MYD88 L265P Somatic Mutation

Its Usefulness in the Differential Diagnosis of Bone Marrow Involvement by B-Cell Lymphoproliferative Disorders

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ABSTRACT

Objectives: *To examine the usefulness of the* MYD88 *L265P somatic mutation in identifying cases of lymphoplasmacytic lymphoma (LPL) from other lymphoplasmacytic neoplasms in bone marrow biopsy specimens.*

Methods: *We studied 64 bone marrow biopsy specimens with involvement by various small B-cell lymphomas or plasma cell myeloma.*

Results: *The* MYD88 *L265P somatic mutation was present in 13/13 cases of LPL, 1/13 cases of hairy cell leukemia, and absent in the other mature B-cell neoplasms tested. A test set of diagnostically challenging bone marrow cases with lymphoplasmacytoid morphology (B-cell lymphoma, not otherwise specified) was selected for additional review and reclassified, without knowledge of the* MYD88 *L265P status. Of those 16 cases, 7 were positive for* MYD88, *including 4/4 cases that were reclassified as LPL during the review.*

Conclusions: *Although not entirely specific,* MYD88 *L265P is a useful adjunct for bone marrow diagnosis in separating LPL from other small B-cell lymphomas and plasma cell myeloma.*

Upon completion of this activity you will be able to:
- discuss the differential diagnosis of B-cell lymphomas with plasmacytic differentiation that involve the bone marrow.
- correlate *MYD88* L265P status with histopathologic and immunophenotypic data.
- examine the utility of *MYD88* L265P mutational testing in routine bone marrow biopsy diagnostic practice.

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Questions appear on p 441. Exam is located at www.ascp.org/ajcpcme.

Lymphoplasmacytic lymphoma (LPL) is a neoplasm of small B lymphocytes, plasmacytoid lymphocytes, and plasma cells involving bone marrow (BM), lymph node, and spleen. It is characteristically accompanied by an IgM monoclonal paraprotein, although other types such as IgG or IgA can rarely occur.[1] LPL is associated clinically with Waldenström macroglobulinemia (WM), defined as BM involvement with monoclonal B cells of LPL type and monoclonal IgM paraprotein at any level. In the BM, LPL may exist in 3 morphologic variants, described as lymphoplasmacytoid, lymphoplasmacytic, and polymorphous types, and the infiltrate is most often present in an intertrabecular/nonparatrabecular pattern.[2,3]

Distinguishing LPL/WM from other small B-cell lymphomas (BCLs) and plasma cell myelomas (PCMs) in BM biopsy specimens can be difficult. The constellation of small mature lymphocytes with varying degrees of plasmacytic differentiation can be seen in other mature B-cell

lymphoproliferative disorders such as splenic marginal zone lymphomas (MZL) and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). LPL may be confused with plasma cell myeloma (PCM), particularly those cases of PCM that are CD20 positive with small lymphocyte-like morphology.[4]

Immunophenotypically, LPL is characterized by expression of pan–B-cell markers such as CD19, CD20, CD79a, and PAX5. CD5 may be expressed in a few cases, whereas CD10 is usually negative. Thus, a specific defining immunophenotype for LPL is lacking. Further complicating the pathologists' task in establishing a diagnosis of LPL in the BM is the fact that an IgM paraprotein can be seen in many lymphoid neoplasms and is also not specific for LPL. Therefore, the pathologic diagnosis of LPL is often one of exclusion.[5]

Partial deletion of chromosome 6q is frequently seen in WM.[6,7] However, it is also common among non-IgM/non-WM LPL and MZL.[8] Furthermore, abnormalities such as deletions of 13q14, 11q21-q22 (*ATM*), and *TP53* and trisomies of 3 and 18 can be shared among low-grade BCLs such as LPL/WM, MZL, and CLL.[8,9] Recently, whole-genome sequencing identified a recurrent mutation in the myeloid differentiation primary response 88 gene (*MYD88* L265P), found in 91% of LPLs. It was absent or rarely present in multiple myeloma, MZL, and IgM monoclonal gammopathy of undetermined significance (MGUS).[10] Because the BM biopsy is often the first procedure performed in a patient suspected of having a lymphoproliferative process, the presence or absence of this mutation in BM harboring a lymphoid or lymphoplasmacytic infiltrate might be a useful feature in establishing a specific diagnosis.

