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t(1;14) and t(11;18) in the differential diagnosis of Waldenström's macroglobulinemia

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Waldenström's macroglobulinemia is caused by several B-cell proliferative disorders including lymphoplasmacytic lymphoma, marginal zone B-cell lymphoma, B-cell chronic lymphocytic leukemia and multiple myeloma. Differential diagnosis between lymphoplasmacytic lymphoma and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue is particularly difficult as there is a considerable overlap in histological presentation. We report a case of Waldenström's macroglobulinemia with involvement of the peripheral blood, bone marrow and stomach. Serum chemistry revealed an IgM of 5.4 g/dl, but Bence–Jones protein in urine was negative. Abnormal lymphoid cells were detected in both blood and the bone marrow. Flow cytometry of the bone marrow aspirate showed that majority of cells were CD20$^+$, CD38$^+$, expressing immunoglobulin lambda light chain, but CD5$^-$ and CD10$^-$. Gastric biopsies revealed infiltration of the gastric mucosa by small lymphoid cells showing plasmacytoid differentiation and occasional Dutcher bodies. Lymphoepithelial lesions and *Helicobacter pylori* were not seen. Thus, the differential diagnosis between lymphoplasmacytic lymphoma and mucosa-associated lymphoid tissue lymphoma was raised. To resolve this, we performed BCL10 immunohistochemistry and reverse transcriptional polymerase chain reaction (RT–PCR) for the API2-MALT1 fusion transcript of t(11;18)(q21;q21). Both bone marrow and gastric biopsies showed strong BCL10 nuclear staining, similar to that seen in t(1;14)(p22;q32) positive mucosa-associated lymphoid tissue lymphoma, but absence of the API2-MALT1 fusion transcript. To further ascertain whether the detection of t(1;14)(p22;q32) and t(11;18)(q21;q21) can be reliably used for the differential diagnosis between lymphoplasmacytic lymphoma and mucosa-associated lymphoid tissue lymphoma, we screened for these translocations by BCL10 immunohistochemistry in 58 lymphoplasmacytic lymphomas and RT–PCR for t(11;18)(q21;q21) in 40 lymphoplasmacytic lymphomas, respectively. None of the lymphoplasmacytic lymphomas studied harbored these translocations. Thus, detection of t(1;14)(p22;q32) and t(11;18)(q21;q21) is useful in the differential diagnosis between lymphoplasmacytic lymphoma and mucosa-associated lymphoid tissue lymphoma.
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Waldenström's macroglobulinemia is a clinical syndrome caused by several B-cell proliferative disorders including B-cell chronic lymphocytic leukemia, multiple myeloma, lymphoplasmacytic lymphoma and marginal zone B-cell lymphoma, which are capable of producing a large amount of monoclonal IgM.[1] The clinical presentation includes those of the underlying disease and symptoms caused by hyperviscosity of blood due to high levels of IgM. Laboratory features are characterized by presence of monoclonal IgM protein in blood and a monoclonal Ig light chain (Bence–Jones protein) in urine. Clinical diagnosis of Waldenström's macroglobulinemia is usually straightforward, however, differential diagnosis of the underlying diseases could be a challenge. Among the underlying diseases, differential diagnosis between lymphoplasmacytic lymphoma and extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) is particularly difficult.

Lymphoplasmacytic lymphoma is histologically characterized by a diffuse infiltrate of small lymphocytes, plasmacytoid lymphocytes with or without Dutcher bodies and plasma cells. The neoplastic cells are positive for IgM, CD20 and CD38, but negative for CD5 and CD10. t(9;14)(p13;q32) is found in 50% of lymphoplasmacytic lymphomas that lack macroglobulinemia but not in those with paraproteinmia.[2] Currently, there are no specific markers for lymphoplasmacytic lymphoma.

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Mucosa-associated lymphoid tissue (MALT) lymphoma is characterized by neoplastic cells, known as centrocyte-like cells infiltrating around reactive B-cell follicles in a marginal zone distribution. The tumor cells are typically $CD20^+$, IgM^+, $CD5^-$ and $CD10^-$. The lymphoma cells frequently show plasmacytic differentiation and may form sheets of diffuse infiltrate.[3] Occasionally, MALT lymphoma involves bone marrow and spleen, and clinically presents with macroglobulinemia.[4] Thus, MALT lymphoma can mimic lymphoplasmacytic lymphoma in both histological and clinical presentations. Genetically, MALT lymphomas are specifically associated with t(11;18)(q21;q21),[5] t(1;14)(p22;q32)/IgH-BCL10[6] and t(14;18)(q32;q21)/IgH-MALT1.[7] We show that examination of these chromosomal translocations could help the differential diagnosis between MALT lymphoma and lymphoplasmacytic lymphoma.

