TCL1 expression patterns in Waldenström macroglobulinemia

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The oncogenic role of TCL1 in chronic lymphocytic leukemia is well established in transgenic mice. TCL1 expression in other B-cell malignancies has been also described: post-germinal center-derived malignancies, such as multiple myeloma, classically do not express TCL1. Waldenström macroglobulinemia is a post-germinal center malignancy that is known to be similar to chronic lymphocytic leukemia in terms of its gene expression profile. TCL1 expression has not been so far assessed in Waldenström macroglobulinemia. Transcriptomic explorations show that TCL1A expression is linked to signaling pathways and biological functions that are known to be involved in Waldenström macroglobulinemia as well as to gene signatures of interest in B-cell malignancies. We investigated TCL1 expression at the protein level in the bone marrow of a series of 59 patients with Waldenström macroglobulinemia: 76% of patients expressed TCL1, which appeared to be associated with a pejorative prognostic impact. TCL1 could have an oncogenic role in Waldenström macroglobulinemia, and deserves further exploration.
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Waldenström macroglobulinemia is an uncommon B-cell lymphoproliferative disorder characterized by lymphoplasmacytic cells that infiltrate the bone marrow, along with the presence of an immunoglobulin M monoclonal protein in the serum.[1]

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Waldenström macroglobulinemia is thought to be derived from B cells in which differentiation has been arrested after a somatic hypermutation process in the germinal center and before terminal maturation into plasma cells.[2] Oncogenesis may be a result of dysregulation of the NF-κB and PI3K/Akt/mTOR signaling pathways,[3–5] and from the influence of the bone marrow microenvironment.[6,7] Furthermore, whole-genome sequencing of Waldenström macroglobulinemia patients has recently uncovered highly recurring *MYD88*^{L265P} and WHIM-like *CXCR4* mutations that have a potential role in such pathways.[8,9]

The T-cell leukemia/lymphoma 1 (TCL1) protein encoded by the *TCL1A* gene (*TCL1*) is normally expressed during the early steps of B- and T-cell lymphopoiesis.[10] TCL1 has been shown to be overexpressed in chronic lymphocytic leukemia,[11,12] and its oncogenic role has been well established in transgenic mice.[13] It acts as an activator of Akt[14] and NF-kB[15] signaling in the cytoplasm, but may also function as a transcriptional regulator in the nucleus.[16] Increased TCL1 expression has been observed in other B-cell disorders[17,18] but not in multiple myeloma. TCL1 expression level still needs to be investigated in Waldenström macroglobulinemia and is the focus of this report.

Materials and methods

Analysis of Transcriptomic Datasets

Transcriptomic data were obtained from native data taken from two publicly available datasets (GSE6691 and GSE12668 datasets). The GSE6691 GEO dataset (56 samples) provided insight into the differences between cells from Waldenström macroglobulinemia and their cell counterparts in chronic lymphocytic leukemia and multiple myeloma. These data were analyzed to compare respective *TCL1A* expressions in these disease entities using the GEO2R tool from NCBI. The GSE12668 dataset (22 samples) provided high-resolution genomic analysis of Waldenström macroglobulinemia. These data were analyzed to assess *TCL1A*-co-regulated genes in Waldenström macroglobulinemia. We performed a new normalization procedure to check the intrinsic qualities of the native data: gene expression levels were normalized using the GC Robust Multi-array Average algorithm and flags were computed using MAS5. Quality assessment of the chips was performed using the affyQC-Report R package. Cluster analysis was performed by hierarchical clustering using Spearman's correlation of similarity measure and an average-linkage algorithm. Data were analyzed using QIAGEN's Ingenuity Pathway Analysis,[19] which assessed the relationships between genes and proteins, and constructed pathways to unveil the relevant biological processes.