We investigated the sensitivity/specificity of the *MYD88* L265P mutation in various B-cell neoplasms involved in BM biopsies and applied it to a diagnostically problematic subset of BM biopsies originally diagnosed as B-cell lymphoma, not otherwise specified (BCL, NOS), including cases with and without plasmacytic differentiation.

Materials and Methods

Case Selection

This study was carried out with approval from the Cleveland Clinic institutional review board. Pathology archives were searched for BM biopsy specimens that involved various mature BCLs from 2000 to 2012. The validation set was composed of lymphoma subtypes (LPL, MZL, CLL/SLL, PCM, mantle cell lymphoma [MCL], hairy cell leukemia [HCL], hairy cell leukemia-variant, and follicular lymphoma) for which a definitive, specific diagnosis was made based on World Health Organization (WHO) criteria. Plasma cell neoplasms were selected if they were positive for cyclin D1 (SP4, Thermo Fisher Scientific, Waltham, MA) on immunohistochemistry so as to include cases with lymphoplasmacytic morphology.[11] All HCL cases were positive for *BRAF* V600E on polymerase chain reaction (PCR). The test set of problematic cases (designated as BCL, NOS) was identified by selecting BM biopsy specimens with a final diagnosis of "B-cell lymphoproliferative disorder with plasmacytic differentiation," or "B-cell lymphoproliferative disorder," in which LPL was part of the stated differential diagnosis. Cases in the BCL, NOS category then underwent complete examination of the medical record for clinical and laboratory data before and after the BM biopsy.

Available biopsy specimens with successful DNA extraction were analyzed for the *MYD88* L265P mutation by allele-specific PCR. All negative results from cases that displayed any plasmacytic differentiation were repeated and were reproducibly negative. Selected positive results, including all those with ambiguous submitted diagnoses (test set), were confirmed with Sanger sequencing. Cases in the BCL, NOS group were rereviewed with all available prior and subsequent clinical, laboratory, immunophenotypic, and genetic data by 2 expert hematopathologists (E.D.H., J.R.C.) who were blind to the *MYD88* L265P mutational status. If possible, diagnoses were revised to a definitive WHO 2008 classification.

DNA Extraction

Sections cut from formalin-fixed, paraffin-embedded blocks of nondecalcified tissue to obtain 1 cm^2 of tissue were placed in 2 mL of microcentrifuge tubes and deparaffinized using xylene and 100% ethanol. Genomic DNA extraction was performed using the Genepure Kit (Qiagen, Germantown, MD) according to the manufacturer's directions.

Allele-Specific PCR Amplification

PCR amplifications were performed on the *MYD88* mutation using the following primer sets: *MYD88* L265P forward (5′-TAG GTG CCC ATC AGA AGC GAC C-3′) and *MYD88* reverse (5′-GGC CTT GGC AAG GCG AGT CCA G-3′). A 50-ng DNA sample was amplified with the following thermal cycling profile: 94°C for 5 minutes, then 40 cycles of 95°C for 15 seconds and 72°C for 30 seconds, with a final extension at 72°C for 10 minutes. The sensitivity of this assay was 0.1% for *MYD88* L265P mutant DNA (OCI-LY-10 cell line) diluted in wild-type DNA (OCI-LY-19 cell line) **Image 1**.

Results

Validation Set

Results of the *MYD88* L265P test of 64 BM samples are displayed in **Table 1**. Of the 13 cases that were submitted as LPL, all were positive for *MYD88* L265P. The patients

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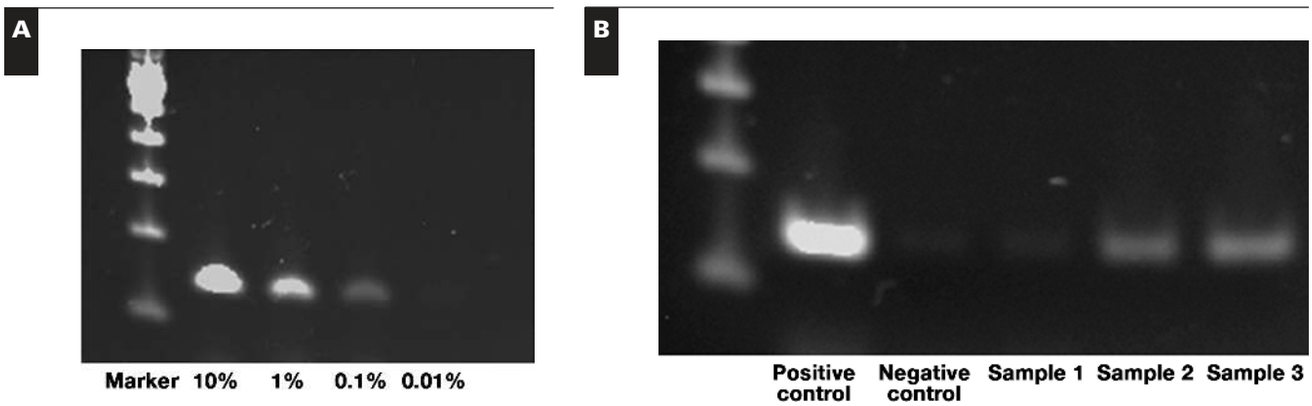

