoid tissues, *BMI1* is primarily expressed in resting cells. In follicle center B cells, the non-neoplastic counterpart of at least part of DLBCL, *BMI1*, was mainly expressed in centrocytes, but not in dividing centroblasts.[22] This was in contrast with aggressive B cell lymphomas in which we have previously observed that *BMI1* is frequently expressed in dividing neoplastic cells, suggesting that aberrant Polycomb group expression contributes to malignant transformation in these lymphomas.[23 24] We also showed that *BMI1* is preferentially expressed in aggressive B cell lymphomas (DLBCL, Burkitt's lymphomas and mantle cell lymphomas), and not in indolent lymphomas (follicular and small lymphocytic lymphomas).[23] However, in this study we did not investigate whether *BMI1* expression predicts clinical outcome.

Thus, *BMI1* is a proto-oncogene that, when aberrantly expressed in mice, is involved in the pathogenesis of lymphomas, possibly by disruption of the *p14ARF* and/or by *p16INK4a* regulated pathways.[24 25] Furthermore, inhibition of apoptosis by disruption of the *p14ARF* pathway may result in reduced sensitivity to chemotherapy-induced cell death and poor outcome in patients with *BMI1*-positive lymphomas.

Therefore, we investigated in primary nodal DLBCL with extensive follow-up whether *BMI1* expression indeed correlates with an ABC-like phenotype and whether expression of *BMI1* predicts poor clinical outcome. Furthermore, to get insight into the possible pathogenic function of *BMI1*, we investigated whether expression of *BMI1* is related to decreased levels of *p14ARF* and/or *p16INK4a* expression.

MATERIALS AND METHODS

Clinical material

Selection of formalin-fixed, paraffin wax-embedded tissue blocks of 60 biopsy specimens of primary nodal DLBCL and the clinical data of these patients were described previously.[8] Table 1 summarises the patient characteristics. Most patients (n = 60) received polychemotherapy, consisting of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) regimens or variants, either alone (n = 31) or in combination with

Abbreviations: ABC, activated B cell; DLBCL, diffuse large B cell lymphomas; GCB, germinal centre B cells

Table 1 Patient characteristics obtained by univariate survival analysis

Patient characteristics	Number	Deaths with lymphoma	5-Year survival*	p Value†
IPI‡				
1	16	5	58	NS
2	21	12	44	
3	14	7	43	
4	8	6	0	
B symptoms				
Yes	19	13	32	0.02
No	41	18	52	
Stage‡				
1+2	25	8	63	0.03
3+4	34	22	34	
ABC/GCB profile§				
GCB	22	8	59	0.06
ABC	36	22	38	

ABC, activated B cell; GCB, germinal centre B cells; IPI, International Prognostic Index.
*As estimated from the Kaplan–Meier curves.
†As determined by χ^2 test, unless stated otherwise.
‡Data concerning stage and therefore IPI could not be retrieved in one patient.
§Data concerning the ABC/GCB profile could not be retrieved in two patients.

involved field radiation (n = 24); in five cases, only involved field radiation was given. The institutional review board of the VU Medical Centre (Amsterdam, The Netherlands) approved the study. Informed consent was provided according to the Declaration of Helsinki.

Antibodies used in this study

Monoclonal *BMI1* antibody (clone 6C9) was generated previously,[26] polyclonal *p14ARF* was obtained from Abcam, Cambridge, UK, and monoclonal *p16Ink4a* (clone E6H4) was a kind gift from Dr R Ridder. Specificity of these antibodies was determined by western blot analysis and bands were found at the predicted height (not shown).

Immunohistochemistry

Slides of paraffin wax-embedded biopsy specimens were used for the detection of *BMI1*, *p14ARF* and *p16INK4a*. Sections were deparaffinised with xylene, endogenous peroxidase was blocked, and antigens were retrieved in either citrate buffer (10 mmol/l, pH 6.0) or TRIS/EDTA buffer (10 mmol/l/1 mmol/l, pH 9.0) in either microwave or autoclave.

