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MYD88 in the driver's seat of B-cell lymphomagenesis: from molecular mechanisms to clinical implications

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ABSTRACT

More than 50 subtypes of B-cell non-Hodgkin lymphoma (B-NHL) are recognized in the most recent World Health Organization classification of 2016. The current treatment paradigm, however, is largely based on 'one-size-fits-all' immune-chemotherapy. Unfortunately, this therapeutic strategy is inadequate for a significant number of patients. As such, there is an indisputable need for novel, preferably targeted, therapies based on a biologically driven classification and risk stratification. Sequencing studies identified mutations in the *MYD88* gene as an important oncogenic driver in B-cell lymphomas. *MYD88* mutations constitutively activate NF- κ B and its associated signaling pathways, thereby promoting B-cell proliferation and survival. High frequencies of the hotspot *MYD88*(L265P) mutation are observed in extranodal diffuse large B-cell lymphoma and Waldenström macroglobulinemia, thereby demonstrating this mutation's potential as a disease marker. In addition, the presence of mutant *MYD88* predicts survival outcome in B-NHL subtypes and it provides a therapeutic target. Early clinical trials targeting *MYD88* have shown encouraging results in relapsed/refractory B-NHL. Patients with these disorders can benefit from analysis for the *MYD88* hotspot mutation in liquid biopsies, as a minimally invasive method to demonstrate treatment response or resistance. Given these clear clinical implications and the crucial role of *MYD88* in lymphomagenesis, we expect that analysis of this gene will increasingly be used in routine clinical practice, not only as a diagnostic classifier, but also as a prognostic and therapeutic biomarker directing precision medicine. This review focuses on the pivotal mechanistic role of mutated *MYD88* and its clinical implications in B-NHL.

Introduction

With the introduction of high-throughput, next-generation sequencing, many studies have aimed to explain the diverse biology, clinical course, prognosis, and therapeutic response of B-cell non-Hodgkin lymphoma (B-NHL). This has increased our knowledge of lymphomagenesis by identifying many novel somatic alterations that affect signaling pathways involved in several B-NHL subtypes. In this rapidly evolving molecular landscape, it is important to translate newly obtained genetic knowledge directly into clinical benefit for patients.¹

Ngo *et al.* were the first to identify an oncogenic, non-synonymous, gain-of-function mutation in myeloid differentiation primary response 88 (*MYD88*), leading to an amino-acid change of leucine to proline at position 265 (NM_002468.5, also referred to as position 273 in NM_001172567) of *MYD88* [*MYD88*(L265P)].² Other recurrent mutations in *MYD88* were likewise identified; however, the impact of these mutations has been difficult to establish due to their low prevalence.³ This review, therefore, focuses on the present understanding of the role of

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MYD88(L265P) in NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) activation and its association with the B-cell receptor (BCR) cascade. In addition, we address the clinical importance of *MYD88(L265P)*, including its prevalence across B-NHL subtypes, its predictive significance in patients' outcome, and its potential as a therapeutic target.

Oncogenic mechanisms of *MYD88(L265P)*

Canonical NF- κ B signaling

In normal physiology, *MYD88* acts as a signaling adaptor in the canonical NF- κ B pathway (Figure 1). This pathway is activated upon recognition of pathogen-associated molecular patterns (PAMP) by receptors containing a toll/interleukin-1 receptor (TIR) domain, such as toll-like receptors (TLR) and the interleukin receptors 1 (IL-1R) and 18 (IL-18R). After ligand binding, the TIR domain of these receptors interacts with the TIR domain of *MYD88*⁴ and this process initiates the formation of the so-called 'myddosome complex'. For this complex, activated *MYD88* recruits IL-1R associated kinase 4 (IRAK4), a serine-threonine kinase, and together they phosphorylate IRAK1 or IRAK2. Phosphorylated IRAK1 and IRAK2 interact with tumor necrosis factor receptor-associated factor 6 (TRAF6), resulting in activation of transforming

growth factor beta-activated kinase 1 (TAK1).⁵ Activated TAK1 continues signaling through the mitogen-activated protein kinase (MAPK) signaling cascade and cooperates with TAK1-binding protein (TAB) to activate the inhibitor of the NF- κ B kinase (IKK) complex.

The IKK complex consists of the kinase subunits IKK α and IKK β and the regulatory subunit NF- κ B essential modulator. After activation, this complex phosphorylates the inhibitor of NF- κ B (I κ B) proteins that are bound to NF- κ B, which prevent migration of NF- κ B to the nucleus. Phosphorylation of these I κ B proteins results in ubiquitylation and proteasomal degradation of I κ B and release of the NF- κ B subunits. Subsequently, the NF- κ B subunits, including RELA (p65)-p50 in the classical pathway and RELB-p52 in the alternative pathway, migrate to the nucleus where they bind to specific DNA-binding sites and induce increased expression of genes involved in B-cell proliferation and survival. In addition, expression of these genes is increased through interactions between the NF- κ B subunits and other transcription factors, such as E1A binding protein P300 (EP300) and CREB binding protein (CREBBP).⁶

In the case of *MYD88(L265P)*, the TIR domain of *MYD88*, in which L265P resides, is more highly activated compared with wildtype *MYD88* and this increases downstream signaling and formation of the myddosome complex.² Henceforth, *MYD88(L265P)* preferentially and