Patients and Samples

Fifty-nine patients who underwent a bone marrow trephine biopsy for Waldenström macroglobulinemia, at Clermont-Ferrand and La Pitié-Salpêtrière University Hospitals between 1998 and 2012, were included in accordance with the Declaration of Helsinki. All included patients presented with Waldenström macroglobulinemia as defined by the international guidelines according to the World Health Organization classification[20] and the Second International Workshop on Waldenström's Macroglobulinemia,[1] ie, bone marrow infiltrated by immunoglobulin M producing clonal lymphoplasmacytic lymphoma. All patients were treated according to the Athens criteria.[1] Their characteristics are shown in Table 1. The median follow-up was 103 months (6–261 months).

Immunohistochemistry and Scoring

Analyses were performed in the Clermont-Ferrand pathology laboratory using monoclonal mouse anti-human TCL1 antibody (Clone 27D6/20; MBL International Corporation, Des Plaines, IL, USA; 1/100 dilution for 1 h) and monoclonal mouse anti-human CD20 antibody (Clone L26; Dako, Carpinteria, CA, USA; 1/100 dilution for 1 h). Immunostaining was assessed by three independent investigators who were blinded to the clinical data. If there was disagreement, a second review was conducted.

To assess the tumor infiltrates (stained by hematoxylin and eosin, and by anti-CD20 antibody), the percentage of TCL1-stained tumor cells was determined using a previously published four-tier scoring system for TCL1 assessment in B-cell malignancies,[11,17] as follows: 0 = negative; 1 = < 5% of tumor cells positive; 2 = positive in up to 50% of tumor cells; and 3 = positive in > 50% of tumor cells. TCL1 staining intensity was also recorded using a previously published three-tier score (0 = no staining; 1 = low intensity; 2 = high intensity)[11] to evaluate the intra-tumoral distribution and level of TCL1 expression within each tumor sample.

Statistical Analyses

Therapeutic requirements and the time until endpoints were defined according to published recommendations.[1,21,22] Pearson Chi-square test, two-sided Fisher test, Mann–Whitney test, Kaplan–Meier method, and log-rank test were applied to the data in appropriate settings, using SPSS Statistics v22 (IBM), PRISM v6.0 (Graphpad), and/or R[23] software.

Results

TCL1A Expression and Related Transcriptome

To investigate the potential role of TCL1 in the biology of Waldenström macroglobulinemia, we first analyzed publicly available transcriptomic datasets. Analyses of data from GSE6691 showed that *TCL1A* mRNA expression levels of Waldenström macroglobulinemia cells ranged from those observed in multiple myeloma to those observed in chronic lymphocytic leukemia ($P < 0.001$) (Figure 1a). In order to analyze TCL1-related transcriptome, we defined two groups of patients according to *TCL1A* expression using the GSE12668 dataset: ie, a $TCL1A^{low}$ group with normalized fluorescence of