Methods for detection of *BMI1* were described previously.[22] For *p14ARF* and *p16INK4a* detection, slides were rinsed in phosphate-buffered saline and primary antibody was applied at optimal dilution. After 1 h at room temperature (20°C), slides were incubated with highly sensitive EnVison horseradish peroxidase system (Dako, Glostrup, Denmark). Bound antibodies were visualised by incubation with diaminobenzidine/H_2O_2. Slides were counterstained with haematoxylin.

Quantification of immunohistochemical staining

For all stainings, percentages of positive tumour cells were evaluated semiquantitatively in approximately the same area; scattered reactive lymphocytes served as an internal positive control. Two observers analysed all stainings independently. In case of disagreement, the staining results were reanalysed by the observers until consensus was reached.

To determine cut-off levels with the most discriminative power, cut-off was tested at 25%, 50% and 75% in relation to prognosis; the statistically most significant cut-off (at 25% for all stainings) was used in this study.

Phenotypic analysis of GCB-like DLBCL versus ABC-like DLBCL

Previously, we divided cases in a GCB-like or an ABC-like subtype,[27] by an algorithm modified from the one previously described by Hans *et al*[4].

Statistical analysis

Survival time was measured from the time of initial diagnosis until death with disease or until the end of follow-up. Patients who died of causes unrelated to the disease were censored at the time of death (n = 2).

Survival curves were constructed with the Kaplan–Meier method. Differences between the curves were analysed using the log-rank test. Multivariate analysis was performed using the Cox proportional hazards model.[28] Qualitative variables were analysed by Pearson χ^2 test or by the Fisher exact test, where appropriate. The Kruskal–Wallis test to compare group means and the Spearman test was used to test correlations between different variables. All p values are based on two-tailed statistical analysis, considering p values <0.05 as significant. Analyses were performed using SPSS V.10.1.

RESULTS

Aberrant expression of *BMI1* in DLBCL as compared with normal follicle centre cells

As described previously, *BMI1* expression in non-neoplastic germinal centres is almost completely restricted to non-dividing cells.[22 29] In DLBCL, *BMI1* was detected in DLBCL in all patients, but in highly varying percentages of dividing lymphoma cells. The percentage of *BMI1*-positive tumour cells ranged from <5% to almost 100% (fig 1); 25% of the cases had <25% whereas 75% had >25% *BMI1*-positive tumour cells.

Low levels of *BMI1* expression are related to a favourable clinical outcome

Using log-rank and Cox regression analysis, different thresholds of percentages of *BMI1*-positive tumour cells were tested; the threshold with optimal discriminative power with regard to overall survival was 25% (table 2, fig 2A). Using multivariate analysis including GCB/ABC phenotype and *BMI1* expression as categorical variables did not yield additional significant prognostic value for expression of *BMI1* above GCB versus ABC phenotype (fig 2B,C). However, expression of *BMI1* tended to be related to poor outcome, especially in GCB-like DLBCL (90% 5-year overall survival time *v* 55% 5-year overall survival time for *BMI1* negative and positive cases, respectively).

BMI1 expression is not restricted to activated B cell-like DLBCL

Microarray studies have shown that *BMI1* is one of the genes that distinguishes a GCB-like DLBCL from an ABC-like DLBCL. Using immunohistochemical analysis, cases with many *BMI1* positive tumour cells more often showed an ABC-like phenotype, although this relation was not significant (table 3).

