MYD88 L265P Somatic Mutation in Waldenström’s Macroglobulinemia

Steven P. Treon, M.D., Ph.D., Lian Xu, M.S., Guang Yang, Ph.D., Yangsheng Zhou, M.D., Ph.D., Xia Liu, M.D., Yang Cao, M.D., Patricia Sheehy, N.P., Robert J. Manning, B.S., Christopher J. Patterson, M.A., Christina Tripsas, M.A., Luca Arcaini, M.D., Geraldine S. Pinkus, M.D., Scott J. Rodig, M.D., Ph.D., Aliyah R. Sohani, M.D., Nancy Lee Harris, M.D., Jason M. Laramie, Ph.D., Donald A. Skifter, Ph.D., Stephen E. Lincoln, Ph.D., and Zachary R. Hunter, M.A.

From the Dana–Farber Cancer Institute (S.P.T., L.X., G.Y., Y.X., Y.C., P.S., R.J.M., C.J.P., C.T., Z.R.H.), Brigham and Women’s Hospital (G.S.P., S.J.R.), and Massachusetts General Hospital (A.R.S., N.L.H.), Harvard Medical School; and the Department of Pathology and Laboratory Medicine, Boston University School of Medicine, Boston; the Department of Hematology Oncology, University of Pavia Medical School and Fondazione Istituto Di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia, Italy (L.A.); and Complete Genomics, Mountain View, CA (J.M.L., D.A.S., S.E.L.). Address reprint requests to Dr. Treon at the Bing Center for Waldenström’s Macroglobulinemia, Dana–Farber Cancer Institute, M547, 450 Brookline Ave., Boston, MA 02115, or at steven_treon@dfci.harvard.edu.


Copyright © 2012 Massachusetts Medical Society.

ABSTRACT

BACKGROUND
Waldenström’s macroglobulinemia is an incurable, IgM-secreting lymphoplasmacytic lymphoma (LPL). The underlying mutation in this disorder has not been delineated.

METHODS
We performed whole-genome sequencing of bone marrow LPL cells in 30 patients with Waldenström’s macroglobulinemia, with paired normal-tissue and tumor-tissue sequencing in 10 patients. Sanger sequencing was used to validate the findings in samples from an expanded cohort of patients with LPL, those with other B-cell disorders that have some of the same features as LPL, and healthy donors.

RESULTS
Among the patients with Waldenström’s macroglobulinemia, a somatic variant (T→C) in LPL cells was identified at position 38182641 at 3p22.2 in the samples from all 10 patients with paired tissue samples and in 17 of 20 samples from patients with unpaired samples. This variant predicted an amino acid change (L265P) in MYD88, a mutation that triggers IRAK-mediated NF-κB signaling. Sanger sequencing identified MYD88 L265P in tumor samples from 49 of 54 patients with Waldenström’s macroglobulinemia and in 3 of 3 patients with non–IgM-secreting LPL (91% of all patients with LPL). MYD88 L265P was absent in paired normal tissue samples from patients with Waldenström’s macroglobulinemia and in 3 of 3 patients with non–IgM-secreting LPL (91% of all patients with LPL). MYD88 L265P was absent in paired normal tissue samples from patients with Waldenström’s macroglobulinemia or non-IgM LPL and in B cells from healthy donors and was absent or rarely expressed in samples from patients with multiple myeloma, marginal-zone lymphoma, or IgM monoclonal gamopathy of unknown significance. Inhibition of MYD88 signaling reduced IκBα and NF-κB p65 phosphorylation, as well as NF-κB nuclear staining, in Waldenström’s macroglobulinemia cells expressing MYD88 L265P. Somatic variants in ARID1A in 5 of 30 patients (17%), leading to a premature stop or frameshift, were also identified and were associated with an increased disease burden. In addition, 2 of 3 patients with Waldenström’s macroglobulinemia who had wild-type MYD88 had somatic variants in MLL2.