Table 1 Patients' characteristics

	ALL (n = *59)*	*Neg./weak TCL1A* (n = *16)*	*Mod./strong TCL1A* (n = *43)*	P
Sex ratio (M/F)	37/22	12/4	25/18	0.23
Age at diagnosis, median (range), years	60 (36–83)	64 (36–83)	59 (37–80)	0.15
*ISS-WM (*n = *54)*				
Low risk, *N* (%)	14 (26)	4 (29)	10 (25)	1
Int/High risk, *N* (%)	40 (74)	10 (71)	30 (75)	
Biology				
Hb, median (range), g/dl	10.5 (5.4–16.5)	9.8 (5.4–14.5)	10.6 (5.6–16.5)	0.24
Hb level ≤ 11.5 g/dl, *N* (%)	41 (69)	12 (75)	29 (67)	0.75
Platelet count, median (range), $\times 10^9$/l	213 (18–430)	190 (37–422)	224 (18–430)	0.25
Platelet count $< 100 \times 10^9$/l, *N* (%)	4 (7)	3 (19)	1 (2)	0.057
β2 microglobulin, median (range), mg/l (*n* = 40)	3.3 (1.4–9.8)	2.9 (2.1–9.8)	3.5 (1.4–6.3)	0.73
β2 microglobulin > 3 mg/l, *N* (%) (*n* = 46)	27 (46)	4 (36)	23 (66)	0.16
Monoclonal component, median (range), g/l	26.2 (2.3–87)	33.1 (8.3–55.2)	25.5 (2.3–87)	0.54
Monoclonal component > 70 g/l, *N* (%)	2 (3)	0	2 (5)	1
Lymphocytosis, median (range), $\times 10^9$/l	1.5 (0.42–6.1)	1.5 (0.54–6.1)	1.74 (0.42–4.9)	0.5
Absolute neutrophil count, median (range) $\times 10^9$/l	3 (0.06–10)	3.7 (0.06–10)	2.7 (1.7–9.6)	0.064
LDH > Normal, *N* (%) (*n* = 52)	6 (10)	1 (8)	5 (13)	1
Clinical features, N *(%)*				
Hyperviscosity	10 (17)	3 (19)	7 (13)	1
Adenopathy	27 (46)	9 (56)	18 (42)	0.32
Splenomegaly	13 (22)	1 (6)	12 (28)	0.09
Hepatomegaly	11 (19)	1 (6)	10 (23)	0.26
Neuropathy	11 (19)	4 (25)	7 (16)	0.47
Treatment, N *(%)*				
Rituximab	5 (9)	1 (6)	4 (9)	
Chlorambucil	27 (46)	5 (31)	22 (51)	
Fludarabine	12 (20)	4 (25)	8 (19)	
Fludarabine, cyclophosphamide	2 (3)	0	2 (5)	
Rituximab, fludarabine, cyclophosphamide	3 (5)	2 (13)	1 (2)	
Dexamethasone, rituximab, cyclophosphamide	9 (15)	4 (25)	5 (12)	
Bendamustine, rituximab	1 (2)	0	1 (2)	

285 ($<$ 25th percentile) and a *TCL1A*^{high} group with normalized fluorescence of 3186 ($>75^{th}$ percentile).

We checked that median *TCL1A* expression in the *TCL1A*^{low} group was 10-fold higher than the background noise and that *TCL1A* expression was significantly different between both groups (mean *TCL1A*^{high}/*TCL1A*^{low} ratio of 11, and a mean delta (*TCL1A*^{high} – *TCL1A*^{low}) of 2901) ($P < 0.001$). We then compared the gene expression profile related to each group (*TCL1A*^{high} *vs TCL1A*^{low}).

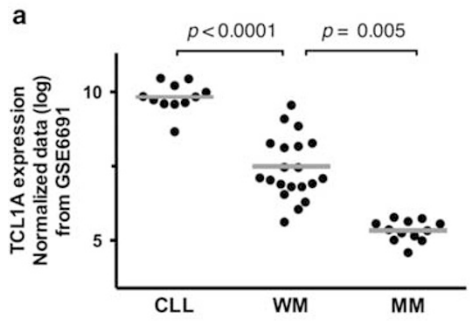
Consistent with the oncogenic role of TCL1, most relevant signaling pathways associated with *TCL1A*^{high} expression are known to be involved in cell cycle, apoptosis, and the DNA-damage response. Interestingly, *TCL1A*^{high} expression was associated with signaling pathways previously shown to be relevant in Waldenström macroglobulinemia (ie, PI3K/AKT, mTOR, BCR signal[3–5]) (Figure 1b). The highlighted biological functions related to *TCL1A* agreed with these results (Supplementary Table 1). In addition, *TCL1A*^{high} was associated with gene signatures of interest in tumor B-cell progression or resistance (eg, activated MYC, CCND1, SOX11 signatures, and an inhibited TP53 signature) (Supplementary Table 2).

By computing these data and using published data on *TCL1A*, we explored the putative interactions of *TCL1A* in Waldenström macroglobulinemia tumor cells. *TCL1A* could be linked to crucial actors in the pathophysiology of Waldenström macroglobulinemia within a tightly coupled network (Figure 1c).

Impact of TCL1 Protein Expression on Survival

Immunostaining of bone marrow tumor cells revealed that TCL1 was expressed at the protein level in 45 (76%) Waldenström macroglobulinemia patients. This expression was weak in 2, moderate in 17, and strong in 26 patients (Figure 2a). TCL1 was preferentially detected in the cytoplasm compared with the nucleus.