Aberrant expression of *p16INK4a* and *p14ARF* in DLBCL as compared with normal follicle centre cells

To determine whether *BMI1* expression is related to (lowered) *p14ARF* or *p16INK4a* expression, we subsequently investigated *p14ARF* and *p16INK4a* expression in normal lymphoid tissue and DLBCL cases. In non-neoplastic lymphoid tissue *p14ARF* and *p16INK4a* are expressed in a limited number of cells of the

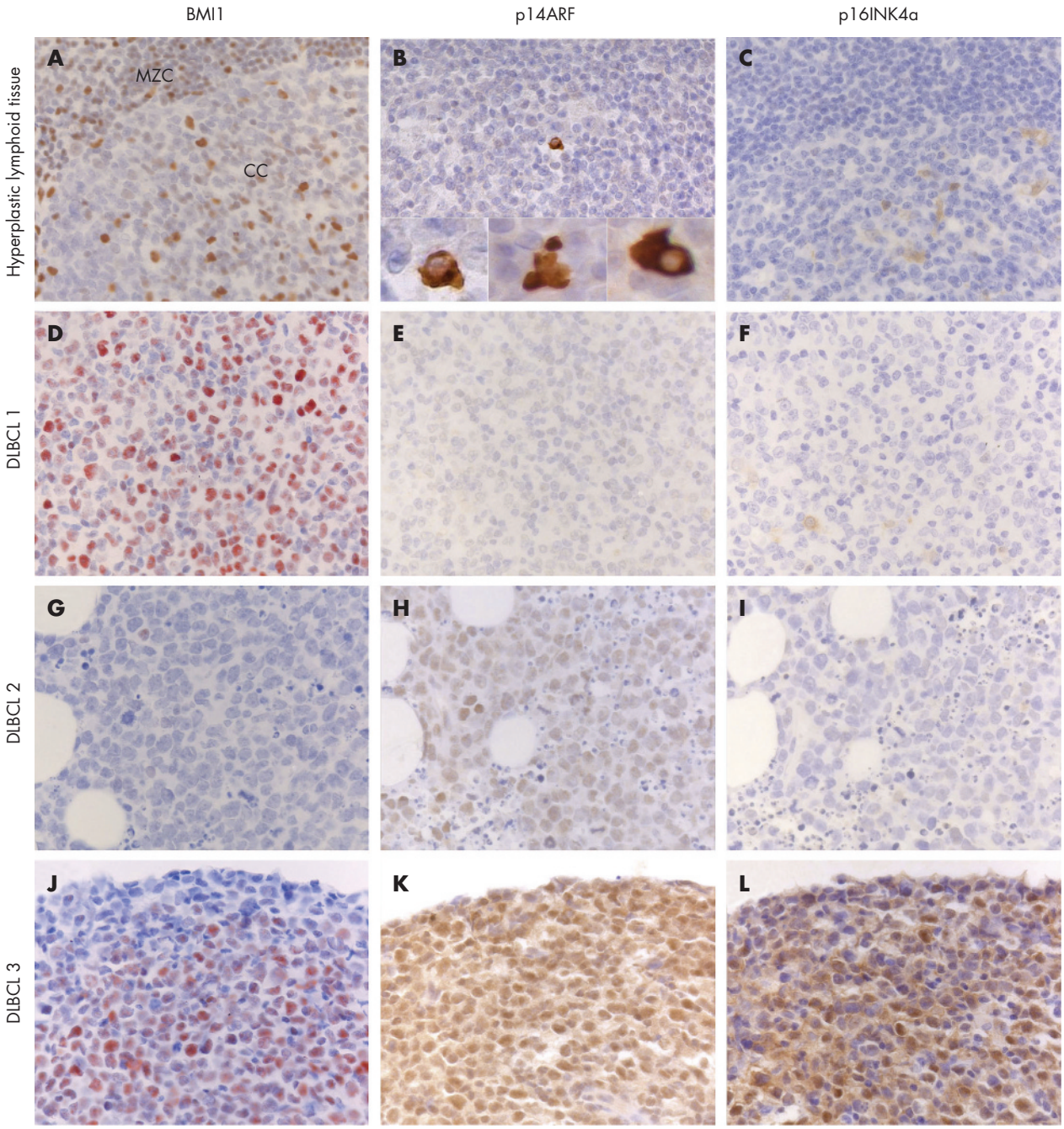

Figure 1 Expression of *BMI1*, *p14ARF* and *p16INK4a* in non-neoplastic lymphoid tissue and in three primary nodular DLBCL. (A–C) hyperplastic lymphoid tissue. *BMI1* staining is observed in resting mantle zone cells, centrocytes and T cells including germinal centre T cells, but not in centroblasts.[29] *p14ARF* and *p16INK4a* expression is detectable only in sporadic cells. Some of the *p14ARF*-positive cells show morphological features of apoptosis (fig B insets). (D–L) *BMI1*, *p14ARF* and *p16INK4a* staining patterns in three different DLBCL cases. DLBCL #1: this case shows *BMI1*-positive staining in >25% of tumour cells, but no expression of *p14ARF* and *p16INK4a*. DLBCL #2: expression of *p14ARF* is observed in the absence of *BMI1* and *p16INK4A* expression. DLBCL #3: this case shows expression of *BMI1*, *p14ARF* and *p16INK4a*.

germinal centre (fig 1). Nuclear expression of *p16INK4a* was found mainly in centroblasts. Interestingly, cytoplasmic *p14ARF* expression was also detected in sporadic interfollicular cells with apoptotic characteristics, consistent with its apoptosis-inducing function.[20]

In contrast, in *p14ARF* and *p16INK4a* positive tumours, expression was observed in the nucleus and cytoplasm of most neoplastic cells of DLBCL. In addition, in DLBCL, *p14ARF*-positive cells did not show apoptotic characteristics (fig 1).

Expression of *BMI1* in DLBCL is not consistently related to low levels of *p16INK4a* or *p14ARF* expression