Materials and methods

Case Report

A 78-year-old female patient had a 1-year history of gouty arthritis in her right ankle and recently complained of dizziness and low back pain for 1 month. Physical examination showed no lymphadenopathy or hepatosplenomegaly. A complete blood count revealed a hemoglobin of 7.8 g/dl, a white blood cell count of 10.1×10^3 cells/μl with 66% being atypical lymphocytes, and a platelet account of 214×10^3 cells/μl. Serum chemistry revealed an IgM of 5.4 g/dl, but Bence–Jones protein in urine was negative. Flow cytometry of the bone marrow aspirate showed that majority of cells expressed CD20, CD38 and immunoglobulin (Ig) lambda light chain, but not CD5 or CD10. Bone marrow trephine biopsy showed a heavy infiltrate by small lymphocytes, which expressed CD20, IgM and Igλ. A diagnosis of Waldenström's macroglobulinemia was made. After three months, the patient received gastric endoscopy, which revealed giant folds in the gastric cardia and a submucosal tumor-like lesion with surface ulceration at the greater curvature of the cardia. Histological examination of gastric biopsies showed that the mucosa was infiltrated by small lymphoid cells showing plasmacytoid differentiation and occasional Dutcher bodies. A differential diagnosis between gastric MALT lymphoma and lymphoplasmacytic lymphoma was raised. The patient was treated with endoxan, oncovin and prednisolone, showed a partial response but died of gastric bleeding and septic shock 24 months later.

Materials

Formalin-fixed and paraffin-embedded tissues blocks from the above case and 58 cases (38 from the bone marrow, 18 from the lymph node and two from the spleen) of lymphoplasmacytic lymphoma were retrieved from the authors' institutions.

RNA Extraction

Total RNA was extracted using an Ambion RNA isolation kit (Ambion Ltd. Huntingdon, Cambridgeshire, UK).[8] Briefly, five to 10 5 μm paraffin sections were deparaffinized in xylene. The tissue was digested with proteinase K (1 mg/ml) for 2 h at 45°C and solubilized in a guanidinium-based buffer. RNA was extracted with acid phenol:chloroform and precipitated in isopropanol. The precipitated RNA was washed in 75% ethanol and redissolved in 20μl RNA Storage Solution™.

Detection of t(11;18)(q21;q21) by RT-PCR

cDNA was synthesized using SuperScript Preamplification System (Invitrogen, Paisley, UK) with a mixture of gene specific primers including one primer for *G6PD*, which were designed to allow amplification of samples prepared from formalin-fixed and paraffin-embedded tissues.[8] Three sets of PCR primers with a common sense primer covering 93% of the known breakpoints on the *API2* gene (p-S:5′-GGAAGAGGAGAGAGAAAGAGCA) and three antisense primers (p-AS1:5′-CCAAGACTGC CTTTGACTCT; p-AS2:5′-GGATTCAGAGACGCCAT CAA and p-AS3:5′-CAAAGGCTGGTCAGTTGTTT) targeting all four breakpoints on the *MALT1* gene were used for PCR of the *API2-MALT1* fusion transcript as described previously.[8] *G6PD* was amplified in parallel as a control. PCR was performed separately with each primer set in duplicate. PCR products were analyzed by electrophoresis on 10% polyacrylamide gels.

Immunohistochemistry

BCL10 was immunostained using a mouse monoclonal antibody (clone 151, Zymed Laboratories Inc., CA, USA) on formalin-fixed and paraffin-embedded tissues as described previously.[9] BCL10 expression was considered positive when it was expressed in more than 5% of tumor cells.

Interphase Fluorescence *In Situ* Hybridization (FISH)

Interphase FISH for detection of BCL10 involved chromosomal translocation was carried out as described previously.[10]

Results

Case Study

The clinical and laboratory investigations clearly supported the diagnosis of Waldenström's macro-

