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Clone-specific MYD88 L265P and CXCR4 mutation status can provide clinical utility in suspected Waldenström macroglobulinemia/lymphoplasmacytic lymphoma

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ABSTRACT

MYD88 L265P, a diagnostic marker for lymphoplasmacytic lymphoma (LPL)/Waldenström macroglobulinemia (WM) can also be detected in other hematopoietic malignancies. We demonstrate a novel approach to increase the specificity of this marker for WM/LPL diagnosis by combining flow cytometric cell sorting with molecular analysis.

Clonal B-lymphocyte and co-occurring clonal plasma cell populations of low-grade B-cell lymphomas were sorted by flow cytometry and analyzed for immunoglobulin gene rearrangements (PCR), and for *MYD88* and *CXCR4* mutations.

Identical clonal origin was confirmed by PCR for 21 LPL/WM cases and *MYD88* L265P was detected in both B-cell and plasma cell fractions. 9/20 other B-cell lymphomas with identical light chain restriction on B-cells and plasma cells were genotypically identical by PCR and *MYD88* L265P was detected in both cell fractions in 7/9 whereas in 11/20 specimens with different clonal origin, *MYD88* L265P was absent (5/11), or only found in B-lymphocytes (4/11), or plasma cells (2/11). *CXCR4* mutations were detected in 17/39 cases, but missed in 63% of these without cell sorting.

Confirming *MYD88*L265P in both B-cells and plasma cell fractions can provide a novel and powerful discriminator to distinguish LPL/WM from phenotypically similar disorders. Furthermore, this approach significantly increases *CXCR4* detection sensitivity.

1. Introduction

Lymphoplasmacytic lymphomas (LPL) are CD5-negative, CD10-negative low-grade B-cell lymphomas with small-size cells and with plasmacytic differentiation, but this immunophenotype is not specific for LPL. The clinical syndrome of Waldenström macroglobulinemia (WM) is defined by the WHO classification as an LPL with an associated monoclonal IgM gammopathy. LPL is a diagnosis of exclusion because other mature B-cell lymphomas may be associated with plasmacytic differentiation. The distinction between LPL and marginal zone lymphoma may be particularly difficult in bone marrow biopsies because the morphologic features of marginal zone differentiation may not be readily apparent.

B-cell lymphomas have monoclonal populations of mature B lymphocytes, [1–6] and those with plasmacytic differentiation would also have monoclonal plasma cells. In addition, plasma cell neoplasms are among the most common marrow disorders and include monoclonal gammopathy of undetermined significance, smoldering myeloma, primary amyloidosis, and multiple myeloma [7]. Given this high incidence, a plasma cell neoplasm could occur by chance in a marrow that also has a B-cell lymphoma.

Recently, the p.L265P mutation in the myeloid differentiation primary response gene (88) (*MYD88*) was described in the vast majority of LPL/WM (85–90%)[8–13] and emerged as a valuable diagnostic marker for LPL/WM. However, by testing larger patient cohorts with more sensitive detection methods the *MYD88* L265P has now also been reported in a substantial proportion of

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IgM monoclonal gammopathy of undetermined significance (IgM-MGUS), in marginal zone lymphomas (10–21%) and other small B-cell disorders, such as chronic lymphocytic leukemia (2–4%), which significantly limits the specificity of this marker for LPL/WM [11,12,14–20]. Previously published *MYD88* studies are summarized in Table 1.

A variety of somatic frameshift and nonsense mutations in the C-X-C chemokine receptor type 4 (*CXCR4*) were reported in 27–35% of patients with *MYD88* L265P and LPL/WM. [21–24] *CXCR4* nonsense mutations resulting in c-terminal truncation and impaired cellular protein translocation have previously been associated with warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome [25–27]. In LPL/WM, mutations of *CXCR4* are associated with disease presentation but do not impact overall survival [21,24]. However, functional studies of the *CXCR4* p.S338X mutation and more recently of *CXCR4* frameshift mutations suggest that these variants promote resistance to small molecule inhibitors, such as the BTK (Bruton tyrosinase kinase) inhibitor ibrutinib [28–30].

We previously sorted B lymphocytes and plasma cells in low-grade B-cell lymphomas and demonstrated that flow cytometric cell sorting followed by analysis for immunoglobulin gene rearrangements by PCR is a powerful tool to demonstrate if these clonal populations expressing identical light chains are indeed clonally related [31].