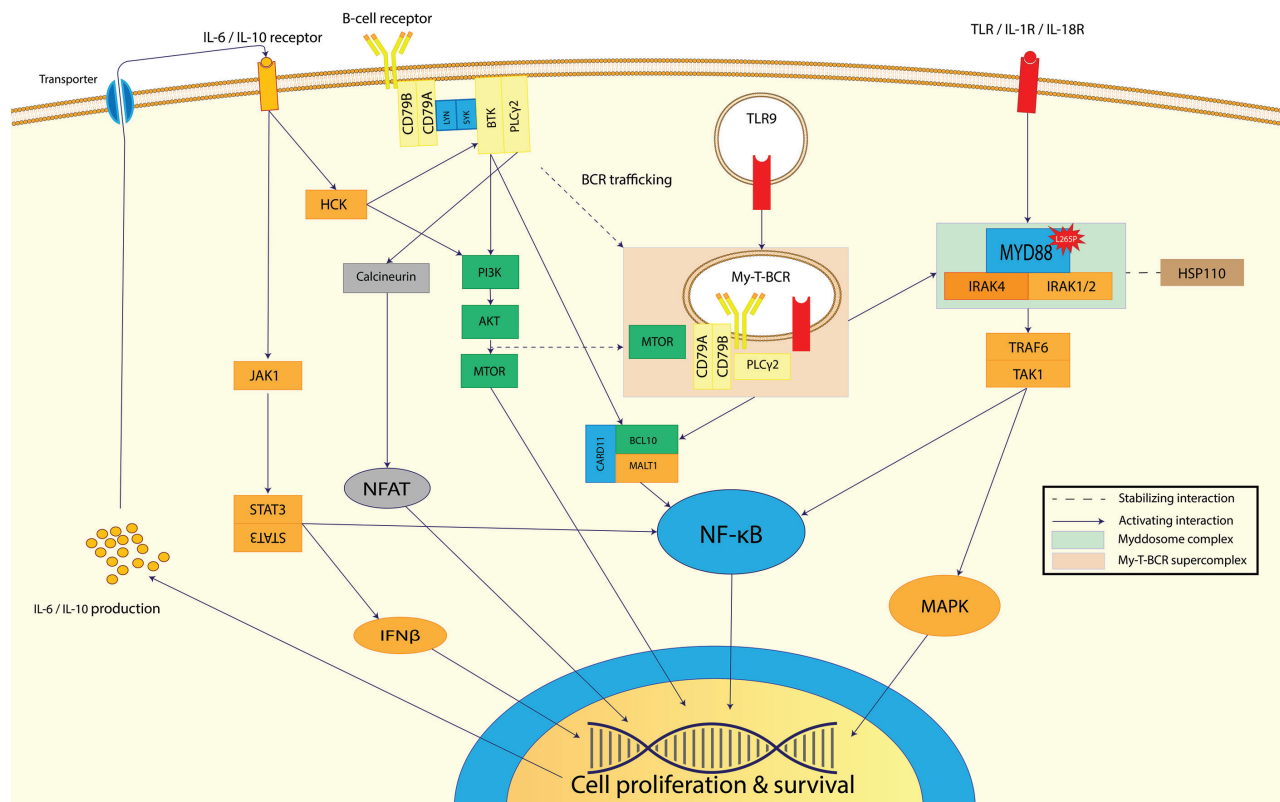


Figure 1. The role of *MYD88* signaling in normal physiology and lymphomagenesis. Recognition of pathogens by TLR, IL1R, and IL-18R induces an immune response through activation of *MYD88* and generates the myddosome complex with IRAK4 and IRAK1 or IRAK2, which is stabilized by HSP110. IRAK1 and IRAK2 activate the MAPK and NF- κ B pathways through TRAF6 and TAK1, causing proliferation and survival of B cells. *MYD88(L265P)* allows for increased formation of the myddosome complex, preferentially with IRAK1, and constitutively activates the NF- κ B pathway. In addition, the formation of the My-T-BCR supercomplex leads to increased activation of mTOR and the CBM complex, promoting lymphomagenesis. Lastly, constitutively active NF- κ B increases autocrine signaling of IL-6 and IL-10, which further promote B-cell proliferation and survival via the alternative JAK/STAT signaling cascade.

constitutively recruits IRAK1 for the myddosome and, together with IRAK4, was found to be essential for survival of activated B-cell (ABC) diffuse large B-cell lymphoma (DLBCL) cell lines with *MYD88*(L265P).^{2,7,8} In addition, IRAK1 was shown to be co-immunoprecipitated with MYD88 in chronic lymphocytic leukemia (CLL) cells with *MYD88*(L265P) and stimulation of IL-1R and TLR induced a 5-fold to 150-fold increase of cytokine secretion compared to that of CLL cells with wildtype *MYD88*.⁹ However, Ansell *et al.*⁷ identified that in Waldenström macroglobulinemia (WM) cell lines, the myddosome complex consisted of IRAK4, TRAF6, and MYD88, but not IRAK1. The authors hypothesized that this difference in complex formation was instigated by the heterozygous nature of *MYD88*(L265P) in WM and the homozygous nature in DLBCL, which was strengthened by the finding that downstream signaling of TAK1 phosphorylation was highest in the DLBCL cell line with homozygous *MYD88*(L265P).⁷ Furthermore, the stabilizing effect of heat shock protein 110 (HSP110) on the myddosome complex, due to interference with the proteasomal degradation of MYD88, is stronger in ABC-DLBCL cell lines with *MYD88*(L265P) than in those with wildtype *MYD88*.¹⁰ As *MYD88*(L265P) constitutively activates the NF- κ B pathway, it is regarded as an important oncogenic driver in B-NHL.^{2,7-12}

B-cell receptor signaling

In addition to the canonical NF- κ B pathway, the BCR pathway plays an important role in B-cell survival and proliferation and oncogenesis of B-NHL with *MYD88* mutations (Figure 1). In normal physiology, stimulation of the BCR activates NF- κ B, as well as the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR), and nuclear factor of activated T cells (NFAT) pathways. After antigen recognition by the BCR, Lck/Yes-related novel protein tyrosine kinase (LYN) is released from its inactive state through dephosphorylation of the C-terminal regulatory tyrosine by cluster of differentiation 45 (CD45) or an exogenous ligand for the Src-homology 2 (SH2) and SH3 domains of LYN, such as CD19. Activated LYN consecutively phosphorylates the immunoreceptor tyrosine-based activation motif (ITAM) domains of the coupled CD79A and CD79B heterodimers. These double-phosphorylated ITAM domains provide a docking site for the SH2 domains of spleen tyrosine kinase (SYK), which is activated by autophosphorylation or through transphosphorylation by LYN. LYN and SYK then activate Bruton tyrosine kinase (BTK) by phosphorylation, which is recruited to the membrane through interaction between the pleckstrin homology (PH) domain of BTK and phosphatidylinositol-3, 4, 5-triphosphate (PIP3) of the PI3K pathway or through interaction between the SH2 domain of BTK with the B-cell linker protein (BLNK) adapter molecule that also recruits phospholipase C γ 2 (PLC γ 2) to the membrane.¹³ BTK activates PLC γ 2, initiating activation of the NF- κ B pathway through formation of CBM complex, consisting of caspase recruitment domain family member 11 (CARD11), BCL10, and mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1). In addition, BTK activates the MAPK and PI3K pathways¹⁴ and PLC γ 2 triggers the NFAT pathway through calcineurin. The CBM complex subsequently attracts TRAF6, TAK1, and TAB, and promotes the degradation of I κ B, which leads to the