CONCLUSIONS
MYD88 L265P is a commonly recurring mutation in patients with Waldenström’s macroglobulinemia that can be useful in differentiating Waldenström’s macroglobulinemia and non-IgM LPL from B-cell disorders that have some of the same features. (Funded by the Peter and Helen Bing Foundation and others.)
Waldenström’s macroglobulinemia is an IgM-secreting lymphoplasmacytic lymphoma (LPL). Clinical manifestations of Waldenström’s macroglobulinemia include cytopenia resulting from bone marrow infiltration by lymphoplasmacytic cells, paraprotein-related cryoglobulinemia, the cold agglutinin syndrome, demyelinating neuropathy, and symptomatic hyperviscosity. The oncogenic basis of Waldenström’s macroglobulinemia has not been defined. Familial clustering of Waldenström’s macroglobulinemia and other B-cell disorders suggests that genetic factors play a role in the pathogenesis of Waldenström’s macroglobulinemia in certain patients. IgM monoclonal gammopathy of unknown significance (MGUS) is characterized by the presence of a monoclonal IgM protein and the absence of bone marrow disease involvement on histologic examination. IgM MGUS can progress to Waldenström’s macroglobulinemia or other B-cell lymphoproliferative disorders, with an estimated probability of progression of 1.5 to 3.0% per year. The oncogenic events responsible for the progression of IgM MGUS to Waldenström’s macroglobulinemia are unknown. Knowledge of the genetic events responsible for the pathogenesis of Waldenström’s macroglobulinemia may permit advancements in diagnostic testing and the development of targeted therapies.

METHODS

PATIENTS AND CELL SAMPLES

We performed whole-genome sequencing in 30 patients who met the diagnostic criteria for Waldenström’s macroglobulinemia. Their participation was approved by the institutional review board at the Dana–Farber Cancer Institute, and we have applied for deposition of their whole-genome-sequencing data in the database of Genotypes and Phenotypes (dbGaP) of the National Center for Biotechnology Information (NCBI). The first and last authors vouch for the accuracy and completeness of the data and analyses. All the participants provided written informed consent. The characteristics of the patients are summarized in Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org. Two patients had a blood relative with Waldenström’s macroglobulinemia, and 7 patients had a blood relative with a different B-cell cancer. Bone marrow and peripheral-blood mononuclear cells were sorted with the use of magnetic beads (Miltenyi). The purity of isolated B cells (CD19+) was more than 90%, and the median clonal B-cell population, as assessed by light-chain-restriction analysis, was 96%. CD19-depleted peripheral-blood mononuclear cells were used as normal paired samples.

WHOLE-GENOME SEQUENCING

High-molecular-weight DNA was isolated with the use of the AllPrep DNA/RNA Mini Kit (Qiagen). Library construction and whole-genome sequencing of paired-end clones were performed by Complete Genomics, as described previously. Read sequences were aligned to reference genome NCBI Build 37. The Complete Genomics Analysis Tool (CGAT), version 1.3, was used to identify high-confidence somatic variants as those with a somatic score of 0.1, indicating an estimated one false somatic single-nucleotide variant per 17.7 Mb of DNA. The copy number was estimated on the basis of the percent guanine–cytosine (GC) normalized read-depth, and acquired uniparental disomy was identified as copy-number–neutral loss of heterozygosity. Allele imbalance was determined according to the percentage of reads that mapped to the minor allele at heterozygous single-nucleotide polymorphisms and was averaged over 500 Kb.