Regarding the clinical relevance of TCL1 expression in Waldenström macroglobulinemia, we failed to demonstrate any significant correlation between TCL1 expression pattern and the Waldenström macroglobulinemia features known to affect the prognosis (Table 1). However, patients with moderate/strong TCL1 expression had a significantly shorter time to next treatment compared with patients with negative/weak expression (16.4 *vs* 59.4 months, respectively;



b

	Canonical Pathways	-log(p-value)	Ratio
Cell cycle	Cell Cycle: G2/M DNA Damage Checkpoint Regulation	1.23E01	4.49E-01
	Mitotic Roles of Polo-Like Kinase	1.09E01	3.64E-01
	Cell Cycle Control of Chromosomal Replication	5.9E00	4.07E-01
	Cyclins and Cell Cycle Regulation	5.66E00	2.44E-01
DDR	Role of CHK Proteins in Cell Cycle Checkpoint Control	6.72E00	3.09E-01
	Mismatch Repair in Eukaryotes	6.41E00	5.62E-01
	DNA damage-induced 14-3-3σ Signaling	5.59E00	4.74E-01
	ATM Signaling	5.52E00	2.71E-01
BCR signaling	B Cell Receptor Signaling	5.45E00	1.76E-01
PI3K/AKT	PI3K Signaling in B Lymphocytes	5.33E00	1.95E-01
	PI3K/AKT Signaling	5.13E00	1.95E-01
mTOR and protein synthesis	mTOR Signaling	9.27E00	2.13E-01
	EIF2 Signaling	1.72E01	2.81E-01
	Regulation of eIF4 and p70S6K Signaling	1.16E01	2.6E-01
	p70S6K Signaling	5.39E00	2.02E-01
	DNA Methylation and Transcriptional Repression Signaling	6.43E00	5.00E-01
	RAN Signaling	6.11E00	5.29E-01
	fMLP Signaling in Neutrophils	5.62E00	2.13E-01
	Telomere Extension by Telomerase	5.51E00	5.33E-01
	Glucocorticoid Receptor Signaling	5.25E00	1.53E-01
	Telomerase Signaling	5.16E00	2.12E-01
	Protein Ubiquitination Pathway	1.06E01	2.00E-01
	Oxidative Phosphorylation	5.00E+00	2.02E-01