release of NF- κ B subunits.^{4,5,14,15}

BTK is an integral protein in the BCR signaling cascade and has been found to be preferentially complexed to MYD88 in WM cells with *MYD88*(L265P) and not in *MYD88* wildtype cells. Inhibition of BTK resulted in a decrease of the formation of this MYD88-BTK complex, but lacked effect on IRAK4/IRAK1 activity and *vice versa*, indicating a potential necessity of dual inhibition of IRAK and BTK for WM with *MYD88*(L265P).¹⁶⁻¹⁸ *MYD88* is frequently mutated in patients who also harbor a mutation in the 196 tyrosine residue in the ITAM domain of CD79B (NM_000626) and these patients seem to benefit most from BTK-inhibition treatment.¹⁹ The exact consequence of these double mutations in B-NHL is unclear, but Phelan *et al.*⁸ recently provided new insight into the mechanism of combined MYD88 and BCR-pathway activation as they identified a MYD88-TLR9-BCR (My-T-BCR) supercomplex. This supercomplex is generated by constitutive trafficking of the BCR towards endolysosomes that contain TLR9 and interacts with mTOR and the CBM complex, thereby promoting lymphomagenesis by activation of the mTOR and NF- κ B pathways. Its presence was demonstrated in cell lines and biopsies of ABC-DLBCL, primary DLBCL of the central nervous system, and lymphoplasmacytic lymphoma and correlated with responsiveness to BTK inhibition. On the other hand, the supercomplex was not identified in CLL or mantle cell lymphoma, suggesting a different mechanism of BCR signaling in these entities. Therefore, the My-T-BCR supercomplex could potentially be used as a biomarker for predicting the efficacy of BTK inhibitors, as a classifier of B-NHL subtypes, or as a novel therapeutic target via inhibition of TLR9.⁸

Autocrine signaling

As described, increased formation of the myddosome complex with IRAK1, as well as activation of the BCR pathway, caused by interactions of BTK with *MYD88*(L265P), *CD79B* mutations, and the My-T-BCR supercomplex, result in constitutive activation of the NF- κ B pathway. NF- κ B not only activates the transcription of genes involved in cell survival and proliferation, but also results in autocrine signaling with IL-6 and IL-10. One consequence of this autocrine signaling loop is the phosphorylation of Janus kinase 1 (JAK1) and, subsequently, signal transducer and activator of transcription 3 (STAT3) with the assembly of a STAT3/STAT3 complex. This complex increases transcription of genes involved in several signaling cascades, including the PI3K/AKT/mTOR, E2F/G2M cell-cycle checkpoint, JAK/STAT, and NF- κ B pathways. In addition, STAT3 activity represses the proapoptotic type I interferon (IFN) signaling pathway by downregulating IFN-regulatory factor 7 (IRF7), IRF9, STAT1, and STAT2 expression.^{2,3,20}

Another consequence of IL-6 signaling is the aberrant expression of hematopoietic cell kinase (HCK), as identified in primary WM cells and B-NHL cell lines.²¹ Increased levels of HCK promote lymphomagenesis, as HCK knock-down in B-NHL cell lines reduces survival and lowers the activity of the BCR, PI3K/AKT, and MAPK/ERK (extracellular signal-regulated kinases) pathways. Furthermore, BTK- and HCK-inhibition treatment of ABC-DLBCL and WM cells with *MYD88*(L265P) decreased HCK expression, whereas mutant *HCK*(T333M) (NM_002110.4) attenuated this effect. These findings suggest that HCK is

Table 1. (A, B) Overview of reported frequencies of *MYD88(L265P)* in B-cell neoplasms according to the 2016 World Health Organization classification of lymphoid neoplasms¹¹⁰ (A) and other mature B-cell neoplasms with specific disease locations (B).**1A**