VALIDATION BY SANGER SEQUENCING

Polymerase-chain-reaction primers were designed to amplify a 726-bp fragment covering myeloid differentiation primary response gene (88) (MYD88) L265P (forward primer, 5′-GGGATATGCTGAAC- TAAGTTGCCAC3′; reverse primer, 5′-GAGCTGTCTGTGAAGTTGGCA-TC TGTGAAGTTGGCATCTC3′). Amplified fragments were isolated with the use of the QIAquick Gel Extraction Kit (Qiagen) and were sequenced with the use of the forward primer 5′-GCTGTGTTGTAAACCTGAGGTTGGAAG3′ and the reverse primer 5′-GACGTGTCTGTGTAAGTTGGGATCATC3′. Sanger sequencing was used to validate the results of whole-genome sequencing and to evaluate MYD88 L265P expression in tumor samples from 24 additional patients with Waldenström’s macroglobulinemia, 3 patients with non-IgM LPL (2 with IgG LPL and 1 with IgA LPL), 10 patients with myeloma, and 46 patients with marginal-zone lymphoma (21 with a splenic subtype, 20 with an extranodal subtype, and 5 with a nodal subtype).
CD19+ bone marrow mononuclear cells isolated from 21 patients with IgM MGUS were also sequenced, with cloning and sequencing of at least 100 clones performed by Genewiz for 7 patients with IgM MGUS in whom direct Sanger sequencing did not reveal MYD88 L265P. Samples with sufficient DNA from 11 patients with IgM MGUS were evaluated by means of an IgH chain rearrangement assay (In Vivo Scribe Technologies). CD19+ peripheral-blood mononuclear cells from 15 healthy donors and from BCWM.1, MWCL-1, WM-WSU, Ramos, MM1.S, RPMI 8226, MEC-1, and U266 cell lines were also sequenced for MYD88 L265P.

**INHIBITORS OF MYD88 SIGNALING**

BCWM.1 and MWCL-1 Waldenström’s macroglobulinemia cell lines expressing MYD88 L265P, bone marrow cells from a patient with Waldenström’s macroglobulinemia, and Ramos, MM1.S, RPMI 8226, and U266 cell lines expressing wild-type MYD88 were incubated overnight with 100 μM of a control peptide, 100 μM of an inhibitor of MYD88 homodimerization (IMG-2005-5, IMGENEX), a dimethylsulfoxide vehicle control, or 10 μM of a dual interleukin-1 receptor–associated kinase (IRAK) 1 and IRAK4 kinase inhibitor (407601, EMD). A median of 3419 somatic variants (range, 2540 to 4011) were identified, and MYD88 L265P was the most common. It was also expressed in 16 of 20 patients with Waldenström’s macroglobulinemia who had unpaired samples. MYD88 L265P was therefore expressed in 26 of the 30 patients in whom whole-genome sequencing was performed. The median percentage of reads supporting the MYD88 L265P variant for these 26 patients was 46.3% (range, 23.4 to 95.7); this percentage may have reflected the presence of polyclonal B cells or contaminating non–CD19+ cells in the samples sequenced or, alternatively, the presence of MYD88 L265P in subpopulations of clonal lymphoplasmacytic cells in some patients. In contrast, MYD88 L265P was not expressed in any reads from paired normal tissue samples subjected to whole-genome sequencing.

The frequency of the MYD88 L265P mutation among patients with a positive family history was 100% (9 of 9 patients), and the frequency among patients with sporadic cases was 86% (18 of 21) (P=0.60). In 22 of the 26 patients with MYD88 L265P expression, the variant was heterozygous, and in the other 4 patients, an acquired uniparental disomy event (median, 49.5 MB [range, 48.5 to 50.0]) at 3p22.2 resulted in a single nucleotide change, T→C, in MYD88.

**RESULTS**

**IDENTIFICATION OF MYD88 L265P AS THE MOST COMMON SOMATIC ALTERATION**

Tumor and normal genomes were sequenced to an average of 66-times coverage (range, 60 to 91) of mapped individual reads. The average gross mapped yield for these genomes was 186.89 Gb (range, 171.56 to 262.03). In all 10 patients with paired tissue samples, we identified a variant at position 38182641 in chromosome 3p22.2 resulting in a single nucleotide change, T→C, in MYD88. This change predicted a switch of leucine to proline at amino acid position 265 (L265P). A median of 3419 somatic variants (range, 2540 to 4011) were identified, and MYD88 L265P was the most common. It was also expressed in 16 of 20 patients with Waldenström’s macroglobulinemia who had unpaired samples. MYD88 L265P was therefore expressed in 26 of the 30 patients in whom whole-genome sequencing was performed. The median percentage of reads supporting the MYD88 L265P variant for these 26 patients was 46.3% (range, 23.4 to 95.7); this percentage may have reflected the presence of polyclonal B cells or contaminating non–CD19+ cells in the samples sequenced or, alternatively, the presence of MYD88 L265P in subpopulations of clonal lymphoplasmacytic cells in some patients. In contrast, MYD88 L265P was not expressed in any reads from paired normal tissue samples subjected to whole-genome sequencing.

