nucleotide polymorphisms have been related to cancer, like rs78378222 in basal cell carcinoma, but most are not.[4] miRNAs act as gene regulators through a translational repression or mRNA degradation via binding to target sites in the 3′UTR of protein-coding transcripts. To date, 11 miRNAs are known to target the human *TP53* gene, including miR-125b, miR-15a, and miR-16.

In this issue, Li et al[1] present data regarding newly identified single nucleotide variants (nSNVs) targeting the TP53 3′UTR that alter the translation of TP53 in patients with DLBCL treated with cyclophosphamide, doxorubicin, vincristine, and prednisone plus the monoclonal antibody rituximab regimen. They correlate these new genetic alterations with the outcome of the patients (see figure). To date, this is the first report of such TP53 mutation in cancer. The presence of these nSNVs disrupts the binding of miRNA to the 3′UTR decreasing the miRNA suppression effect and thus increasing the p53 protein expression. Compared with wild-type (WT) CDS, mutated p53 CDS is associated with a shorter survival.[5,6] The influence of the nSNVs on the outcome of DLBCL patients depends on the status of TP53 CDS, WT or mutated. In the case of WT TP53 nSNVs, miRNA suppression is decreased and WT p53 expression increases, and outcome of patients improves compared with WT p53 without nSNVs. In the case of mutated *TP53* and the presence of nSNVs, the expression of mutated p53 increases, leading to an increase in resistance to chemotherapy and the poorest outcome. Interestingly, the authors demonstrated that some nSNVs located within the seed match site in the *TP53* 3′UTR could significantly alter p53 protein levels in vitro. Approximately 50% of DLBCLs display 3′UTR alterations that are predicted to disrupt miRNA binding sites, a much higher frequency than observed within the CDS.

Thus, what is important to foresee for outcome after treatment is not the presence of mutated p53 but the existence of nSNVs and p53 status.

There are several genetic mechanisms associated with refractoriness to therapy in DLBCL patients, mutation of *TP53* being one that is present in ~20% of the patients. Genetic alterations of molecules upstream or downstream of the p53 pathway, such as MDM2, ATM, p21, CDKN2A, or p73, are also implicated in the refractoriness to therapy.[2,6] Mutations or hyperexpression of c-MYC, hyperexpression of bcl-2, or survivin are other frequent alterations found in refractory patients, but not all genetic alterations are currently described. We know that patients who expressed these modifications at the time of DLBCL diagnosis are more prone not to respond well to monoclonal antibody rituximab, but it is not a clear-cut figure. To propose a different regimen for these patients is not possible currently because we do not have drugs/regimens allowing better response in these patients. Some small molecules targeting p53 are currently in development, but their exact activity is not known.[7] According to the present work published in this issue of *Blood*, a complete and accurate knowledge of the genomic status of both TP 53 CDS and 3′UTR appears mandatory before their clinical usage.

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● ● ● LYMPHOID NEOPLASIA

Comment on Poulain et al, page 4504

A new era for Waldenstrom macroglobulinemia: MYD88 L265P

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In this issue of *Blood*, Poulain et al demonstrate the high prevalence of the MYD88 L265P somatic mutation in patients with Waldenstrom macroglobulinemia (WM) and provide insight into its biological relevance in the growth and survival of WM.[1]

Since the initial report by Treon et al[2] of the existence of the MYD88 L265P somatic mutation in WM patients using whole genome sequencing (WGS), a flurry of recent studies have confirmed the high frequency of the MYD88 L265P somatic mutation in patients with WM and IgM monoclonal gammopathy of unknown significance (IgM MGUS) by Sanger, polymerase chain reaction (PCR), and allele-specific PCR (AS-PCR) assays[3-8] (see table). These studies have also contributed to our understanding of how MYD88 L265P enables the expansion of WM cells and the potential for use in the diagnosis, treatment, and response assessment of WM.

The discovery of a mutation in MYD88 is significant given its role as an adaptor molecule in Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) signaling. Following TLR or IL-1R stimulation, MYD88 is recruited to the activated receptor complex as a homodimer, which complexes with IL-1R–associated kinase (IRAK) 4 to activate IRAK1. Tumor necrosis factor receptor–associated factor 6 is then activated leading to nuclear factor κB (NF-κB) activation via IκBα phosphorylation. Similar to the findings by Ngo et al[9] in MYD88

L265P–expressing activated B-cell–like (ABC) diffuse large B-cell lymphoma (DLBCL) cell lines, inhibition of MYD88/IRAK signaling attenuates NF-κB signaling and survival of MYD88 L265P–expressing WM cells.[1,2,8,10] Correspondingly, WM cell survival is enhanced by MYD88 L265P overexpression. Additionally, Yang et al[10] have shown that inhibition of MYD88 in L265P–expressing WM cells is accompanied by decreased activation of Bruton tyrosine kinase (BTK), whereas overexpression of MYD88 L265P enhances BTK phosphorylation. These studies also show that IRAK and BTK independently direct downstream NF-κB activation and combined use of IRAK and BTK inhibitors leads to synergistic tumor cell killing in MYD88 L265P–expressing WM cells. These studies would suggest a model wherein MYD88 L265P triggers NF-κB via dual, but independent pathways, which signal through BTK and/or IRAK1/IRAK4.