Mature B-cell neoplasms	<i>MYD88(L265P)</i> prevalence	<i>MYD88(L265P)</i> incidence	Total sequenced	Range	Number of studies	References
Chronic lymphocytic leukemia/small lymphocytic lymphoma	2.5%	221	8773	0 – 25%	41	18, 22-24, 28, 38-53
Monoclonal B-cell lymphocytosis	0%	0	75	N.A.	2	53, 54
B-cell prolymphocytic leukemia	Unknown*					
Splenic marginal zone lymphoma	7.0%	59	840	0 – 50%	19	18, 23, 29, 55, 56
Hairy cell leukemia	1.1%	1	89	0 – 8%	5	22, 30, 57-59
Splenic B-cell lymphoma/leukemia, unclassifiable	16.7%	1	6	N.A.	1	60
Lymphoplasmacytic lymphoma	85.5%	337	394	0 – 100%	16	18, 22-30
Non-IgM lymphoplasmacytic lymphoma	55.0%	33	60	42 – 100%	7	18, 23, 31, 33, 61
Waldenström macroglobulinemia	85.3%	1888	2213	57 – 100%	34	18, 22, 23, 31-37
Monoclonal gammopathy of undetermined significance, IgM	52.7%	301	571	0 – 100%	13	18, 22, 23, 62
Monoclonal gammopathy of undetermined significance, IgG/A	0%	0	41	N.A.	3	18, 22, 23, 34
Plasma cell myeloma	1.5%	3	205	0 – 30%	14	18, 22, 23, 30, 43, 63, 106, 107
Solitary plasmacytoma of bone	Unknown*					
Extrasosseous plasmacytoma	Unknown*					
Monoclonal immunoglobulin deposition diseases	Unknown*					
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)	3.9%	15	383	0 – 13%	9	18, 22, 23, 64, 65
Nodal marginal zone lymphoma	10.3%	16	156	0 – 71%	9	18, 22, 23, 66
Follicular lymphoma	1.9%	5	264	0 – 50%	10	18, 22, 23, 67, 68
Pediatric-type follicular lymphoma	0%	0	27	N.A.	2	69, 70
Large B-cell lymphoma with <i>IRF4</i> rearrangement	Unknown*					
Primary cutaneous follicle center lymphoma	0%	0	60	N.A.	3	71-73
Mantle cell lymphoma	6.7%	2	30	0 – 50%	6	30, 43, 74
Diffuse large B-cell lymphoma (DLBCL), NOS	15.6%	853	5457	0 – 71%	43	3, 18, 22, 23, 67, 75-84, 113
Germinal center B-cell type	5.3%	81	1520	0 – 57%	21	3, 22, 23, 79-81, 85
Activated B-cell type	22.9%	492	2151	8 – 61%	21	3, 22, 23, 79-81, 85
T-cell/histiocyte-rich large B-cell lymphoma	Unknown*					
Primary DLBCL of the central nervous system	60.8%	382	628	33 – 100%	21	18, 22, 23, 86-88, 96
Primary cutaneous DLBCL, leg type	62.2%	138	222	40 – 75%	9	22, 71, 89-91
EBV ⁺ DLBCL, NOS	4.4%	4	90	0 – 22%	4	22, 83, 92
EBV ⁺ mucocutaneous ulcer	0%	0	14	N.A.	1	93
DLBCL associated with chronic inflammation	Unknown*					
Lymphomatoid granulomatosis	Unknown*					
Primary mediastinal (thymic) large B-cell lymphoma	0%	0	68	N.A.	3	2, 3, 94
Intravascular large B-cell lymphoma	44.0%	11	25	N.A.	1	95
ALK ⁺ Large B-cell lymphoma	Unknown*					
Plasmablastic lymphoma	Unknown*					
Primary effusion lymphoma	Unknown*					
HHV8 ⁺ DLBCL, NOS	Unknown*					
Burkitt lymphoma	1.5%	1	67	0 – 2%	2	2, 74
Burkitt-like lymphoma with 11q aberration	Unknown*					
High-grade B-cell lymphoma, with <i>MYC</i> and <i>BCL2</i> and/or <i>BCL6</i> rearrangements	11.1%	1	9	N.A.	1	83
High-grade B-cell lymphoma, NOS	Unknown*					
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma	Unknown*					

1B

Other mature B-cell neoplasms with	<i>MYD88</i> (L265P) prevalence	<i>MYD88</i> (L265P) incidence	Total sequenced	Range	Number of studies	References
Ocular adnexal marginal zone lymphoma	9.0%	23	255	36 – 71%	6	22, 23, 105
Primary bone DLBCL	5.8%	3	52	0 – 15%	3	100-102
Primary breast DLBCL	54.3%	38	70	35 – 71%	3	22, 99
Primary cutaneous marginal zone lymphoma	2.0%	2	100	0 – 4%	3	103, 104
Primary DLBCL of the thyroid	0%	0	21	N.A.	1	22
Primary testicular lymphoma	68.4%	65	95	14 – 82%	6	22, 23, 96, 108
Vitreoretinal lymphoma	72.7%	88	121	50 – 82%	9	22, 97, 98

* No data found in a literature search of articles published from January 2011 until August 2019. Terms used: 'WHO terms' (MeSH terms) AND *MYD88* 'WHO terms' (MeSH Terms) AND Genetic. Additionally, all articles found by the 'WHO terms' (MeSH terms) were screened for lymphomas with unknown status of the *MYD88* L265P mutation. DLBCL: diffuse large B-cell lymphoma; NOS: not otherwise specified; EBV: Epstein-Barr virus; ALK: anaplastic lymphoid kinase; HHV8: human herpes virus 8.

downstream of *MYD88*(L265P) and that HCK should be regarded as a potential therapeutic target in B-NHL with *MYD88*(L265P).

Prevalence

The described oncogenic mechanisms largely depend on the prevalence of *MYD88*(L265P) in B-NHL. Several studies, using Sanger sequencing, allele-specific polymerase chain reaction (PCR) analysis, or (targeted) next-generation sequencing, have demonstrated that the occurrence of *MYD88*(L265P) varies highly among the different subtypes of B-NHL (Table 1).^{2,3,18,22-108} The highest prevalence of *MYD88*(L265P) is found in lymphoplasmacytic lymphoma/WM, with approximately 85% of the patients being affected.^{18,22-37} In DLBCL, the prevalence of *MYD88*(L265P) is highest (range, 44% to 73%) in extranodal DLBCL, in immune-privileged sites,⁹⁶ such as primary DLBCL of the central nervous system^{18,22,23,86-88,96} and primary testicular lymphoma,^{22,23,96,108} primary cutaneous DLBCL, leg type,^{22,71,89-91} orbital/vitreoretinal DLBCL,^{22,97,98} intravascular large B-cell lymphoma,⁹⁵ and primary breast DLBCL.^{22,99} The high prevalence of *MYD88*(L265P) in extranodal site-specific lymphomas, lymphoplasmacytic lymphoma, and WM may provide an indication for the origin of these lymphomas. Interestingly, B-NHL entities with a high prevalence of *MYD88*(L265P) are characterized by a monoclonal immunoglobulin M. Furthermore, the high occurrence of *MYD88*(L265P) in extranodal DLBCL may imply that B cells need to gain this mutation for survival and manifestation in extranodal sites.