Image 1 Representative figures on agarose gel analysis of the allele-specific polymerase chain reaction (PCR) for the *MYD88* L265P mutation. **A**, Serial dilution of positive control cell line (OCI-LY-10) into a background of cells negative for *MYD88* L265P (OCI-LY-19). The limit of detection is 0.1%. **B**, Representative output of a typical allele-specific PCR on samples extracted from formalin-fixed paraffin-embedded samples. Samples 2 and 3 would be considered positive for the mutation.

were 6 men and 7 women, with a median age of 62 years (range, 48-88 years). Twelve patients with LPL had serum protein electrophoresis and immunofixation data, and all 12 had an IgM monoclonal paraprotein (9 κ, 2 λ, 1 biclonal), with a median monoclonal protein concentration of 1.23 g/dL (range, 0.30-4.26 g/dL). Involvement by LPL accounted for a median of 40% of BM cellularity (range, 10%-70%) and was predominantly in an interstitial pattern, but 1 case was paratrabecular and 1 was both interstitial and paratrabecular.

BM samples involving other subtypes of small BCLs were negative for the *MYD88* L265P mutation with the exception of 1 case of HCL **Image 2D**, **Image 2E**, **Image 2F**, and **Image 2G**. In that case, the BM aspirate smear showed cytologic and flow cytometric features typical of HCL (expression of CD11c, CD19, bright CD20, CD22, CD25, CD103, CD123, and monotypic κ surface immunoglobulin light chains). The BM trephine biopsy showed sheets of lymphoid cells with abundant clear cytoplasm and oval nuclear contours replacing the majority of the BM space and was positive on immunohistochemistry for CD20, CD25, and cyclin D1 (a subset with weak expression). Monoclonal serum protein electrophoresis analysis was not conducted. However, in addition to the *MYD88* L265P mutation, the *BRAF* V600E mutation was noted. The patient was treated with cladribine and showed a good hematologic response, with sustained remission at the last available follow-up (10 months). These data confirm that *MYD88* L265P is characteristic of LPL (sensitivity 100%, specificity 98% in our validation set of small BCLs involving the BM) and highlight the fact that the mutation is not entirely specific for LPL.

Table 1
Results of the *MYD88* L265P Mutation Test of 64 Bone Marrow Samples (Validation Set)

Submitted Diagnosis	No. Positive for *MYD88* L265P/Total
LPL	13/13[a]
SMZL	0/6[b]
CLL/SLL	0/9
PCM	0/8[c]
Mantle cell lymphoma	0/7
Hairy cell leukemia	1/13
Hairy cell leukemia variant	0/2
Follicular lymphoma	0/6

CLL, chronic lymphocytic leukemia; LPL, lymphoplasmacytic lymphoma; PCM, plasma cell myelomas; SLL, small lymphocytic lymphoma; SMZL, splenic marginal zone lymphoma.
[a] 12/12 with available data had an IgM monoclonal paraprotein.
[b] No serum protein electrophoresis/immunofixation data were available.
[c] All with available data (7/8) were positive for *IGH-CCND1* on fluorescence in situ hybridization, 1/8 was positive for CD20 on immunohistochemistry, and 1/8 had an IgM κ monoclonal paraprotein. All were positive for cyclin D1 on immunohistochemistry.

