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 \(\text{MYD88}\) gene as an important oncogenic driver in B-cell lymphomas. \(\text{MYD88}\) mutations constitutively activate NF-\(\kappa\)B and its associated signaling pathways, thereby promoting B-cell proliferation and survival. High frequencies of the hotspot \(\text{MYD88}(L265P)\) 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 \(\text{MYD88}\) predicts survival outcome in B-NHL subtypes and provides a therapeutic target. Early clinical trials targeting \(\text{MYD88}\) have shown encouraging results in relapsed/refractory B-NHL. Patients with these disorders can benefit from analysis for the \(\text{MYD88}\) 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 \(\text{MYD88}\) 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 \(\text{MYD88}\) and its clinical implications in B-NHL.
MYD88(L265P) in NF-κB (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-κB signaling

In normal physiology, MYD88 acts as a signaling adaptor in the canonical NF-κB 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 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). 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-κB kinase (IKK) complex. The IKK complex consists of the kinase subunits IKKα and IKKβ and the regulatory subunit NF-κB essential modulator. After activation, this complex phosphorylates the inhibitor of NF-κB (IκB) proteins that are bound to NF-κB, which prevent migration of NF-κB to the nucleus. Phosphorylation of these IκB proteins results in ubiquitylation and proteasomal degradation of IκB and release of the NF-κB subunits. Subsequently, the NF-κB 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-κB subunits and other transcription factors, such as E1A binding protein F300 (EP300) and CREB binding protein (CREBBP).

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. 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-κB 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-κB 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-κB 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). 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. However, Ansell et al. 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). 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. As MYD88(L265P) constitutively activates the NF-κB pathway, it is regarded as an important oncogenic driver in B-NHL.

**B-cell receptor signaling**

In addition to the canonical NF-κB 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-κB, 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 SH5 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 Cγ2 (PLCγ2) to the membrane. BTK activates PLCγ2, initiating activation of the NF-κB pathway through formation of CBM complex, consisting of caspase recruitment domain family member 11 (CARD11), BLCL10, and mucosa-associated lymphoid tissue lymphoma translocation protein1 (MALT1). In addition, BTK activates the MAPK and PI3K pathways and PLCγ2 triggers the NFAT pathway through calcineurin. The CBM complex subsequently attracts TRAF6, TAK1, and TAB, and promotes the degradation of IκB, which leads to the release of NF-κB subunits.

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). 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. The exact consequence of these double mutations in B-NHL is unclear, but Phelan et al. 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 the CBM complex, thereby promoting lymphomagenesis by activating the mTOR and NF-κB 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.

**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-κB pathway. NF-κB not only activates the transcription of genes involved in cell survival and proliferation, but is also responsible for 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-κB 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.

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. Increased levels of HCK promote lymphomagenesis, as HCK knockdown 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 (T555M) (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 neoplasms¹³⁴ (A) and other mature B-cell neoplasms with specific disease locations (B).

| Mature B-cell neoplasms                                                                 | MYD88(L265P) prevalence | MYD88(L265P) 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*                 |                         |                 |         |                   |                                   |
| Splenial 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 gamopathy of undetermined significance, IgM                                 | 52.7%                    | 301                     | 571             | 0 – 100%| 13                | 18, 22, 23, 62                   |
| Monoclonal gamopathy 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*                 |                         |                 |         |                   |                                   |
| Extramedial 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 IRF4 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%                    | 653                     | 547             | 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 1q aberration                                                | Unknown*                 |                         |                 |         |                   |                                   |
| High-grade B-cell lymphoma, with MYC and BCL2 and/or BCL6 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*                 |                         |                 |         |                   |                                   |
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).2,3,11,12,106 The highest prevalence of MYD88(L265P) is found in lymphoplasmacytic lymphoma/WM, with approximately 85% of the patients being affected.16,22,23 In DLBCL, the prevalence of MYD88(L265P) is highest (range, 44% to 73%) in extranodal DLBCL, in immune-privileged sites,16 such as primary DLBCL of the central nervous system,12,22,23,88-90 primary cutaneous lymphoma,12 and primary cutaneous DLBCL, leg type.22-24,49 orbital/vitreoretinal DLBCL,12 orbital lymphoma,12 and primary breast DLBCL.22-24 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.,79 comprising 18 studies with a total of 2002 DLBCL patients, demonstrated 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 (GC) DLBCL. Large sequencing studies, such as those by Reddy et al.,80 Schmitz et al.,81 Chapuy et al.,77 and Intlekofer et al.,81 have compared with 44 of 766 (6%) cases of GC 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 Sujbert 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 classification).110,111 Furthermore, MYD88(L265P) is absent in primary mediastinal large B-cell lymphoma,2,3,12 and primary cutaneous follicle center lymphoma,12-13 and rarely present in hairy cell leukemia (1.1%),22,30,35-39 plasma cell myeloma (1.5%),22,23,40,101,107 Burkitt lymphoma (1.5%),74 follicular lymphoma (1.9%),18,22,23,47,48 and CLL (2.5%),18,22-24,26,18-22

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.112 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.80 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 type,113 and immune-privileged DLBCL.22-24,112 On the other hand, in a study by Xu et al.,104 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 survival.