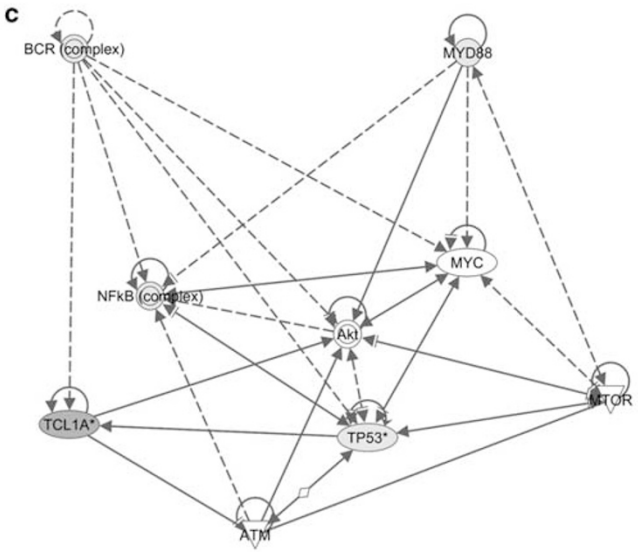


Figure 1 *TCL1A* transcriptomic expression: comparative exploration and biological relevance. (**a**) *TCL1A* transcriptomic expression was compared within three different B-cell malignancies: chronic lymphocytic leukemia (CLL), multiple myeloma (MM), and Waldenström macroglobulinemia (WM). *TCL1A* transcription level in WM was intermediate between CLL and MM, as was expected regarding the classical precocious post-germinal center normal counterpart in WM. (**b**) Main canonical pathways were positively linked to a high *TCL1A* transcription level. In order to show only the strongest and most reliable data, we show all pathways applicable to hematological malignancies that had a –log(*P*-value) >5 and were linked to biological function. It is noteworthy that the main pathways implicated in the pathophysiology of WM are among the canonical pathways linked to *TCL1A*'s high transcription level (BCR, PI3K/Akt, mTOR, and ATM). (**c**) *TCL1A* could be directly (full line) or indirectly (dotted line) linked to the main biological actors in WM (actors significantly more expressed in *TCL1A*^{high} cells are colored (red or light grey)). Of note, in this study, MYD88 and TCL1 transcription levels were correlated (MYD88 ratio 1.23; delta: 142; $P=0.024$). A full color version of this figure is available at the *Modern Pathology* journal online.

HR 2.07 (95% CI: 1.037–3.692), $P=0.036$) (Figure 2b). No significant impact was observed in terms of progression-free survival or overall survival.

Discussion

Herein, for the first time, we have highlighted the expression of the *TCL1* oncogene in Waldenström macroglobulinemia. The TCL1 transcriptomic targets were in line with the biology of Waldenström macroglobulinemia, and high TCL1 expression was associated with a poor outcome.

Using analyses from transcriptomic datasets, we found that *TCL1* expression levels ranged from those observed in multiple myeloma to those observed in chronic lymphocytic leukemia. Moreover, gene expression profiles related to *TCL1* gene expression were similar to the gene signatures of interest in B-cell malignancies, and corresponded to the biological pathways involved in cell cycle, apoptosis, and the DNA-damage response, which are known to be implicated in Waldenström macroglobulinemia oncogenesis. Altogether, these results suggest that TCL1 could have an oncogenic role in Waldenström macroglobulinemia.