Test Set

To further explore the usefulness of *MYD88* L265P in a set of diagnostically problematic cases, we evaluated a series of 16 BM biopsies containing a B-cell lymphoproliferative disorder of small lymphocytes. This series was from the same time frame as the previous validation set. A specific WHO diagnosis was not rendered in this series because of the uncertainty regarding proper subclassification (diagnosed as BCL, NOS). After gathering all clinical, laboratory, and follow-up information available at the time of rereview, cases were evaluated by 2 hematopathologists, and specific WHO 2008 consensus diagnoses were made if possible. Reviewers were blind to the *MYD88* mutational status. This rereview yielded the results shown in **Table 2**.

The revised diagnostic categories were LPL (n = 4), MZL (n = 6), and PCM with small lymphocyte-like morphology (n = 2). The MZL cases consisted of 5 splenic MZL and 1 extranodal MZL of mucosa-associated lymphoid tissue. A separate category of BCL, other, was created for 2 cases (T13, T14).

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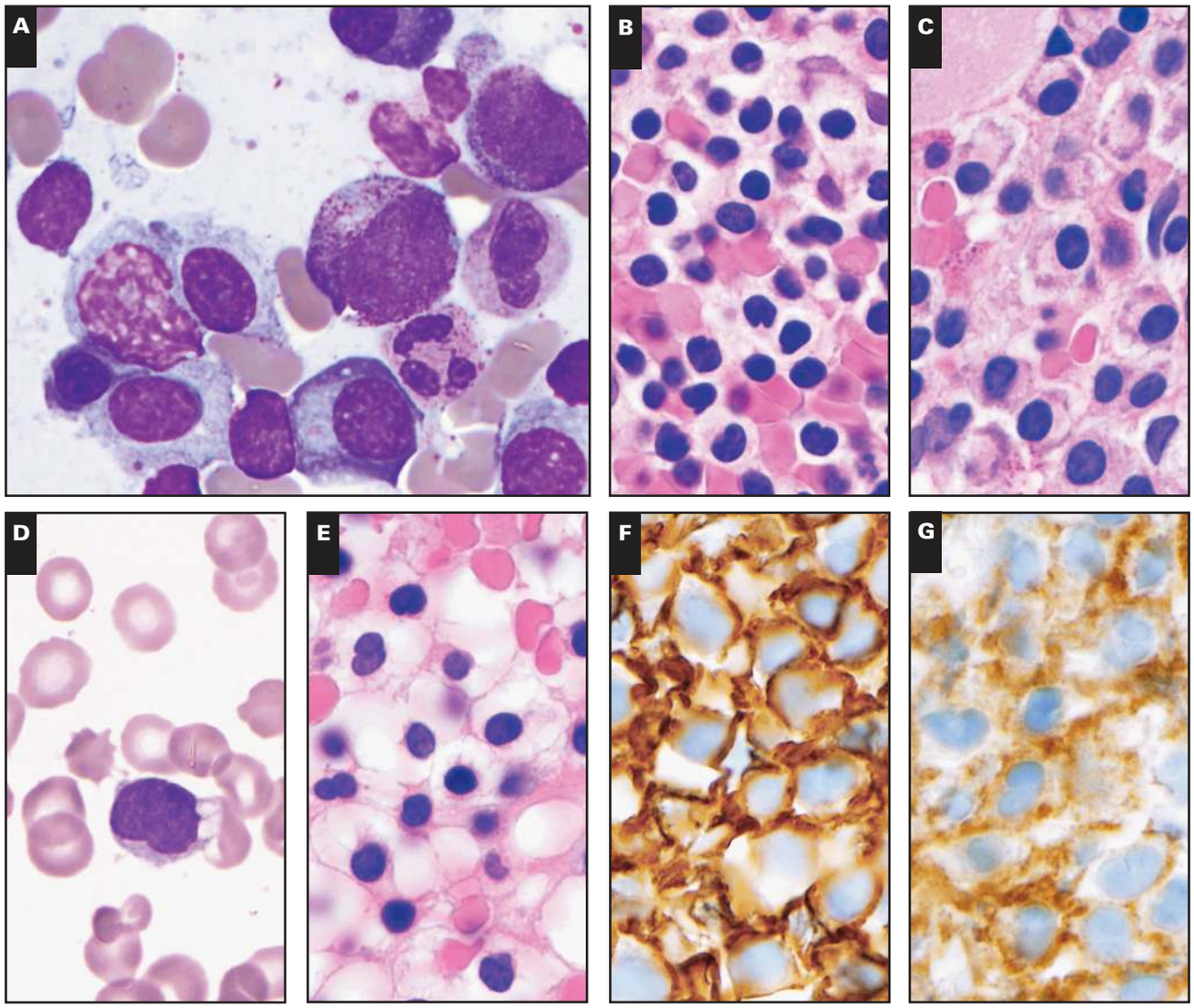