The frequency of the MYD88 L265P mutation among patients with a positive family history was 100% (9 of 9 patients), and the frequency among patients with sporadic cases was 86% (18 of 21) (P=0.60). In 22 of the 26 patients with MYD88 L265P expression, the variant was heterozygous, and in the other 4 patients, an acquired uniparental disomy event (median, 49.5 MB [range, 48.5 to 50.0]) at 3p22.2 resulted in homozygous MYD88 L265P expression in at least a subset of tumor cells. These uniparental disomy events were absent in paired normal samples. No distinguishing clinical or laboratory features were observed in patients who were homozygous for MYD88 L265P, as compared with patients who were heterozygous, though patients who were ho-
mozygous for MYD88 L265P had a longer mean interval since the diagnosis of Waldenström's macroglobulinemia (56.4 months vs. 11.1 months, P=0.21). Among the 4 patients who were homozygous for MYD88 L265P, 2 had a positive family history (accounting for 22% of all patients with a positive family history), and 2 had sporadic cases (accounting for 11% of all patients with sporadic cases) (P=0.84).

No recurring somatic variants were identified in other genes encoding toll-like receptor or NF-κB signaling pathways in the 10 patients with paired samples. The next most common somatic variants, after MYD88, occurred in the AT-rich interactive domain 1A gene (ARID1A) and the histone cluster 1, H1e gene (HIST1H1E), with variants occurring in each of these genes in 2 of 10 patients with paired samples (20%). Two nonrecurring single-nucleotide variants were identified by whole-genome sequencing in each gene and were validated by Sanger sequencing (see Table S2 in the Supplementary Appendix). Three additional patients with unpaired samples had variants in ARID1A, and the somatic status was confirmed by Sanger sequencing of normal tissue. Therefore, 5 of 30 patients (17%) had a mutation in ARID1A, including three single-nucleotide variants leading to premature protein truncation (one each at R173, Q547, and Q934), and two frameshift changes (a deletion at position 27057944 and an insertion at position 27107136 in chromosome 1). Patients with both ARID1A and MYD88 L265P mutations, as compared with patients who did not have ARID1A mutations, had greater involvement of bone marrow disease (90% vs. 50%, P=0.08), a lower hematocrit (26% vs. 32%, P=0.03), and a lower platelet count (168,000 per cubic millimeter vs. 207,000 per cubic millimeter, P=0.08). No somatic variants in HIST1H1E were identified in any of the patients with unpaired samples. Additional somatic variants were identified in nonrecurring genes and occurred only in single patients (see Table S2 in the Supplementary Appendix).

**SANGER SEQUENCING FOR MYD88 L265P**

Sanger sequencing confirmed MYD88 L265P expression in all 26 tumor samples in which it had been revealed by whole-genome sequencing and in 1 sample from an additional patient, in whom lymphoplasmacytic cells had read-level evidence for MYD88 L265P but the variant did not meet the criterion for high confidence. In this patient, approximately 10% of bone marrow lymphoplasmacytic cells expressed MYD88 L265P. Sanger sequencing therefore identified MYD88 L265P in tumor samples from 27 of 30 patients with Waldenström's macroglobulinemia (90%) and confirmed the absence of MYD88 L265P in normal tissue from 10 patients with paired samples and from 9 patients with unpaired samples for whom normal tissue was available. For the 3 patients with Waldenström's macroglobulinemia who did not have MYD88 L265P expression, a review of all the map reads and sequencing of the entire coding region revealed no other MYD88 variants. In addition, we sought to identify variants exclusive to patients with wild-type MYD88. In 2 of 3 patients with wild-type MYD88, variants in the mixed-lineage leukemia 2 gene (MLL2) were identified and were confirmed to be somatic after Sanger sequencing of normal tissue was performed in these patients. One of these 2 patients had a single-nucleotide variant in chromosome 12 at position 49425599 (A→G), and the other patient had a deletion at position 49448413 (C) resulting in a frameshift mutation. No other variants in recurring genes were identified in these patients.