In DLBCL in general, a recent meta-analysis by Lee *et al.*,²² comprising 18 studies with a total of 2002 DLBCL patients, documented that 255 of 1236 (21%) cases of ABC-DLBCL harbored *MYD88*(L265P), compared with 44 of 766 (6%) cases of germinal center B-cell-like (GCB) DLBCL. Large sequencing studies, such as those by Reddy *et al.*,⁸⁰ Schmitz *et al.*,⁸¹ Chapuy *et al.*,⁷⁷ and Intlekofer *et al.*,⁷⁹ have compared with 44 of 766 (6%) cases of GCB DLBCL with archaic cell-of-origin classification, based on immunohistochemistry or gene expression profiling, and have shown that *MYD88*(L265P) and other mutations transcend these classifications and should be put into context with emerging genomic classification systems. These large sequencing studies underscore the need to evaluate the status of not only *MYD88*, but also

other genes involved in B-cell lymphomagenesis for diagnosis and during treatment with targeted therapies, as proposed by Sujobert *et al.*¹⁰⁹

Overall, these results identify *MYD88*(L265P) as a diagnostic classifier for specific B-NHL subtypes. This is supported by a recent study by our group that identified *MYD88* mutations as an independent marker, in a cohort of 250 patients with DLBCL, in addition to the routinely used *MYC* and *BCL2* and/or *BCL6* rearrangements and Epstein-Barr virus status (according to the 2016 World Health Organization classification¹¹⁰).⁸³ Furthermore, *MYD88*(L265P) is absent in primary mediastinal large B-cell lymphoma^{2,3,94} and primary cutaneous follicle center lymphoma,⁷¹⁻⁷³ and rarely present in hairy cell leukemia (1.1%),^{22,30,57-59} plasma cell myeloma (1.5%),^{18,22,23,43,106,107} Burkitt lymphoma (1.5%),^{2,74} follicular lymphoma (1.9%),^{18,22,23,67,68} and CLL (2.5%).^{18,22-24,28,38-52}

Prognostic impact

In addition to its role as a diagnostic classifier, the prognostic value of *MYD88*(L265P) has been a topic of many studies involving B-NHL patients. Lee *et al.* performed a meta-analysis of three studies with accurate multivariate hazard ratios to investigate the prognostic value of *MYD88*(L265P) in DLBCL.²² This analysis, involving a total of 275 DLBCL patients, showed that DLBCL patients with *MYD88*(L265P) had a statistically significant inferior overall survival compared with DLBCL patients with wildtype *MYD88*. In addition, *MYD88*(L265P) was significantly associated with older age, high International Prognostic Index (IPI)-score risk groups, and extranodal localization. We also demonstrated this association of *MYD88*(L265P) with an inferior survival in our recent study in which we evaluated *MYD88* status, together with *CD79B*, *MYC*, *BCL2*, *BCL6* and Epstein-Barr virus status and clinical characteristics in 250 DLBCL patients.⁸³ Additionally, we showed that the performance of the IPI score is improved by adding *MYD88*(L265P) as a poor risk factor.

The correlation of *MYD88* mutations with an inferior overall survival is also observed in several subtypes of extranodal DLBCL, such as primary cutaneous DLBCL, leg type¹¹¹ and immune-privileged DLBCL.^{22,83,112} On the other hand, in a study by Xu *et al.*,⁸⁴ *MYD88* mutations were significantly more frequent in DLBCL patients who

were refractory to chemotherapy with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) (28%) compared with DLBCL patients who were chemosensitive (15%), but no statistically significant correlation with overall survival was found. The actual prognostic value of MYD88 in DLBCL requires further investigation, as other studies identified no effect of MYD88(L265P) on the survival of DLBCL patients.^{22, 112-114}

In other subtypes of B-NHL, such as CLL, splenic marginal zone lymphoma, and WM, MYD88(L265P) is associated with a superior survival compared with wildtype MYD88.^{45,115,116} In WM, approximately 30-40% of patients present with concomitantly mutated MYD88 and CXCR4, a gene involved in homing of B cells in the bone marrow, and these patients present with a greater disease burden and reduced progression-free and overall

Table 2. Overview of several (ongoing) clinical trials with novel therapies targeting BTK, PI3K, mTOR and XPO1 in B-cell non-Hodgkin lymphomas in which MYD88(L265P) is frequent.

Medication	Target	Overall response rate	B-NHL	N. of patients Ref. of trial registration
Ibrutinib	BTK	33%	Relapsed ABC	12 (NCT01325701)
Ibrutinib	BTK	93%	WM	55 (NCT01614821)
Ibrutinib	BTK	83%	PCNSL	6 ¹²⁰
Ibrutinib	BTK	37%	ABC-DLBCL	38 (NCT01325701)
Ibrutinib	BTK	5%	GCB-DLBCL	20 (NCT01325701)
Ibrutinib	BTK	68-88%	Relapsed MCL	16 (NCT02169180) 139 (NCT01646021) 111 (NCT01236391)
Acalabrutinib	BTK	Ongoing	CLL	~306 (NCT02029443)
Acalabrutinib	BTK	Ongoing	MCL	~124 (NCT02213926)
Acalabrutinib	BTK	Ongoing	DLBCL	~39 (NCT03571308)
Acalabrutinib	BTK	Ongoing	ABC-DLBCL	~21 (NCT02112526)
Acalabrutinib	BTK	Ongoing	MCL	~70 (NCT02717624)
Zanubrutinib	BTK	Ongoing	B-cell lymphoma	~44 (NCT03189524)
Zanubrutinib	BTK	Ongoing	Relapsed MCL	~86 (NCT03206970)
Zanubrutinib	BTK	Ongoing	Relapsed WM	~40 (NCT03332173)
Zanubrutinib	BTK	Ongoing	Relapsed CLL	~91 (NCT03206918)
Zanubrutinib	BTK	Ongoing	Relapsed MZL	~65 (NCT03846427)
Zanubrutinib	BTK	Ongoing	WM	~210 (NCT03053440)
Enzastaurin	PKC		MCL	61 (NCT00088205)
Enzastaurin	PKC		Relapsed DLBCL	55 (NCT00042666)
Enzastaurin	PKC		Relapsed WM	46 (NCT00718419)
Buparlisib	PI3K	11.5%	Relapsed DLBCL	26 (NCT01693614)
Buparlisib	PI3K	22.7%	Relapsed MCL	22 (NCT01693614)
Buparlisib	PI3K	25%	PCNSL	4 (NCT02301364)
Idelalisib	PI3K	40%	Relapsed MCL	40 (NCT01090414)
Idelalisib	PI3K	47%	Relapsed MZL	15 (NCT01282424)
Idelalisib	PI3K	80%	Relapsed LPL/WM	10 (NCT01282424)
Parsaclisib	PI3K	30%	Relapsed DLBCL	23 (NCT02018861)
Parsaclisib	PI3K	67%	Relapsed MCL	14 (NCT02018861)
Parsaclisib	PI3K	78%	Relapsed MZL	9 (NCT02018861)
Everolimus	mTOR	20-32%	Relapsed MCL	35 (NCT00516412) 19 ¹⁴¹
Everolimus	mTOR	70%	Relapsed WM	51 (NCT00436618)
Everolimus	mTOR	30%	Relapsed DLBCL	47 ¹⁴¹
Temsirolimus	mTOR	32-47%	Relapsed MCL	47 (NCT01180049) 141 (NCT01646021)
IMO-8400	TLR7/8/9		Relapsed DLBCL	6 (NCT02252146)
IMO-8400	TLR7/8/9		Relapsed WM	5 (NCT02363439)