Image 2 Illustrative *MYD88* L265P–positive cases (test set). **A-C**, B-cell lymphoma, not otherwise specified, with plasmacytic differentiation and IgM paraprotein 1.12 g/dL. The reviewers could not distinguish between splenic marginal zone lymphoma and lymphoplasmacytic lymphoma (LPL) after all available data in the medical record were reviewed; perhaps the presence of *MYD88* L265P could be evidence to favor a diagnosis of LPL (**A**, Wright stain, ×1,000; **B** and **C**, H&E, ×1,000). **D-G**, Hairy cell leukemia. Flow immunophenotype: CD11c, CD19, bright CD20, CD22, CD25, CD103, CD123, and monotypic κ sIg light chains. The patient showed a good hematologic response to cladribine, with sustained remission (≥13 months) (**D**, Wright stain, ×1,000; **E**, H&E, ×1,000; **F**, CD20, ×1,000; **G**, CD25, ×1,000).

Both cases were being evaluated for a suspected myeloid disorder without lymphadenopathy or splenomegaly and were thus incidentally discovered B-cell processes. The supposition was that these cases might represent coexisting monoclonal B-cell lymphocytosis–type proliferations. Of the final 2 cases, 1 (T15) was a CD5-negative case with plasmacytic differentiation, for which the reviewers could not distinguish between splenic MZL and LPL after all available data in the medical record were reviewed **Image 2A**, **Image 2B**, and **Image 2C**. The remaining case (T16) was a staging BM biopsy in a patient recently diagnosed with a retroperitoneal "double hit" aggressive diffuse large BCL (DLBCL) that contained both *BCL2* and *MYC* rearrangements and a BM biopsy showing a small B-cell lymphoproliferative disorder but no evidence of "double hit" lymphoma **Image 2H**, **Image 2I**, and **Image 2J**. Within this test cohort, the *MYD88* L265P mutation was identified in each of the 4 LPL cases, 1 of the 5 splenic MZL cases, and both cases of BCL, NOS (T15 and T16).

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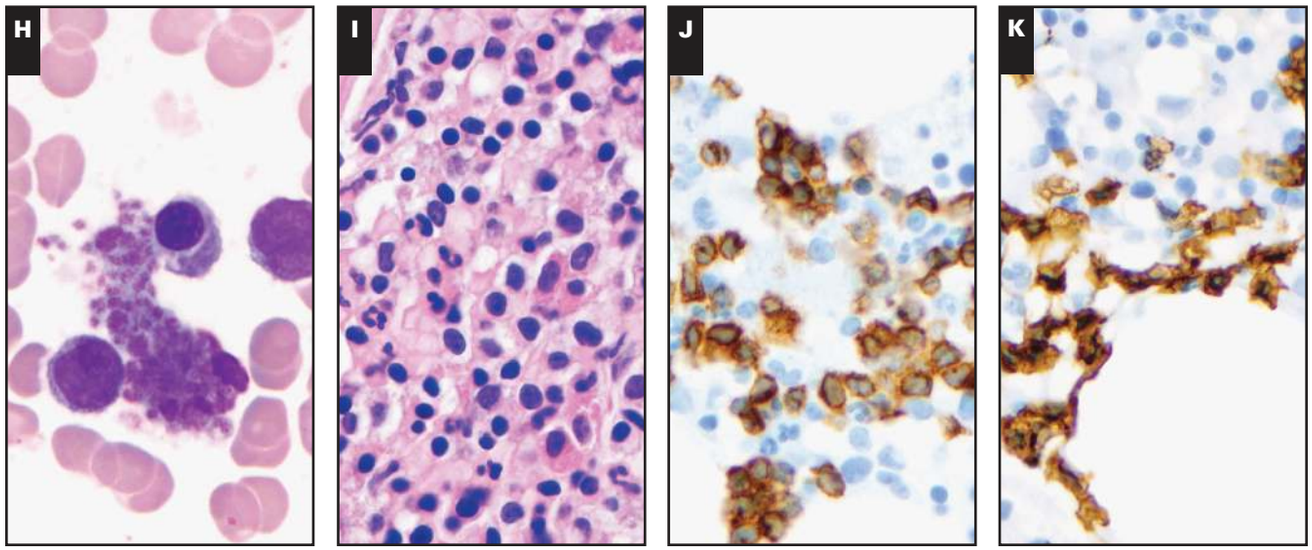