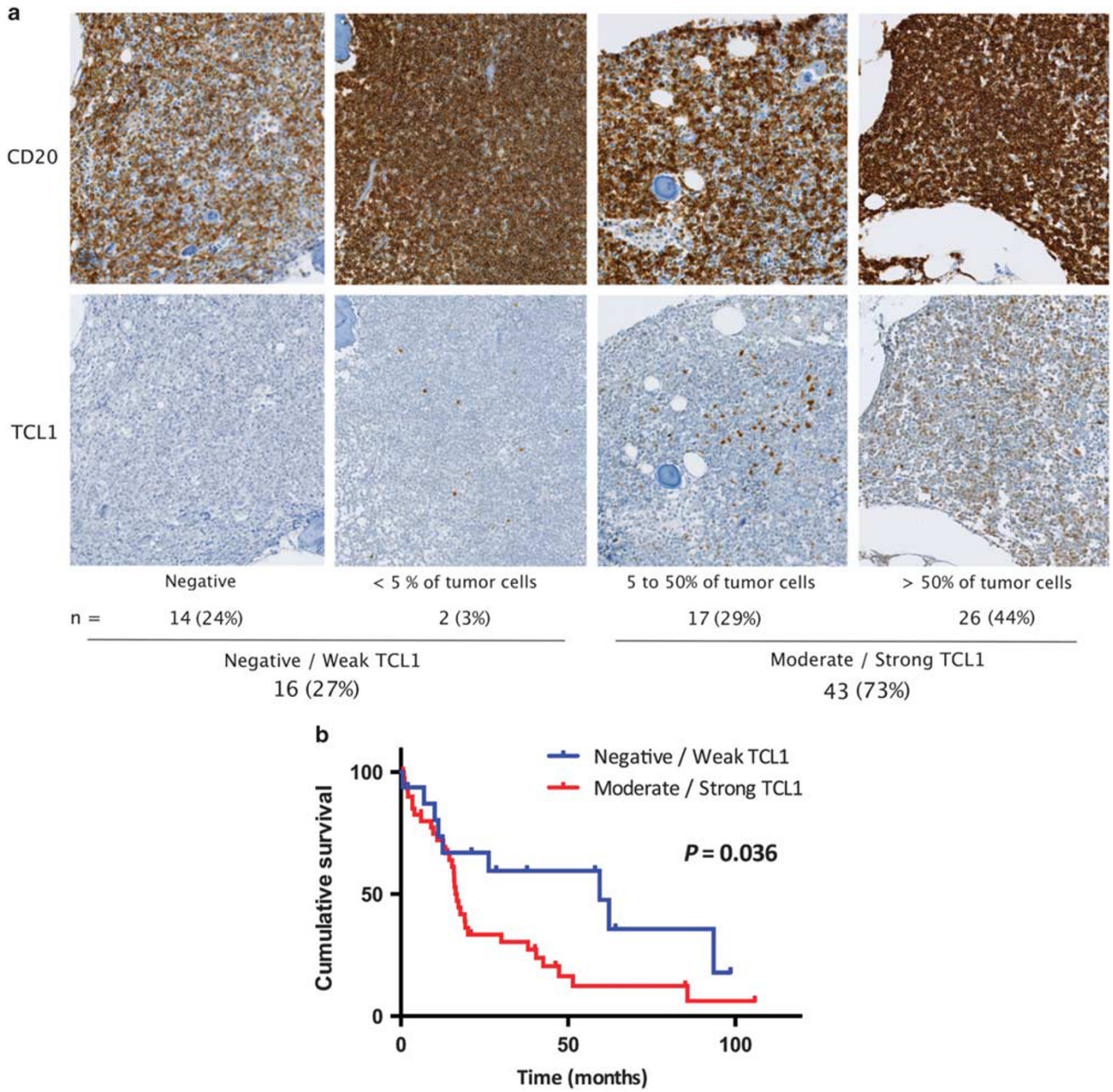


Figure 2 TCL1 protein expression: characteristics and clinical impacts on the time to next treatment. (**a**) TCL1 protein expression was assessed with a previously published four-tier scoring system that focused on the percentage of CD20+ tumor cells positive for TCL1 staining. Most patients showed 'moderate to strong' expression of TCL1. (**b**) Patients with moderate/strong TCL1 expression had a shorter time to next treatment compared with those with negative/weak expression (respectively 16.4 *vs* 59.4 months, HR 2.07 (1.037–3.692), $P = 0.036$).

Most patients expressed TCL1 at a protein level. Moreover, the high percentage of tumor cells that expressed TCL1 was linked to a poor outcome but was not associated with classical agressive features. Interestingly, staining intensity was heterogeneous within the tumor infiltrates, as has been described in chronic lymphocytic leukemia,[11] which questions the mechanisms underlying TCL1 overexpression in Waldenström macroglobulinemia.

TCL1 was preferentially detected in the cytoplasm compared with the nucleus: this has also been observed in Burkitt's lymphoma, but both cytoplasmic and nuclear localization were then seen in pre-germinal center B-cell lymphoma.[17]

On the basis of these observations, two major issues have to be addressed. First, the published data indicate that TCL1 is not expressed in patients with marginal-zone lymphoma.[17,24] Differential diagnosis of Waldenström macroglobulinemia with marginal-zone lymphoma can be challenging, and its diagnosis directly impacts on the choice of therapeutic options. Our data suggest that TCL1 expression could be a differential marker between Waldenström macroglobulinemia and marginal-zone lymphoma, although

this remains to be explored further. In addition, if TCL1 expression can clearly distinguish Waldenström macroglobulinemia from other B-cell malignancies, including marginal-zone lymphoma and multiple myeloma, such a difference, as demonstrated by the *MYD88*^{L265P} mutation, could provide important insights regarding the specific patterns of tumor development in Waldenström macroglobulinemia.

Overall, our results indicate that the TCL1 oncogene is significantly expressed in most of Waldenström macroglobulinemia cases, in which it could drive important pathways and adversely impact on prognosis. Added value of TCL1 staining for Waldenström macroglobulinemia diagnosis remains to be investigated.

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Disclosure/conflict of interest

The authors declare no conflict of interests.

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Supplementary Information accompanies the paper on Modern Pathology website (http://www.nature.com/modpathol)