The likely role of MYD88 L265P as an early oncogenic event for the development of WM is raised by studies showing this somatic mutation in up to 80% of individuals with IgM MGUS[2,4–7] (see table). The presence of MYD88 L265P in patients with IgM MGUS significantly increases the risk of malignant evolution as reported by Varettoni et al.[6] IgM-MGUS patients with MYD88 L265P have higher levels of IgM, lower levels of IgG and IgA, and a higher incidence of Bence-Jones proteinuria at diagnosis.[6] Other recurring somatic mutations have been revealed by WGS in WM patients, which could serve as triggers for progression from IgM MGUS to symptomatic WM.[2] A multihit model could also explain why no significant differences in treatment response and progression-free and overall survival based solely on MYD88 mutation status determination were observed by Jimenez et al[7] in a large series of WM patients. MYD88 mutation status does, however, separate patients across a few clinical characteristics including serum IgM (sIgM) levels, bone marrow (BM) disease involvement, peripheral blood lymphocytosis, and/or tumor cell expression of CD27.[1,2,4,7]

The diagnostic discrimination of WM/lymphoplasmacytic lymphoma (LPL) vs marginal zone lymphomas (MZLs), IgM secreting myeloma (MM), and chronic lymphocytic leukemia (CLL) with plasmacytic differentiation can sometimes be difficult due to overlapping clinicopathological characteristics. MYD88 L265P can help distinguish WM/LPL from MZL, MM, and CLL, which show no or infrequent expression (3% to 9%) of this somatic mutation, with the possibility that many of these cases are WM/LPL.[1–4,6–8] The expression of MYD88 L265P should, however, be considered supportive and not exclusively diagnostic of WM/LPL because its presence has also been demonstrated in other lymphomas including DLBCL (ABC subtype)[10] and also does not discriminate IgM MGUS from WM, the latter requiring the presence of an infiltrate by histologic examination. However, higher levels of MYD88 L265P in BM B cells are associated with evolution to WM,[4] and larger studies are needed clarify if quantitative PCR can be used to delineate IgM MGUS from WM, as well as IgM MGUS patients at higher risk of malignant progression.

The potential for MYD88 L265P to be exploited therapeutically in WM is suggested by studies showing that inhibition of MYD88 and downstream targets including IRAK1, IRAK4, BTK, and TAK1 can suppress downstream NF-κB signaling and/or induce WM cell killing.[1,2,8,10] High rates of response were observed in an ongoing clinical trial with a BTK inhibitor in relapsed/refractory WM patients and may correlate with MYD88 status. These clinical findings may reflect the dependence of tumor growth and survival on MYD88 L265P–directed BTK activity as suggested by the work of Yang et al.[10] Investigation of MYD88 mutation status may therefore help identify patients who could benefit with MYD88 path–targeted therapies.

The potential use of MYD88 L265P as a response tool was shown by both Xu et al[4] and Jimenez et al[7] using quantitative AS-PCR to follow changes in BM disease burden after treatment. Response assessment can often be difficult in WM patients due to dependence on serial sIgM measurements. Many agents used to treat WM discordantly affect sIgM levels relative to the underlying disease burden. Repeat BM biopsies are used to clarify such discordant responses and determine the need for changing therapeutic management. The use of quantitative PCR for MYD88 L265P could provide a faster and more cost-effective determination of such discordant findings relative to immunohistochemistry. Peripheral blood testing for MYD88 L265P may also be possible and adapted to a quantitative approach providing a noninvasive means for underlying tumor burden assessment.

In summary, MYD88 L265P is a widely prevalent somatic mutation in patients with WM and IgM MGUS, which can help differentiate these entities from overlapping B-cell disorders. MYD88 L265P promotes the growth and survival of WM cells and represents a novel target for WM therapy. Indeed, a new era is upon us with the opportunity to advance this new genomic finding into tools for diagnosis and response assessment, as well as therapies that will improve the lives of WM patients.

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MYD88 L265P expression in WM and IgM MGUS

Reference	Method	Tissue	WM	IgM MGUS
2	WGS/Sanger	BM CD19$^+$	91%	10%
3	PCR	BM	70%	ND
4	AS-PCR	BM CD19$^+$	93%	54%
5	Sanger	BM		54%
6	AS-PCR	BM	100%	47%
7	AS-PCR	BM	86%	87%
1	PCR	BM CD19$^+$	80%	ND
8	Sanger	NA	70%	ND

BM, unselected bone marrow cells; BM CD19$^+$, CD19$^+$-selected BM cells; NA, not available; ND, not done.

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