globulinemia. However, it was a challenge to make a histological diagnosis. The gastric biopsy revealed infiltration of the mucosa by small lymphoid cells showing plasmacytoid differentiation and occasional Dutcher bodies. These cells expressed CD20 and IgM but not CD3 or IgD, and showed Ig lambda light-chain restriction. Lymphoepithelial lesions and *Helicobacter pylori* were not seen as demonstrated by immunohistochemistry. The differential diagnosis was between gastric MALT lymphoma and lymphoplasmacytic lymphoma. To resolve this, we attempted to detect the MALT lymphoma-associated translocations: t(11;18)(q21;q21) and t(1;14)(p22;q32). RT-PCR of the API2-MALT1 fusion transcript was used for detection t(11;18)(q21;q21) and both the bone marrow and gastric biopsies were negative for the fusion transcript. BCL10 immunohistochemistry was performed to attempt to identify evidence of t(1;14)(p22;q32) and both bone marrow and gastric biopsies showed strong homogeneous BCL10 nuclear expression in most if not all tumor cells (Figure 1a), similar to that seen in MALT lymphoma with t(1;14)(p22;q32) or its variants.[9,10] To verify whether the tumor cells harbor a BCL10 involved chromosomal translocation, interphase fluorescence *in situ* hybridization (FISH) was performed.[10] Although various pretreatments were tried, interphase FISH failed due to poor quality of the biopsy. Nonetheless, the strong BCL10 nuclear expression supported a MALT lymphoma diagnosis.

Lack of t(11;18)(q21;q21) and t(1;14)(p22;q32) in Lymphoplasmacytic Lymphoma

To further investigate whether t(11;18)(q21;q21) and t(1;14)(p22;q32) can be reliably used for differential diagnosis between MALT lymphoma and lymphoplasmacytic lymphoma, we investigated whether these translocations occur in lymphoplasmacytic lymphoma. A total of 40 cases of lymphoplasmacytic lymphoma were studied for t(11;18)(q21;q21). Control G6PD RT-PCR showed expected sized products in all cases, confirming that the RNA samples from these cases were adequate for RT-PCR of the API2-MALT1 fusion transcript. However, none of the lymphoplasmacytic lymphomas examined showed the API2-MALT1 fusion.

Strong BCL10 nuclear expression was used as presumptive evidence of t(1;14)(p22;q32) or its variant in 58 cases of lymphoplasmacytic lymphoma. None of these cases studied showed strong BCL10 nuclear expression, similar to that seen in MALT lymphoma with t(1;14)(p22;q32). However, 17 cases showed a weak BCL10 nuclear expression in 40–80% of tumor cells, and the remaining cases showed either a weak cytoplasmic BCL10 expression (Figure 1b) or negative staining.

Discussion

Several B-cell lymphomas including lymphoplasmacytic lymphoma, B-cell chronic lymphocytic leukemia, multiple myeloma and marginal zone B-cell lymphoma may show maturation to plasmacytoid or plasma cells containing cytoplasmic immunoglobulin and clinically present with Waldenström's macroglobulinemia. Diagnosis of lymphoplasmacytic lymphoma is restricted to those that lack features of other lymphomas. Among these B-cell proliferative diseases, the differential diagnosis between MALT lymphoma and lymphoplasmacytic lymphoma is frequently difficult as there is a considerable overlap in histological features and occasionally in clinical presentation. This is particularly true when the primary MALT lymphoma lesion is inconspicuous and not clinically recognizable. Even if it is recognized, the lymphoepithelial lesion and reactive lymphoid follicles, characteristic features of MALT lymphoma, may not be detectable in those presenting with primary macroglobulinemia. Here we showed that t(11;18)(q21;q21) and t(1;14)(p22;q32) were absent in lymphoplasmacytic lymphoma and detection of those translocations was useful in the differential diagnosis between lymphoplasmacytic lymphoma and MALT lymphoma.

Both t(11;18)(q21;q21) and t(1;14)(p22;q32) are significantly associated with advanced cases of MALT lymphoma. For example, t(11;18)(q21;q21) has been found in 78% of gastric MALT lymphomas at stage II_E or above.[8,11] MALT lymphoma with Waldenström's macroglobulinemia is usually at an advanced stage, typically showing bone marrow and peripheral blood involvement.[4,12] Thus, it is most likely that the incidence of these translocations in MALT lymphoma with Waldenström's macroglobulinemia is high. In this regard, it is noteworthy that t(11;18)(q21;q21) has been observed in several MALT lymphomas with Waldenström's macroglobulinemia.[4,13] Thus, detection of these translocations will play a significant role in the differential diagnosis between lymphoplasmacytic lymphoma and MALT lymphoma.

T(11;18)(q21;q21) can be detected by RT-PCR or interphase FISH.[8,13] These molecular assays can be applied to both frozen and formalin-fixed and paraffin-embedded tissues. T(1;14)(p22;q32) and its variants can be detected by interphase FISH. The tumor cells with t(1;14)(p22;q32) are characterized by strong uniformed BCL10 nuclear staining.[10] We previously showed that 5/5 cytogenetically proven t(1;14)(p22;q32) cases displayed strong BCL10 nuclear expression[9] (Du *et al*, unpublished data). In a recent study, we further confirmed t(1;14)(p22;q32) or its variants in 5/7 cases showing strong BCL10 nuclear expression by interphase FISH.[10] Thus, BCL10 immunohistochemistry can be used as screening test for t(1;14)(p22;q32) or its variants.