Expression of *p14ARF* and *p16INK4a* was detected as nuclear staining in the tumour cells in 21 of 42 and in 22 of 59 cases, respectively. Expression of *p14ARF* and *p16INK4a* showed no direct correlation with expression of *BMI1*. However, *p16INK4a* tended to be more frequently absent in cases with many *BMI1*-positive tumour cells: 29 of 43 patients with high numbers of *BMI1*-positive tumour cells showed no expression of *p16INK4a*

Table 2 Patient characteristics in relation to *BMI1* expression

Patient characteristics	% *BMI1*-positive tumour cells		p Value*
	<25% (n = 15)	≥25% (n = 45)	
Median age (range)	62.3 (24–94)	63 (34–94)	NS†
Sex			
M	12	27	NS
F	3	18	
Stage‡			
1+2	5	20	NS
3+4	10	24	
B symptoms			
Yes	5	14	NS
No	10	31	
IPI§			
1+2	8	29	NS
3+4	7	15	
Complete remission			
Yes	13	29	NS
No	2	16	
Relapse¶			
Yes	4	9	NS
No	9	20	
Death			
Yes	4	27	0.04
No	11	18	

F, female; IPI, International Prognostic Index; M, male.
*As determined by χ^2 test, unless stated otherwise.
†As determined by Mann–Whitney U test.
‡Data concerning the staging could not be retrieved in one case.
§Data concerning IPI could not be retrieved in one case.
¶These cases involve patients who did reach complete remission.

as compared with 8 of 16 patients with few *BMI1*-positive tumour cells (p = 0.24; not shown). Also, when the analysis was restricted to cases without a proven genetic loss in the CDKN2A locus as determined by CGH analysis (n = 27; Oudejans JJ *et al*, manuscript in preparation), no correlation between expression of *p14ARF* and *p16INK4a*, and *BMI1* was observed (table 3).

DISCUSSION

In this study, we have shown that *BMI1* and its presumed target genes *p14ARF* and *p16INK4a* are frequently aberrantly expressed in malignant cells of DLBCL when compared with normal follicle centre B-lymphocytes, and that expression of *BMI1* is related to an unfavourable clinical outcome in primary nodal DLBCL.