Sanger sequencing also revealed MYD88 L265P expression in 22 of 24 patients with Waldenström's macroglobulinemia in a separate cohort, including 2 with homozygous expression. MYD88 L265P was expressed in both CD19+ cells and CD138+ cells from 12 of these patients for whom both cell sorts were available, a finding that was consistent with the known distribution of the malignant Waldenström's macroglobulinemia clone. Three patients with LPL who had IgG-secreting disease or IgA-secreting disease also had MYD88 L265P expression, signifying that MYD88 L265P is expressed in non–IgM-secreting LPL. MYD88 L265P was thus expressed in 52 of the 57 patients with LPL (91%).

MYD88 L265P was also detected as a heterozygous variant in BCWM.1 and MWCL-1 Waldenström's macroglobulinemia cell lines and was not expressed in IgM-secreting WSU and Ramos cell lines, which carry an 8;14 translocation, nor in any myeloma cell lines. MYD88 L265P was absent in peripheral-blood B cells from 15 healthy donors and in tumor samples from 10 patients with myeloma, including 2 with IgM myeloma, including 2 with IgM myeloma (P<0.001 for the comparison of samples from both groups of patients with samples...
from patients with Waldenström’s macroglobulinemia. Of the 46 patients with marginal-zone lymphoma, 3 patients (1 with a splenic subtype, 1 with an extranodal subtype, and 1 with a nodal subtype) had MYD88 L265P expression (7%, P<0.001 for the comparison with Waldenström’s macroglobulinemia). All 3 patients were heterozygous for MYD88 L265P, and 2 of them (1 with a splenic subtype and 1 with a nodal subtype) had extensive bone marrow involvement, a monoclonal IgM protein, and clinicopathological features that overlapped with those of Waldenström’s macroglobulinemia. As determined by means of direct Sanger sequencing, MYD88 L265P was present in CD19+ cells from 2 of 21 patients with IgM MGUS (10%, P<0.001 for the comparison with Waldenström’s macroglobulinemia). Cloning and sequencing of at least 100 clones showed that MYD88 L265P was not present in the patients with IgM MGUS in whom direct sequencing did not reveal MYD88 L265P. In addition, a clonal IgH rearrangement was present in at least a subset of transcripts in 7 of 11 patients with IgM MGUS, none of whom had MYD88 L265P expression, indicating the presence of clonal B cells in most samples used for Sanger sequencing.

**INHIBITION OF MYD88 SIGNALING IN CELLS EXPRESSING L265P**

After culture with an inhibitor of MYD88 homodimerization, Waldenström’s macroglobulinemia cells expressing MYD88 L265P showed a marked decrease in nuclear staining of NF-κB p65 (Fig. 1), as well as decreased IκBα and NF-κB p65 phosphorylation (see Fig. S1 and S2 in the Supplementary Appendix). Similar results were obtained when cells expressing MYD88 L265P were incubated with an IRAK 1/4 kinase inhibitor (see the Supplementary Appendix). In contrast, cells expressing wild-type MYD88 either showed no IκBα, NF-κB p65 activity, or nuclear NF-κB p65 staining or remained unaffected by the inhibition of MYD88 signaling.