B-NHL: B-cell non-Hodgkin lymphomas; BTK: Bruton tyrosine kinase; PKC: protein kinase C; PI3K: phosphoinositide 3-kinase; mTOR: mammalian target of rapamycin; TLR: toll-like receptor; ABC: activated B-cell; DLBCL: diffuse large B-cell lymphoma; WM: Waldenström macroglobulinemia; PCNSL: primary DLBCL of the central nervous system; GCB: germinal center B-cell like; MCL: mantle cell lymphoma; CLL: chronic lymphocytic leukemia; MZL: marginal zone lymphoma; LPL: lymphoplasmacytic lymphoma.

survival.^{117,118} With regards to CLL, Impropio *et al.*⁵⁹ stated that *MYD88*(L265P) occurs mainly in patients with mutated *IGHV* or chromosome 13q deletions and both alterations are associated with a superior survival. Furthermore, WM patients with wildtype *MYD88* had an increased risk of disease transformation, ibrutinib resistance and shorter overall survival.^{9,117, 118}

Targeted therapies

The oncogenic activity of *MYD88*(L265P), as well as its high frequency in several B-NHL subtypes, ensure that *MYD88* and its affiliated signaling pathways are very interesting for targeted therapeutic strategies. As reviewed by Yu *et al.*¹⁸ and Weber *et al.*,¹¹⁹ several targets are conceivable for direct or indirect inhibition, such as IRAK1 and IRAK4 in the myddosome-complex, TAK1 in downstream signaling, BTK in the BCR pathway, TLR9 in the My-T-BCR supercomplex, and components of the concurrently activated PI3K/AKT/mTOR and HCK pathways (Figure 2).

Of these targets, inhibition of BTK has been the most extensively studied, regardless of the fact that BTK is not a *MYD88*(L265P)-specific target and is not directly involved with the myddosome complex. The BTK inhibitor ibrutinib is approved as treatment for CLL, mantle cell lymphoma, relapsed/refractory marginal zone lymphoma, and WM by the United States Food and Drug Administration (FDA). Additionally, the FDA permitted the combined use of ibrutinib and rituximab as the first non-chemotherapeutic regimen for WM patients. In early clinical trials in patients with relapsed/refractory DLBCL

and primary DLBCL of the central nervous system, ibrutinib elicited an overall response rate of 80-85% in those with *MYD88*(L265P) alone or in combination with mutated *CD79B*.^{19,120} Furthermore, in a randomized phase III trial, ibrutinib plus R-CHOP improved the overall survival of DLBCL patients younger than 60 years regardless of the cell-of-origin.¹²¹ Nonetheless, ibrutinib tends to produce many off-target effects and acquisition of resistance to the drug is common. For instance, ibrutinib resistance can be caused by the C481S mutation in *BTK* (NM_000061), which hampers the interaction between ibrutinib and BTK,¹²² but also by mutations in *PLCγ2*,¹²³ *CARD11*,¹²⁰ and *CXCR4*.¹²⁴ Given these drawbacks of ibrutinib, next-generation BTK inhibitors, such as acalabrutinib and zanubrutinib, are being developed and used for research. Studies demonstrated that acalabrutinib achieved an overall response rate of 95% in relapsed CLL¹²⁵ and 81% in relapsed mantle cell lymphoma,¹²⁶ and this medicine is now approved as treatment for mantle cell lymphoma by the United States FDA. Zanubrutinib achieved an overall response rate of 90% in WM, and was also shown to be well tolerated and to overcome the ibrutinib resistance mechanism induced by *CXCR4* mutations.¹²⁷

In addition to studies on BTK inhibition, several phase I/II clinical trials have investigated the response of novel therapeutic targets (in)directly involved with *MYD88* in patients with B-NHL. In relapsed/refractory WM, mTOR inhibition with everolimus produced an overall response rate of 50%.¹²⁸ In several subtypes of relapsed/refractory B-NHL, PI3K inhibition with piasclisib produced overall response rates ranging between 20% and 78%, with a low risk of adverse events and improved long-term out-