It was previously shown and confirmed by us that DLBCL with an ABC-like phenotype have a relatively unfavourable clinical outcome and that one of the genes involved in the designation of this profile is *BMI1*[4] (supplement of studies by Alizadeh *et al*[11], de Boer *et al*[13] and Muris *et al*[27]). Although we found that *BMI1* expression is indeed more frequently present in ABC-like DLBCL, *BMI1* expression was not restricted to this group of DLBCL. This might be explained by the fact that, also in the microarray study, *BMI1* alone does not determine the GCB-like versus ABC-like phenotype.[11 13] Expression of *BMI1* was also not significantly correlated with expression of *MUM-1* (data not shown), which was previously shown to be related to a poor prognosis.[27] This indicates that the prognostic effect of *BMI1* expression is probably not directly related to *MUM-1* expression.

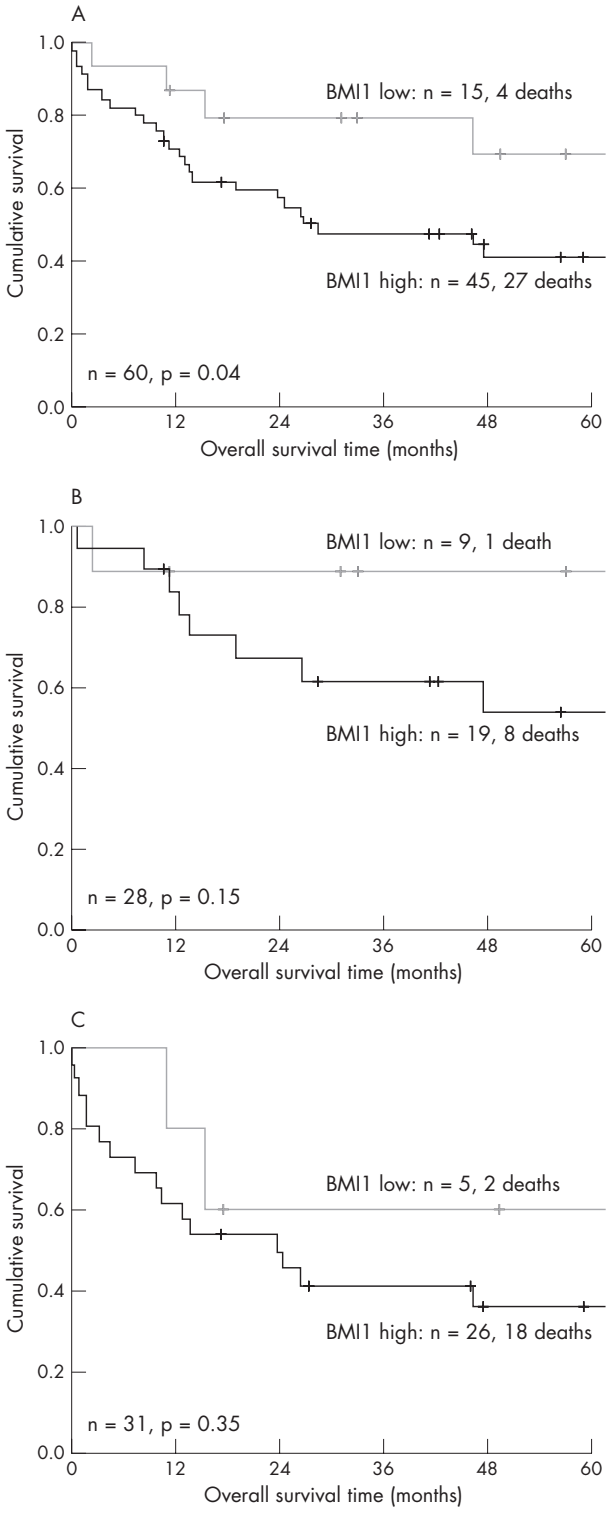

Figure 2 Comparison of overall survival in patients with primary nodal DLBCL according to *BMI1* expression. High numbers of *BMI1*-positive tumour cells are correlated with poor prognosis (A). *BMI1* expression has no additional significant prognostic value above GCB (B) versus ABC (C) phenotype.