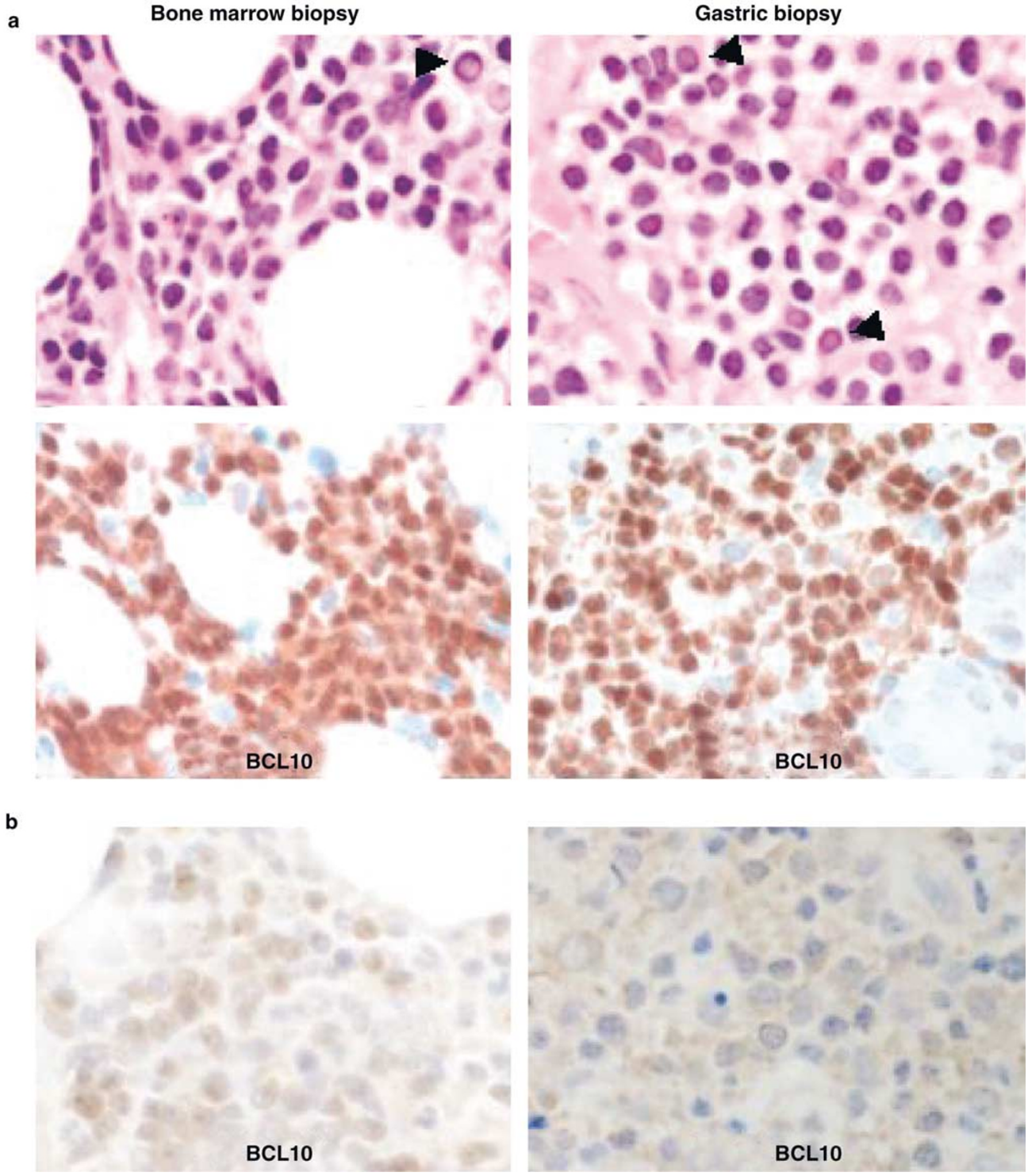

Figure 1 (**a**) Histology and BCL10 immunohistochemistry of the index case. Both bone marrow and gastric biopsies reveal infiltration by small lymphoid cells showing plasmacytoid differentiation and occasional Dutcher bodies (indicated by arrow head). BCL10 immunohistochemistry shows strong nuclear staining in the tumor cells of both biopsies. (**b**) BCL10 expression pattern in lymphoplasmacytic lymphoma. The case on the left shows a weak nuclear expression pattern, while the case on the right displays a weak cytoplasmic expression pattern.