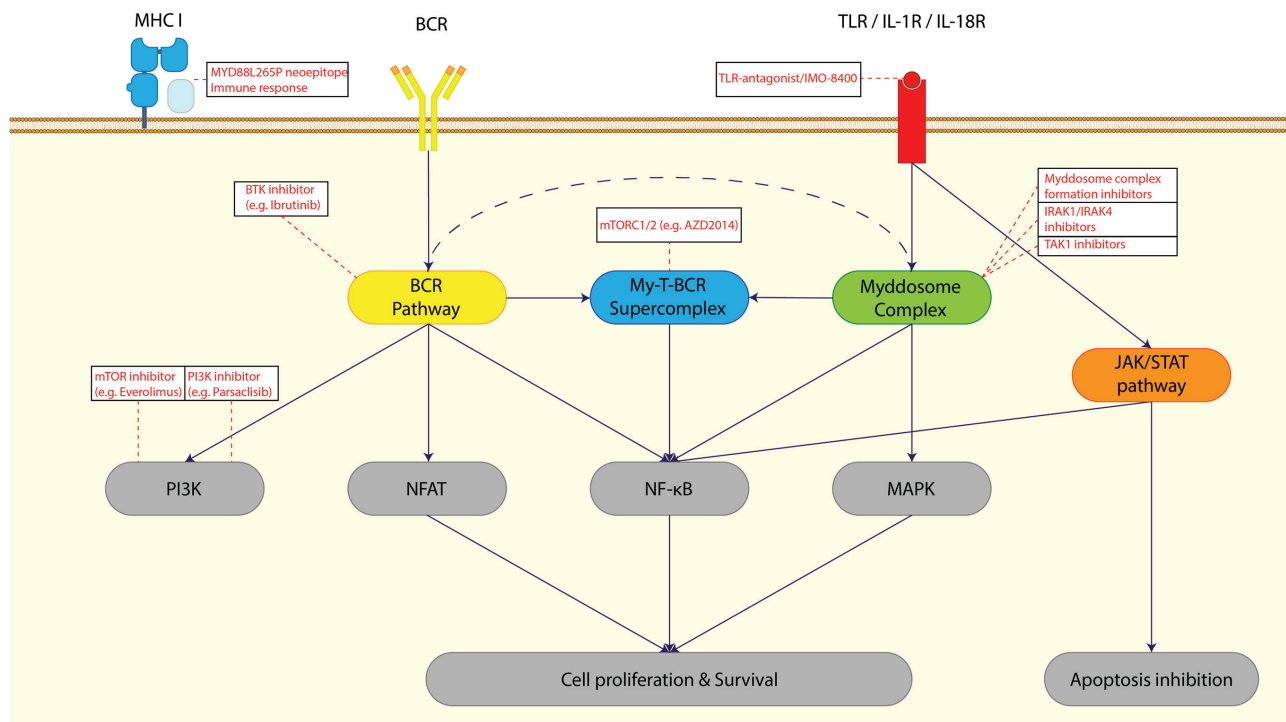


Figure 2. Signaling cascades in mutated *MYD88* B-cell non-Hodgkin lymphoma can be inhibited by several targeted therapeutic strategies. A combination of several therapies might increase efficacy and reduce the risk of relapse, depending on the molecular profile of the B-cell non-Hodgkin lymphoma.

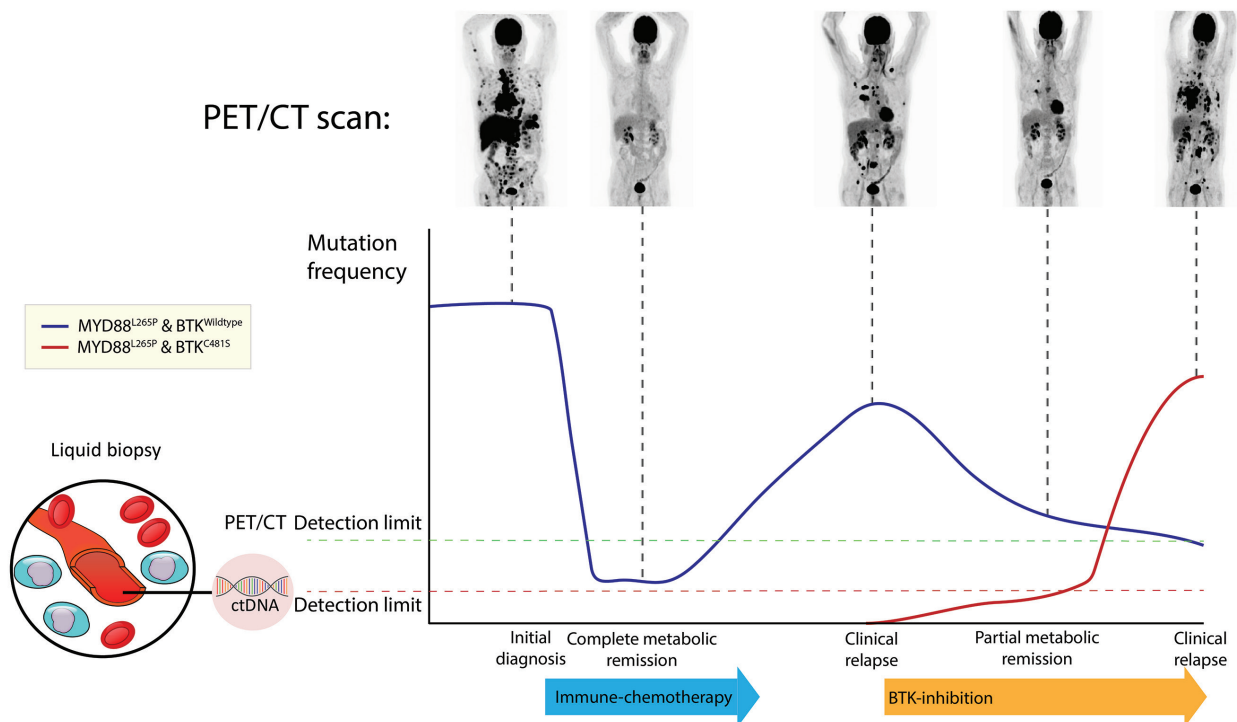


Figure 3. Schematic representation of the potential use of liquid biopsies during disease progression in B-cell non-Hodgkin lymphoma. After diagnosis, a hypothetical patient was treated with immune-chemotherapy. During therapy, the lymphoma was significantly reduced, as evidenced by a complete metabolic remission on positron emission tomography-computed tomography (PET/CT) scans and minimal residual disease by the analysis of circulating tumor DNA (ctDNA) mutation frequency. Thereafter, the residual B-cell lymphoma developed again, gradually increased, and induced a significant clinical relapse. Following comprehensive (genetic) diagnostic procedures, including histological confirmation, liquid biopsies, and PET/CT scans, the patient was treated with BTK inhibition as a second-line therapy, consequently reducing the lymphoma and leading to a partial metabolic remission. Lastly, residual lymphoma cells harboring a *BTK*(C481S) mutation gained resistance to the BTK inhibition therapy; these cells expanded unimpeded and resulted in another clinical relapse. In this schematic representation, the mutation frequency throughout the course of the patient's disease is plotted. The two detection limits indicate the sensitivity of PET/CT and the liquid biopsy (e.g., ctDNA with digital droplet polymerase chain reaction analysis).

comes.¹²⁹ In *in vitro* assays, enzastaurin, a protein kinase C inhibitor, reduced proliferation and viability of DLBCL cells by regulation of the PI3K, MAPK, and JAK/STAT pathways; however, it also increased phosphorylation of BTK, suggesting the need for simultaneous treatment of enzastaurin with BTK inhibition.¹³⁰ Patients with DLBCL are currently being recruited into a phase III clinical trial in which enzastaurin is combined with R-CHOP (NCT03263026).