H-K, Low-grade component of "double hit" lymphoma with atypical, slightly clefted morphology and a monotypic B-cell population on flow cytometry. Immunohistochemistry for CD10 and MYC was negative. Fluorescence in situ hybridization for a *BCL2* translocation was negative. The retroperitoneal lymphoma was negative for *MYD88* L265P (**H**, Wright stain, ×1,000; **I**, H&E, ×500; **J**, CD79a, ×500; **K**, CD20, ×500).

Discussion

MYD88 is a signaling adaptor molecule that activates the NF-KB pathway in the course of the normal immune response.[12] The *MYD88* L265P mutation was initially discovered in DLBCL, where it was shown to promote cell survival in the activated B-cell–like subtype of DLBCL via an IRAK kinase-protein complex, leading to JAK-STAT3 activation, NF-KB signaling, and interleukin/interferon secretion. The mutation was detected in 29% of activated B-cell–like DLBCLs, 1.4% of germinal center B DLBCLs, 1.7% of Burkitt lymphomas, and 9% of gastric mucosa–associated lymphoid tissue lymphoma biopsies.[13] A recent study of Burkitt lymphoma using RNA sequencing and interference screening found *MYD*88 gene mutations in 4.8% of samples, but the location(s) was not specified.[14] Other large BCLs with a postgerminal center phenotype frequently harbor the *MYD88* L265P mutation, including primary central nervous system lymphoma (36%) and primary cutaneous DLBCL, leg type (69%).[15,16]

Considering small BCLs, *MYD88* gene mutations were discovered in CLL using whole-genome sequencing[17] and were present in a validation set (9/91 cases of CLL), 6 of which had the *MYD88* L265P mutation.[18] In our study, whole-genome sequencing of BM WM/LPL cells identified the *MYD88* L265P somatic variant, which was present in tumor samples of 49 (91%) of 54 patients with WM, 3 (100%) of 3 patients with non-IgM LPL, 3 (7%) of 46 patients with MZL, and 2 (10%) of 21 patients with IgM MGUS and absent from tumor samples with PCM, including IgM PCM, and from paired normal and nonpaired normal tissue samples.[10] Notably, in that study, the MZL samples that were positive for *MYD88* L265P (1 splenic MZL, 1 extranodal, and 1 nodal subtype) had features that overlapped with WM, including a monoclonal IgM paraprotein. Although the association of this mutation with LPL/WM was confirmed in a recent series, its frequency was slightly lower (67%), possibly because of methodologic or definitional differences.[19] Recently, it was suggested that the proportion of IgM MGUS cases harboring the mutation was higher (56%), calling into question the nosology of IgM MGUS and its relationship to LPL.[20]

In practice, it can be challenging to separate LPL from other small B-cell neoplasms that may be seen in BM biopsy specimens. Discovery of *MYD88* L265P in the majority of LPL cases represents a potentially useful diagnostic feature that might be exploited in the diagnosis of small BCLs with plasmacytic differentiation on BM biopsy.[10] Some histologic features, such as sinusoidal infiltration and a nodular pattern with a CD23+ meshwork in splenic MZL, are reported to be helpful in separating splenic MZL from LPL in the BM.[21,22] However, these features are not uniform and lack sensitivity. For example, Inamdar and colleagues[23] reported a focal sinusoidal pattern in only 30% of splenic MZL cases, whereas others demonstrated that a sinusoidal pattern can be seen in MCL[24] and other lymphoma subtypes.[25] In our total set of cases, a sinusoidal pattern of infiltration was present in 4 of 12 cases of MZL, in 1 case of MCL, and in 1 case of LPL. Nodular meshworks were present only in 1 case of splenic MZL and 1 case of MCL. Thus, in our experience, these

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Table 2
Clinical and Pathologic Features of the BCL, NOS Group (Test Set)