Although none of the lymphoplasmacytic lymphoma cases studied showed strong BCL10 nuclear staining similar to that seen in t(1;14)(p22;q32) positive MALT lymphoma, 29% of cases displayed a weak nuclear staining in 40–80% of tumor cells. The significance of BCL10 nuclear expression in lymphoplasmacytic lymphoma is unknown. Interestingly, moderate BCL10 nuclear staining was

recently described in nasal NK/T-cell lymphomas in the absence of t(1;14)(p22;q32) and t(11;18)(q21;q21), and BCL10 nuclear expression pattern correlated with NFκB p65 nuclear localization.[14]

In summary, t(1;14)(p22;q32) and t(11;18)(q21;q21) are not associated with lymphoplasmacytic lymphoma, further supporting the previous evidence that these chromosomal translocations are MALT lymphoma specific. Detection of these translocations is useful in the differential diagnosis between lymphoplasmacytic lymphoma and MALT lymphoma.

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References

1 Berger F, Isaacson PG, Piris MA, *et al.* Lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia. In: Jaffe ES, Harris NL, Stein H, Vardiman JW (eds). World Health Organization Classification of Tumours, Pathology & Genetics: Tumours of Haematopoietic and Lymphoid Tissues. IARC Press: Lyon, 2001, pp 132–134.

2 Offit K, Parsa NZ, Filippa D, *et al.* t(9;14)(p13;q32) denotes a subset of low-grade non-Hodgkin's lymphoma with plasmacytoid differentiation. Blood 1992; 80:2594–2599.

3 Isaacson PG, Muller-Hermelink HK, Piris MA, *et al.* Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In: Jaffe ES, Harris NL, Stein H, Vardiman JW (eds). World Health Organization Classification of Tumours. Pathology & Genetics: Tumours of Haematopoietic and Lymphoid Tissues. IARC Press: Lyon, 2001, pp 157–160.

4 Hirase N, Yufu Y, Abe Y, *et al.* Primary macroglobulinemia with t(11;18)(q21;q21). Cancer Genet Cytogenet 2000;117:113–117.

5 Ott G, Katzenberger T, Greiner A, *et al.* The t(11;18)(q21;q21) chromosome translocation is a frequent and specific aberration in low-grade but not high-grade malignant non-Hodgkin's lymphomas of the mucosa-associated lymphoid tissue (MALT-) type. Cancer Res 1997;57:3944–3948.

6 Wotherspoon AC, Soosay GN, Diss TC, *et al.* Low-grade primary B-cell lymphoma of the lung. An immunohistochemical, molecular, and cytogenetic study of a single case. Am J Clin Pathol 1990;94: 655–660.

7 Streubel B, Lamprecht A, Dierlamm J, *et al.* t(14;18(q32;q21) involving IGH and MALT1 is a frequent chromosomal aberration in MALT lymphoma. Blood 2003;101:2335–2339.

8 Liu H, Ye H, Ruskone-Fourmestraux A, *et al.* T(11;18) is a marker for all stage gastric MALT lymphomas that will not respond to *H. pylori* eradication. Gastroentorology 2002;122:1286–1294.

9 Ye H, Dogan A, Karran L, *et al.* BCL10 expression in normal and neoplastic lymphoid tissue: nuclear localization in MALT lymphoma. Am J Pathol 2000;157:1147–1154.

10 Ye H, Liu H, Attygalle A, *et al.* Variable frequencies of t(11;18)(q21;q21) in MALT lymphomas of different sites: significant association with CagA strains of *H. pylori* in gastric MALT lymphoma. Blood 2003; 102:1012–1018.

11 Liu H, Ye H, Dogan A, *et al.* T(11;18)(q21;q21) is associated with advanced mucosa-associated lymphoid tissue lymphoma that expresses nuclear BCL10. Blood 2001;98:1182–1187.

12 Valdez R, Finn WG, Ross CW, *et al.* Waldenstrom macroglobulinemia caused by extranodal marginal zone B-cell lymphoma: a report of six cases. Am J Clin Pathol 2002;116:495–496.

13 Kobayashi Y, Nakata M, Maekawa M, *et al.* Detection of t(11;18) in MALT-type lymphoma with dual-color fluorescence in situ hybridization and reverse transcriptase–polymerase chain reaction analysis. Diagn Mol Pathol 2001;10:207–213.

14 Shen L, Liang ACT, Lu L, *et al.* Aberrant BCL10 nuclear expression in nasal NK/T-cell lymphoma. Blood 2003;102:1553–1554.