In the current study, this approach was applied to test for the prevalence of *MYD88* L265P in sorted populations of B lymphocytes and plasma cells. Assessing the *MYD88* L265P status in sorted cell fractions significantly enhanced the specificity of this marker for LPL/WM. Given the potential clinical and therapeutic implications associated with *CXCR4* mutations in LPL/WM, this study also documents improved sensitivity for sequence analysis of *CXCR4* variants.

2. Patients and methods

2.1. Patients

Case selection was based on clinical history and immunophenotyping results by flow cytometry. Cohort 1 is comprised of 21 specimens submitted to our laboratory with an established diagnosis of Waldenström macroglobulinemia. Cohort 2 and 3 consists of 20 bone marrow specimens sequentially submitted for analysis with suspicion for LPL/WM. Final diagnostic information of cases from cohort 2 + 3 was not available. These 41 bone marrow biopsies were from 31 males and 10 females ranging in age from 45 to 90 years with a median age of 71 years.

Control biopsies included multiple myelomas enriched for plasma cells (14 biopsies), chronic lymphocytic leukemias (8), diffuse large B-cell lymphomas (3), mantle cell lymphomas (2), follicular lymphomas (2), and Burkitt lymphoma (1). Four control biopsies were marginal zone lymphomas that had not been sorted into separate B lymphocyte and plasma cell populations. Three additional control biopsies had B lymphocytes and plasma cells restricted for different immunoglobulin light chains by flow cytometry. All specimens were accessioned for human subjects research in compliance with IRB review exemption issued by the Western Institutional Review Board (Olympia, WA 98502-5010).

2.2. Flow cytometry and cell sorting

Antibody combinations, their sources, and the detailed procedures for flow cytometric analysis were reported previously [31,32]. CD19-positive B lymphocytes and CD38-positive plasma cells were sorted by flow cytometry to separate fractions.

Table 1
Previously reported *MYD88*L265P positive findings in hematopoietic malignancies.

Refs.	Method	WM	LPL	MGUS	MZL/MALT	CLL/SLL	Other
Ngo et al. [8]	mRNA sequencing				9% (6/67)		29% ABC-DLBCL (55/192)
Treon et al. [9]	WGS Sanger	91% (49/54)	100% (3/3)				
Xu et al. [13]	Sanger AS-PCR	93% (97/104)		54% (13/24)	10% (2/20)	4% (1/26)	
Varettoni et al. [12]	Sanger AS-PCR	100% (58/58)		47% (36/77)	6% (5/84)		4% B-CLPD (3/52)
Poulain et al. [13]	Sanger	79% (53/67)	100% (1/1)	50% (1/2)	6% (1/16)		4% FCL (1/23)
Puente et al. [14]	WGS					3% (9/310)	
Fernandez-Rodriguez et al. [16]	AS-PCR						10% DLBCL (17/175)
Capaldi et al. [17]	Sanger AS-PCR	97% (31/32)	100% (1/1)	43% (9/21)			25% DLBCL (2/8)
Bohers et al. [19]	Sanger						29% DLBCL (18/61)
Ondrejka et al. [39]	AS-PCR		100% (13/13)				8% HCL (1/13)
Mori et al. [40]	Sanger Restriction-Enzyme Digest AS-PCR	76% (19/25)	50% (1/2)				
Jimenez et al. [41]	AS-PCR	86% (101/117)		87% (27/31)	21% (3/14)		19% DLBCL (9/48)
Willenbacher et al. [42]	Sanger	85% (6/7)					
Insuasti-Beltran et al. [46]	Pyro-sequencing		96% (43/45)		4% (2/53)	3% (6/220)	

AS-PCR, allele specific polymerase chain reaction; WGS, whole genome sequencing; WM, Waldenström macroglobulinemia; LPL, lymphoplasmacytic lymphoma; MGUS, monoclonal gammopathy of undetermined significance; MZL/MALT, marginal zone lymphoma/mucosa associated lymphoid tissue lymphoma; CLL/SLL chronic lymphocytic lymphoma/small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma; FCL, follicular lymphoma; B-CLPD B-cell chronic lymphoproliferative disorders; HCL, hairy cell leukemia.