A possible explanation for the observed relationship between *BMI1* expression and prognosis is that *BMI1* inhibits apoptosis via down regulation of the *p14ARF* gene. Experimental model

Table 3 Tumour characteristics in relation to percentages of *BMI1* positive tumour cells

Tumour characteristics	*BMI1*-positive tumour cells (%)		
	<25% (n = 15)	≥25% (n = 45)	p Value*
ABC/GCB profile			
GCB	9	19	NS
ABC	5	26	
p14ARF			
Negative	2	8	NS
Positive	0	11	
p16INK4a			
Negative	2	19	NS
Positive	0	6	

Results of interpretable cases are given. The number of cases for p14ARF and p16INK4a stainings is low as only cases were included, and for these it was confirmed that there are no genetical aberrations present in the CDKN2A locus. An ABC/GCB profile could not be defined in one case due to uninterpretable staining of one of the individual markers.
*As determined by χ^2 test.

systems demonstrated that *BMI1* cooperates with *c-Myc* to induce lymphomas by preventing *c-Myc* and *Ink4a/ARF* induced apoptosis,[18] thus inhibiting the intrinsic apoptosis pathway and thereby explaining poor response to chemotherapy. However, inhibition of apoptosis by high expression of *BMI1* would imply down regulation of *p14ARF*, which was observed only in some *BMI1*-positive cases. In non-neoplastic lymphoid tissues, expression of *p14ARF* in reactive lymph nodes was observed in a few cells frequently, with morphological features of apoptosis (see fig 1). This would be consistent with the normal tumour-suppressive, apoptosis-inducing function of *p14ARF*. However, in *p14ARF*-positive DLBCL, diffuse positive staining was observed in cells without morphological features of apoptosis, suggesting that the normal apoptosis-inducing effect of *p14ARF* is disrupted, consistent with a previous paper by Sanchez-Aguilera *et al*.[30] Thus, in some cases, *p14ARF* is expressed despite expression of *BMI1*, but its apoptosis-inducing effect might be counteracted by downstream apoptosis-inhibiting proteins like Bcl-2 and XIAP.

Alternatively, *BMI1* could be involved in down regulation of the tumour suppressor protein *p16INK4a*, resulting in suppression of senescence and cell cycle control,[15] causing enhanced proliferation of the tumour cells. Indeed, in our study we also found *BMI1*-positive cases that showed absence or low levels of *p16INK4a* expression. However, some *BMI1*-positive patients also showed expression of *p16INK4a* in most rapidly proliferating cells. Similar to *p14ARF*, expression of *p16INK4a* in non-neoplastic reactive lymphoid tissues is restricted to very few follicle centre cells. Thus, the normal cell cycle suppressive function of *p16INK4a* in these lymphomas is probably disrupted. Co-expression of *BMI1* and *p14ARF/p16INK4a* may be explained by phosphorylation of *BMI1* and subsequent de-repression of the *INK4a/ARF* locus, as recently shown by Voncken *et al*[31]

Recently, Glinsky *et al*[32] also investigated the expression of *BMI1* and its putative targets in relation to prognosis. Expression of these 11 *BMI1* target genes was correlated to poor prognosis in patients with lymphoma. Thus, *BMI1* seems to be an important gene in normal and neoplastic tissue, because of its role in regulating the expression of various genes involved in cell cycle control.

We conclude that *BMI1* and *p14ARF* and *p16INK4*a are aberrantly expressed in some primary nodal DLBCL. Expression of *BMI1* is related to unfavourable outcome. Down regulation of tumour suppressor genes *p14ARF* and *p16INK4a* by *BMI1* might explain the relationship between *BMI1* expression and poor outcome in some DLBCL. However, strong expression of *p14ARF* and *p16INK4a* in some DLBCL suggests that the tumour-suppressive effect of these proteins is counteracted by downstream mechanisms.