Case	Revised Diagnosis	Age/Sex	Bone Marrow Immunophenotype
T1	LPL	44/F	CD20+, fewer CD138+ plasma cells with monotypic κ and IgM heavy chain
T2	LPL	68/F	CD19+, CD20+, monotypic κ; CD5–, CD10–
T3	LPL	74/M	CD19+, CD20+, monotypic κ; CD5–, CD10–
T4	LPL	62/F	CD19+, CD20 bright+, CD5+, monotypic λ
T5	SMZL	79/M	CD19+, CD20+, CD23+^{dim}, CD5–, CD10–, CD123–
T6	SMZL	71/M	CD19+, CD20+^{bright}, CD25+^{dim}, monotypic κ; CD5–, CD10–, CD103–, CD123–
T7	SMZL	59/F	CD19+, CD20+, monotypic λ; CD5–, CD10–, CD103–, CD123–; scattered polytypic plasma cells
T8	SMZL	85/M	CD19+, CD20+, monotypic λ; CD5–, CD10–; scattered polytypic plasma cells
T9	SMZL	75/M	CD19+, CD20+, monotypic κ; CD5–, CD10–
T10	MZL-EN	50/M	CD19+, CD20+, monotypic κ; CD5–, CD10–
T11	PCM	65/F	Flow: CD38+, CD138+, and cytoplasmic κ monotypic; IHC: CD20+, MUM1+, PAX5+^{weak}, CD45–, cyclin D1–
T12	PCM	52/F	Flow: CD38+, CD45–,cytoplasmic κ monotypic; IHC: lymphoplasmacytoid cells are CD20+, cyclin D1–
T13	BCL, other (?MBL)	73/F	CD19+, CD20+, CD25+^{dim}, CD10–, CD123–, CD103–, monotypic λ; equivocal for CD5 by flow and IHC
T14	BCL, other (?MBL)	62/M	CD19+, CD20+, monotypic κ, CD5–; IHC: PAX5+, cyclin D1–
T15	BCL, NOS (LPL vs MZL)	64/M	CD19+, CD20+, monotypic λ, CD138+ plasma cells with monotypic λ
T16	BCL, NOS	50/M	IHC: small B-cells, CD20+, MYC–, CD10–

BCL, NOS, B-cell lymphoma, not otherwise specified; IHC, immunohistochemistry; LPL, lymphoplasmacytic lymphoma; MBL, monoclonal B lymphocytosis; MZL, marginal zone lymphoma; MZL-EN, extranodal marginal zone lymphoma; PCM, plasma cell myelomas; SMZL, splenic marginal zone lymphoma; SPE, serum protein electrophoresis.
[a] Reviewers were blind to the *MYD88* L265P status.
[b] Immunofixation analysis not done.

features are not particularly useful in an individual case. Indeed, some authors have written that distinguishing the two may be arbitrary.[26] An objective marker of LPL would help pathologists more reliably diagnose this pathologic entity. The discovery of *MYD88* L265P in more than 90% of LPLs with only rare other small B-cell lymphoproliferative disorders harboring this mutation opens the door for its use in a routine diagnostic setting. We confirm that *MYD88* L265P detection on allele-specific PCR is characteristic of LPL and absent or rarely present in multiple myeloma and other small BCLs. We further identified the first case of HCL harboring this mutation. The test performed well in identifying straightforward cases of WM/LPL and was less often present in splenic MZL, with similar positivity rates as those previously described.[10,13,19,27] Overall, our rate of positivity was 8% in splenic MZLs, similar to the 6% to 13% reported in splenic MZLs with allele-specific PCR.[27,28] Importantly, *MYD88* L265P was absent in other mimickers of LPL in this study, such as IgM myeloma (n = 1), CD20-positive small lymphocyte-like myeloma (n = 2), and CD5-negative monoclonal B lymphocytosis (n = 2). We did not have any *MYD88*-mutated cases of CLL, either because non-L265P variants may have been present or because our numbers of CLL were not high enough to capture any positive findings, given the known prevalence.[18]

With this information, we then applied the test in a set of BM cases involving a monoclonal B-cell population of small lymphocytes that were difficult to subclassify at the time of initial review. We established specific diagnoses in these cases, aided largely by additional clinical and laboratory data that were not present at the time of initial diagnosis. This represents a valuable set of cases in which *MYD88* L265P mutational analysis might be reasonably applied to aid in the specific diagnosis and thus provide a clearer picture of its diagnostic use. *MYD88* L265P was identified in all 4 difficult cases of LPL and 1 case of splenic MZL. Conversely, it was absent in 9 other non-LPL cases. However, it was also found in 2 cases that could not be confidently diagnosed as LPL. In 1 of these cases (T15), the differential diagnosis was splenic MZL vs LPL, and perhaps the presence of *MYD88* L265P might be considered evidence to favor an LPL diagnosis, given the presence of a substantial IgM paraprotein and lack of splenomegaly. This patient would meet criteria for WM set forth by the International Workshop in WM, also in agreement with the National Comprehensive Cancer Network diagnostic protocol.[29,30] Indeed, definitions of LPL/WM may be in flux as further investigation into the clinical and biologic significance of this mutation become clearer.