**DISCUSSION**

We describe here the presence of a widely expressed somatic mutation (MYD88 L265P) in patients with Waldenström’s macroglobulinemia, as identified by means of whole-genome sequencing and confirmed by means of Sanger sequencing. Sanger sequencing also identified MYD88 L265P in other patients with Waldenström’s macroglobulinemia and in patients with non–IgM-secreting LPL. In total, 91% of patients with LPL had MYD88 L265P expression. In contrast, MYD88 L265P was absent in tissue samples from patients with myeloma, including samples from patients with IgM-secreting myeloma, and was expressed in only a small subgroup of patients with marginal-zone lymphoma (7%), which included patients who had features related to those of Waldenström’s macroglobulinemia. MYD88 L265P may therefore be useful in distinguishing LPL from marginal-zone
lymphoma and multiple myeloma — an often difficult task owing to overlapping morphologic, immunophenotypic, cytogenetic, and clinical features.\(^2\,18,19\) As compared with the levels of MYD88 L265P expression in LPL, lower levels of MYD88 L265P expression were reported in diffuse large-cell lymphoma of the activated B-cell type (14 to 29%), mucosa-associated lymphoid-tissue lymphoma (9%), and chronic lymphocytic leukemia (3%).\(^20\,22\)

Of particular interest was the absence of MYD88 L265P in most, but not all, patients with IgM MGUS as evaluated by Sanger sequencing. Only 2 of the 21 patients with IgM MGUS (10%) had MYD88 L265P expression, both of whom had subcentimeter adenopathy but no bone marrow infiltrate, which is required for the clinicopathological diagnosis of Waldenström’s macroglobulinemia.\(^1,2\) One of these patients had progressive disease, as evidenced by serial increases in serum IgM levels and a declining hematocrit; the disease was diagnosed only recently in the other patient, and the follow-up time has been short.

The significance of these findings for determining the relationship of IgM MGUS to Waldenström’s macroglobulinemia remains to be clarified. It is tempting to speculate that acquisition of MYD88 L265P represents a transforming event that facilitates the progression of IgM MGUS to Waldenström’s macroglobulinemia. Many, if not most, cases of IgM MGUS progress to Waldenström’s macroglobulinemia.\(^7\,9\) A large prospective study involving patients with IgM MGUS will be required to evaluate the contribution of MYD88 L265P to the progression to Waldenström’s macroglobulinemia rather than to other B-cell lymphoproliferative disorders. The absence of MYD88 L265P in most of the patients with IgM MGUS could also reflect a lower frequency of clonal B cells in the samples we used, which might not have been detected by Sanger sequencing. However, the presence of clonal IgH rearrangements in most of the samples from these patients that were evaluated suggests that the number of clonal B cells in the samples was probably sufficient.

The expression of MYD88 L265P occurred at

---

**Figure 2. MYD88-Directed NF-κB Signaling.**

After toll-like receptor or interleukin-1 receptor binds to its ligand, the toll–interleukin-1 receptor (TIR) domains activate MYD88 directly, or in the case of toll-like receptor 4 trigger MYD88 through interactions with TIR domain containing adaptor protein (TIRAP) and Bruton’s tyrosine kinase (BTK). MYD88 then dimerizes and triggers autophosphorylation of interleukin-1 receptor–associated kinase (IRAK) 4, with subsequent recruitment and phosphorylation of IRAK1 and cytosolic release of membrane-bound tumor necrosis factor receptor–associated factor 6 (TRAF6) from IRAK1. The TGF-β–activated kinase 1 (TAK1) binding proteins (TAB1 and TAB2) promote binding of TRAF6 to TAK1, thereby triggering phosphorylation of the heterotrimeric IκB kinase (IKK) complex (NEMO/IKKγ, IKKα, and IKKβ). The activation of this heterotrimeric complex leads to IκBα phosphorylation and release of NF-κB p65 and p50, which translocate to the nucleus and drive NF-κB–dependent prosurvival signaling.
mutations in patients with Waldenström's macroglobulinemia may therefore confer a predisposition to IgM MGUS, but not Waldenström's macroglobulinemia itself, particularly since cases of IgM MGUS are present in affected families and progress over time to Waldenström's macroglobulinemia. A larger study comprising familial and sporadic cases of IgM MGUS and Waldenström's macroglobulinemia will be needed to clarify these observations.