Take-home messages

- Expression of *BMI1* in diffuse large B cell lymphomas correlates with poor clinical outcome.
- Strong expression of *p14ARF* and *p16INK4a* in some DLBCL suggests that the tumour-suppressive effect of these proteins is counteracted by downstream mechanisms.
- Expression of *BMI1* is not restricted to activated B cell like DLBCL.

ACKNOWLEDGEMENTS

We thank the following persons for their help in collecting tumour material and clinical data: Professor R Willemze, Professor HC Kluin-Nelemans, Dr LH Siegenbeek van Heukelom, Dr NM Jiwa, Dr MA MacKenzie and Dr JH Krieken. We also thank Dr R Ridder for kindly providing the *p16INK4a* antibody.

Authors' affiliations

Joost C van Galen*, Jettie J F Muris*, Joost J Oudejans, Wim Vos, Cindy P E Giroth, Frank M Raaphorst, Chris J L M Meijer, Departments of Pathology, VU Medical Center, Amsterdam, The Netherlands
Gert J Ossenkoppele, Departments of Haematology, VU Medical Center, Amsterdam, The Netherlands
Arie P Otte, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands
Frank M Raaphorst, Crucell Holland BV,Leiden, The Netherlands

*These authors contributed equally to this study

Competing interests: None.

REFERENCES

1 **The International Non-Hodgkin's Lymphomas Prognostic Factors Project**. A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med* 1993;**329**:987–94.
2 **Kramer MH**, Hermans J, Parker J, *et al*. Clinical significance of Bcl-2 and p53 protein expression in diffuse large B-cell lymphoma: a population-based study. *J Clin Oncol* 1998;**14**:2131–8.
3 **Barrans SL**, Carter I, Owen RG, *et al*. Germinal center phenotype and bcl-2 expression combined with the International Prognostic Index improves patient risk stratification in diffuse large B-cell lymphoma. *Blood* 2002;**99**:1136–43.
4 **Hans CP**, Weisenburger DD, Greiner TC, *et al*. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004;**103**:275–82.
5 **Lossos IS**, Jones CD, Warnke R, *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.
6 **Chang CC**, Liu YC, Cleveland RP, *et al*. Expression of c-Myc and p53 correlates with clinical outcome in diffuse large B-cell lymphomas. *Am J Clin Pathol* 2000;**113**:512–18.
7 **Yamaguchi M**, Seto M, Okamoto M, *et al*. De novo CD5+ diffuse large B-cell lymphoma: a clinicopathologic study of 109 patients. *Blood* 2002;**99**:815–21.
8 **Muris JJ**, Meijer CJ, Cillessen SA, *et al*. Prognostic significance of activated cytotoxic T-lymphocytes in primary nodal diffuse large B-cell lymphomas. *Leukemia* 2004;**18**:589–96.
9 **Muris JJ**, Cillessen SA, Vos W, *et al*. Immunohistochemical profiling of caspase signaling pathways predicts clinical response to chemotherapy in primary nodal diffuse large B-cell lymphomas. *Blood* 2005;**105**:2916–23.
10 **Kuppers R**, Klein U, Hansmann ML, *et al*. Cellular origin of human B-cell lymphomas. *N Engl J Med* 1999;**341**:1520–9.
11 **Alizadeh AA**, Eisen MB, Davis RE, *et al*. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000;**403**:503–11.
12 **Shipp MA**, Ross KN, Tamayo P, *et al*. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat Med* 2002;**8**:68–74.
13 **de Boer WP**, Oudejans JJ, Meijer CJ, *et al*. Analysing gene expressions with GRANK. *Bioinformatics* 2003;**19**:2000–1.