The clinical trials mentioned above focus on therapeutic targets that are directly or indirectly involved with MYD88 activity; however these targets are not specific for *MYD88*(L265P) and patients are selected irrespective of the mutational status of *MYD88*. The lack of biomarkers in these clinical trials is a potential weakness, especially regarding the evolving field of genetics and precision medicine. Novel drugs targeting the oncogenic mechanisms of *MYD88*(L265P), such as inhibition of the interaction between TLR9 and MYD88 in the My-T-BCR supercomplex⁸ and between MYD88 and IRAK4 in the myddosome,¹³¹ or direct inhibition of IRAK4^{11,39} or TAK1,⁷ would be interesting for B-NHL patients with *MYD88*(L265P) and have shown promising results in *in vitro* and *in vivo* studies. In addition, the use of immunomodulatory oligonucleotides (IMO) such as IMO 8400, an antagonist of TLR7, TLR8, and TLR9, could be an interesting targeted treatment for *MYD88*(L265P) B-NHL and especially for ABC-DLBCL

with the My-T-BCR supercomplex. IMO-8400 has mainly been investigated in immune-mediated inflammatory diseases and only two phase I/II clinical trials with *MYD88*(L265P)-positive DLBCL and WM have been performed, showing that IMO-8400 is well tolerated in these patients (NCT02252146, NCT02363439, <https://www.iderapharma.com/wp-content/uploads/2015/12/IMO-8400-WM-ASH-Poster.pdf>). More research is required on the *MYD88*(L265P)-specificity of the above-mentioned targets in order to determine their role in the treatment of B-NHL patients with *MYD88*(L265P) and, thereby, improve personalized medicine.

An alternative therapeutic approach for these patients, as reviewed by Weber *et al.*,¹¹⁹ is the induction of a T-cell mediated immune response towards tumor-specific neoepitopes that are derived from *MYD88*(L265P). In *in vitro* experiments, such neoepitopes, presented by major histocompatibility class I molecules, prompted a cytotoxic CD8⁺ T-cell response.¹³² These tumor-specific T cells can be harvested from healthy donors¹³³ or patients with B-NHL and primed to elicit a tumor-specific cytotoxic effect or theoretically used as a model for chimeric antigen receptor (CAR) T-cell therapy. Furthermore, *in vitro* assays of DLBCL showed that *MYD88*(L265P) tumor cells develop resistance against T-cell mediated cytotoxicity via upregulation of IL-10 and STAT3 and that inhibition of either IL-10 or STAT3 significantly attenuates this gain

of resistance.¹³⁴ To our knowledge, currently no clinical trials are underway to investigate this intriguing treatment concept.

Liquid biopsy

Until now, comprehensive genomic analysis for accurate diagnosis and classification of B-NHL has been based on DNA isolated from lymphoma tissues. For most patients, the collection of this tissue is a highly invasive procedure with the risk of severe complications.¹³⁵ An alternative and less invasive method of sampling is the so-called 'liquid biopsy', using blood plasma or cerebrospinal fluid, instead of lymphoma tissue. These fluids contain circulating tumor DNA (ctDNA) that is secreted or released during apoptosis or necrosis of the tumor cells, and may harbor somatic mutations, such as MYD88(L265P). Besides being a less invasive method of sampling, ctDNA allows detection of spatial differences between lymphoma cells spread throughout the body, which is not possible with tissue biopsies.

The high frequency of MYD88(L265P) in several B-NHL subtypes make this mutation perfectly appropriate for screening by ctDNA, as already demonstrated in DLBCL,¹³⁶ primary DLBCL of the central nervous system,¹³⁷ and intravascular large B-cell lymphoma.⁹⁵ With the highly sensitive and specific method of digital droplet PCR (ddPCR), even low amounts of ctDNA can be detected, potentially providing information about minimal residual disease, clonal evolution over time, and spatial differences between the lymphoma cells. As demonstrated in patients with DLBCL and WM, ddPCR analysis of liquid biopsies can aid in monitoring the disease course, because of the highly sensitive identification and quantification of the variant allele frequency of MYD88(L265P).^{31,138}

An alternative technique for ctDNA analysis is targeted

next-generation sequencing. The benefit of this technique over ddPCR is the possibility of identifying multiple variants at the same time, as was shown by Bohers *et al.*¹³⁹ and Kurtz *et al.*¹⁴⁰ in liquid biopsies from 30 and 217 DLBCL patients, respectively. The mutational burden of most of their patients, with a median of 117 variants per patient, was sufficient for disease monitoring. This novel way of disease monitoring could enhance evaluation of treatment responses (Figure 3). In their studies, the tumor burden, as measured by positron emission tomography-computed tomography scans, was significantly correlated with the variant allele frequency of ctDNA both during and after treatment.^{139,140} Given this recent progress in ctDNA analysis, liquid biopsies are a minimally invasive method for evaluation of the molecular profile and can be used for analysis of tumor burden, disease progression, and treatment efficacy in patients with B-NHL.

Conclusions and future perspectives

Routine diagnostics in B-NHL are moving forward from classical morphology and immunohistochemistry towards the implementation of genetic analysis. In several subtypes of B-NHL subtype, MYD88(L265P) plays a crucial role as a driver of lymphomagenesis and can be used as a diagnostic classifier, as well as a prognostic factor and predictive biomarker. B-NHL with MYD88(L265P) can be (in)directly targeted by several novel therapeutic strategies and prospective clinical trials investigating these strategies are ongoing. We expect that these theranostic strategies will be guided by analysis of MYD88(L265P) in liquid biopsies to monitor disease progression and determine response to therapy. Altogether, given the significant clinical relevance of MYD88(L265P), we advocate evaluation of MYD88 mutational status in routine diagnostics of B-NHL.

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