The other case, T16, is worth further discussion. This patient had a concurrent unclassifiable large BCL, with features intermediate between DLBCL and Burkitt lymphoma, with both *BCL2* and *MYC* rearrangements ("double hit" lymphoma). Because patients with LPL may progress to a large BCL (13% in one series),[31] it is possible that the BCL, NOS diagnosis in this patient could represent low-level underlying involvement by low-grade B-cell process. However, the lymph node was negative for *MYD88* L265P, and the BM cells were negative for *MYC* on immunohistochemistry and negative for *BCL2* translocation on fluorescence in situ hybridization (data not shown). Thus, a "transformation" from BM LPL or follicular lymphoma is unlikely. A second scenario that could relate these 2 processes is the presence of a "*BCL2*-translocated founder clone," either in the form of preexisting follicular lymphoma or in a follicular lymphoma-like t(14;18)

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Spleen	Monoclonal Paraprotein (g/dL)	Sinusoidal Pattern	*MYD88* L265P Status[a]
Normal	Free κ light chains in urine	No	+
Normal	IgM-κ 0.27; free κ light chains in urine	No	+
Normal	Not done	No	+
Normal	IgM-λ 1.19	Yes	+
Splenomegaly	Not done	No	–
Normal	IgM-κ 0.21	No	–
Splenomegaly	Hypogammaglobulinemia; no M protein on immunofixation	No	–
Splenomegaly	No M protein on SPE[b]	No	–
Splenomegaly	IgM-κ and IgG-κ (0.25 and 0.26)	No	+
Normal	IgM-κ 2.38	No	–
Normal	IgG-κ 2.27	No	–
Splenomegaly	IgA-κ 0.08	No	–
Normal	IgG-κ 0.41	No	–
Unknown	Not done	No	–
Normal	IgM-λ 1.12	No	+
Normal	Not done	No	+

memory B cell.[32] This founder B-cell clone then developed subclones, one of which acquired an *MYC* rearrangement and became the "double hit" aggressive lymphoma, and the other of which presented in the BM and acquired an *MYD88* L265P somatic mutation. The possibility of *MYD88* L265P as a secondary event would be inconsistent with the current belief that *MYD88* L265P appears to be a driver mutation in aggressive lymphoma.[13] Also, as noted before, we have no evidence of a *BCL2* translocation in the BM component. Thirdly, the 2 processes could be unrelated. The *MYD88* L265P–positive B-cell clone in the marrow is an incidental process whose biologic potential with regard to clinically evident disease (LPL or otherwise) is still unknown.

The past few years have seen an increase in the variety of immunophenotypic and molecular genetic features that assist in the differential diagnosis of small BCLs in the BM. These include CD10 and BCL2-IGH@ translocations in follicular lymphoma,[33] cyclin D1 and SOX11 expression in MCL,[34,35] *BRAF* V600E mutation and phospho-ERK expression in HCL,[36,37] strong cyclin D1 expression in PCM with small lymphocyte-like morphology,[11] and LEF1 for CLL/SLL.[38,39] Allele-specific PCR shows the *MYD88* L265P mutation to be specific and sensitive for identifying LPL/WM. We emphasize that allele-specific PCR or another sensitive method be used as opposed to Sanger sequencing because of the often sparse and patchy nature of lymphoid infiltrates in the BM. Because it is not entirely specific for LPL, the results should be interpreted in the setting of other clinical, laboratory, and pathologic findings. We recommend testing for this mutation in problematic cases where, based on our data, its presence would strongly favor LPL/WM. However, it should not be used in cases with a clear diagnosis based on current WHO criteria. The presence or absence of the mutation may gain importance outside diagnostic application in the future if it informs individualized treatment decisions in patients with evidence of an overactive *MYD88* pathway.

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