14 **Raaphorst FM**, Otte AP, Meijer CJ. Polycomb-group genes as regulators of mammalian lymphopoiesis. *Trends Immunol* 2001;**22**:682–90.
15 **Jacobs JJ**, Kieboom K, Marino S, *et al.* The oncogene and Polycomb-group gene *BMI1* regulates cell proliferation and senescence through the ink4a locus. *Nature* 1999;**397**:164–8.
16 **van Lohuizen M**, Verbeek S, Scheijen B, *et al.* Identification of cooperating oncogenes in E mu-myc transgenic mice by provirus tagging. *Cell* 1991;**65**:737–52.
17 **Alkema MJ**, Bronk M, Verhoeven E, *et al.* Identification of BMI1-interacting proteins as constituents of a multimeric mammalian polycomb complex. *Genes Dev* 1997;**11**:226–40.
18 **Jacobs JJ**, Scheijen B, Voncken JW, *et al. BMI1* collaborates with c-Myc in tumorigenesis by inhibiting c-Myc-induced apoptosis via INK4a/ARF. *Genes Dev* 1999;**13**:2678–90.
19 **Ruas M**, Peters G. The p16INK4a/CDKN2A tumor suppressor and its relatives. *Biochim Biophys Acta* 1998;**1378**:F115–77.
20 **Park IK**, Morrison SJ, Clarke MF. Bmi1, stem cells, and senescence regulation. *J Clin Invest* 2004;**113**:175–9.
21 **Lowe SW**, Sherr CJ. Tumor suppression by Ink4a-Arf: progress and puzzles. *Curr Opin Genet Dev* 2003;**13**:77–83.
22 **Van Galen JC**, Dukers DF, Giroth C, *et al.* Distinct expression patterns of polycomb oncoproteins and their binding partners during the germinal center reaction. *Eur J Immunol* 2004;**34**:1870–81.
23 **van Kemenade FJ**, Raaphorst FM, Blokzijl T, *et al.* Coexpression of BMI1 and EZH2 polycomb-group proteins is associated with cycling cells and degree of malignancy in B-cell non-Hodgkin lymphoma. *Blood* 2001;**97**:3896–901.
24 **Raaphorst FM**. Of mice, flies, and man: the emerging role of polycomb-group genes in human malignant lymphomas. *Int J Hematol* 2005;**81**:281–7.
25 **Valk-Lingbeek ME**, Bruggeman SW, van Lohuizen M. Stem cells and cancer; the polycomb connection. *Cell* 2004;**118**:409–18.
26 **Hamer KM**, Sewalt RG, den Blaauwen JL, *et al.* A panel of monoclonal antibodies against human polycomb group proteins. *Hybrid Hybridomics* 2002;**21**:245–52.
27 **Muris JJF**, Meijer CJLM, Vos W, *et al.* Immunohistochemical profiling based on Bcl-2, CD10 and MUM1 expression improves risk stratification in patients with primary nodal diffuse large B-cell lymphoma. *J Pathol* 2006;**208**:714–23.
28 **Cox DR**. Regression models and life tables. *J Roy Statist Soc Ser B Methodol* 1972;**34**:187–220.
29 **Raaphorst FM**, van Kemenade FJ, Fieret E, *et al.* Cutting edge: polycomb gene expression patterns reflect distinct B cell differentiation stages in human germinal centers. *J Immunol* 2000;**164**:1–4.
30 **Sanchez-Aguilera A**, Sanchez-Beato M, Garcia JF, *et al.* p14(ARF) nuclear overexpression in aggressive B-cell lymphomas is a sensor of malfunction of the common tumor suppressor pathways. *Blood* 2002;**99**:1411–18.
31 **Voncken JW**, Niessen H, Neufeld B, *et al.* MAPKAP kinase 3pK phosphorylates and regulates chromatin association of the polycomb group protein Bmi1. *J Biol Chem* 2005;**280**:5178–87.
32 **Glinsky GV**, Berezovska O, Glinskii AB. Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. *J Clin Invest* 2005;**115**:1503–21.