After MYD88, ARID1A had the most somatic variants, with variants occurring in 17% of patients. ARID1A is a member of the SWI–SNF family of proteins, which facilitate repositioning of nucleosomes for transcriptional regulation, DNA repair, recombination, and chromosome segregation. ARID1A is a tumor-suppressor gene in breast, ovarian, and gastric cancers, as well as in acute lymphoblastic leukemia, in which loss of expression is associated with resistance to glucocorticoid therapy. ARID1A mutations were present in patients with sporadic cases and those with familial cases and were coexpressed with MYD88 L265P in all cases. Patients with both ARID1A and MYD88 L265P mutations had more aggressive disease features than did patients who did not have ARID1A mutations. The presence of ARID1A mutations in patients with Waldenström's macroglobulinemia may therefore have prognostic as well as treatment implications, given the use of glucocorticoids in many treatment regimens for patients with Waldenström's macroglobulinemia; however, we do not have sufficient data to confirm these relationships.

Among the three patients with wild-type MYD88, two had variants in MLL2, a gene encoding the histone-modifying enzyme histone–lysine N-methyltransferase. No patient with MYD88 L265P expression had MLL2 variants. Both patients with MLL2 variants had high levels of circulating clonal B cells and CD23 expression, features that are uncommon in Waldenström's macroglobulinemia. MLL2 is a frequent target of somatic mutations in follicular lymphomas (89%) and diffuse large B-cell lymphomas (32%). A larger study involving patients with Waldenström's macroglobulinemia or non-IgM LPL who have wild-type MYD88, as well as functional studies, will be needed to validate the significance of these findings with respect to the pathogenesis of Waldenström's macroglobulinemia.

MYD88 is an adaptor molecule in toll-like receptor and interleukin-1 receptor signaling (Fig. 2). After stimulation of the toll-like receptor or interleukin-1 receptor, MYD88 is recruited to the activated receptor complex as a homodimer and forms complexes with IRAK4, leading to activation of IRAK1 and IRAK2. Tumor necrosis factor receptor–associated factor 6 (TRAF6) is then activated by IRAK1, leading to phosphorylation of IκBα and activation of NF-κB. Ngo et al. found that inhibition of MYD88 signaling decreased NF-κB activity and survival of activated B-cell–type diffuse large-cell lymphoma cell lines expressing MYD88 L265P. Our observation that blocking of MYD88 signaling decreased NF-κB activity is consistent with these findings. These observations are of relevance to Waldenström's macroglobulinemia, since NF-κB signaling is important for the growth and survival of Waldenström's macroglobulinemia cells. Blockade of IκBα by proteasome inhibitors is associated with high rates of response in patients with Waldenström's macroglobulinemia, as well as non-IgM LPL. The presence of MYD88 L265P can help differentiate Waldenström's macroglobulinemia, as well as non-IgM LPL, from B-cell disorders that have some of the same features and provides insight into the pathogenesis of Waldenström's macroglobulinemia. It remains to be seen whether MYD88 L265P signaling can be targeted for the therapy of Waldenström's macroglobulinemia and non-IgM LPL.

Presented in part at the Annual Meeting of the American Society of Hematology, Atlanta, December 10–13, 2011. Supported by the Peter and Helen Bing Foundation, the International Waldenström's Macroglobulinemia Foundation, the Coyote Fund for Waldenström's Macroglobulinemia, the Waldenström’s Cancer Fund, the Bailey Family Fund for Waldenström’s Macroglobulinemia, the D’Amato Family Fund for Genomic Discovery, the Edward and Linda Nelson Fund for Waldenström’s Macroglobulinemia Research, the Bauman Family Trust, and the Tannenhauer Family Foundation. Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.
We thank the patients with Waldenström's macroglobulinemia who provided samples for the study; and Drs. Yu-Tzu Tai and Constantine Mitsiades (laboratory of Dr. Ken Anderson, Dana–Farber Cancer Institute) for providing samples from patients with myeloma for these studies.

REFERENCES