Table 2. Overview of several (ongoing) clinical trials with novel therapies targeting BTK, PI3K, mTOR and XP01 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         |
| Ibrutinib  | BTK    | 37%                   | ABC-DLBCL | 38 (NCT01325701) |
| Ibrutinib  | BTK    | 5%                    | GCB-DLBCL | 20 (NCT01325701) |
| Ibrutinib  | BTK    | 68-88%                | Relapsed MCL | 16 (NCT02169180) |
| Acalabrutinib | BTK | Ongoing               | CLL   | ~3-6 (NCT02029445) |
| Acalabrutinib | BTK | Ongoing               | MCL   | ~12-4 (NCT02123926) |
| 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 (NCT03653440) |
| Enzastaurin | PKC   |                       | MCL   | 61 (NCT00088285) |
| Enzastaurin | PKC   |                       | Relapsed DLBCL | 55 (NCT00042666) |
| Enzastaurin | PKC   |                       | Relapsed WM | 46 (NCT00718419) |
| Buparlisib | PI3K  | 11.5%                 | Relapsed DLBCL | 26 (NCT01603614) |
| Buparlisib | PI3K  | 22.7%                 | Relapsed MCL | 22 (NCT01603614) |
| Buparlisib | PI3K  | 25%                   | PCNSL | 4 (NCT02301364) |
| Idelalisib | PI3K  | 40%                   | Relapsed MCL | 40 (NCT01009414) |
| Idelalisib | PI3K  | 47%                   | Relapsed MZL | 15 (NCT01282424) |
| Idelalisib | PI3K  | 80%                   | Relapsed LPL/LM | 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 (NCT05164412) |
| Everolimus  | mTOR  | 70%                   | Relapsed WM | 51 (NCT00436618) |
| Everolimus  | mTOR  | 30%                   | Relapsed DLBCL | 47 (NCT01180049) |
| Temsirolimus | mTOR | 32-47%                | Relapsed MCL | 47 (NCT01180049) |
| IMO-8400   | TLR7/8/9 |                       | Relapsed DLBCL | 6 (NCT02252146) |
| IMO-8400   | TLR7/8/9 |                       | Relapsed WM | 5 (NCT02383439) |

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.
With regards to CLL, Improgo et al. 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.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 parsaclisib 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.
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. 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 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 supercomplex and between MYD88 and IRAK4 in the myddosome, or direct inhibition of IRAK4 or TAK1, 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., 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. These tumor-specific T cells can be harvested from healthy donors 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. 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. 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, primary DLBCL of the central nervous system, and intravascular large B-cell lymphoma. 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).

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. and Kurtz et al. 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. 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.

**References**

1. Vermaat JS, Pals ST, Younes A, et al. Precision medicine in diffuse large B-cell lymphoma: hitting the target. Haematologica. 2015;100(8):989-993.
2. Ngo VN, Young RM, Schmitz R, et al. Oncogenically active MYD88 mutations in human lymphoma. Nature. 2011;470(7332):115-119.
3. Dubois S, Vialy PJ, Bohers E, et al. Biological and clinical relevance of associated genomic alterations in MYD88 L265P and non-L265P-mutated diffuse large B-cell lymphoma: analysis of 361 cases. Clin Cancer Res. 2017;23(9):2232-2244.
4. Deguine J, Barton GM. MYD88: a central player in innate immune signaling. F1000Prime Rep. 2014;6:97.
5. Lin SC, Lo YC, Wu H. Helical assembly in MYD88: hitting the target. Haematologica. 2015;100(8):989-993.
6. Perkins ND. The diverse and complex roles of NF-kappaB subunits in cancer. Nat Rev Cancer. 2012;12(2):121-132.
7. Ansell SM, Hodges LS, Secreto FJ, et al. Activation of TAK1 by MYD88 L265P drives malignant B-cell growth in non-Hodgkin lymphoma. Blood Cancer J. 2014;4:e183.
8. Phelan JD, Young RM, Webster DE, et al. A multiprotein supercomplex controlling oncogenic signalling in lymphoma. Nature. 2018;560(7718):387-391.
9. Puente XS, Finynot M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. Nature. 2011;475(7354):101-105.
10. Boudsocq C, Verhoeven E, Martin L, et al. HSP110 sustains chronic NF-kappaB signalling in activated B-cell diffuse large B-cell lymphoma through MYD88 stabilization. Blood. 2016;127(5):510-520.
11. Ni HW, Shirazi F, Baladandayuthapani V, et al. Targeting myddosome signaling in Waldenstrom's macroglobulinemia with the interleukin-1 receptor-associated kinase 1/4 inhibitor R191. Clin Cancer Res. 2018;24(24):6408-6420.
12. Rousseau S, Martel G. Gain-of-function mutations in the toll-like receptor pathway: TPL2-mediated ERK1/ERK2 MAPK activation, a path to tumorigenesis in lymphoid neoplasms? Front Cell Dev Biol. 2016;4:50.
13. Kurosaki T, Hikida M. Tyrosine kinases and interleukin-1 receptor-associated kinase 1/4 in hematopoietic malignancies. Cancer Res. 2018;78(10):2457-2462.
14. Wilson WH, Young RM, Schmitz R, et al. Synergistic cooperation and crosstalk between MYD88(L265P) and mutations that dysregulate CD79B and surface IgM. J Exp Med. 2017;214(9):2759-2776.
15. Yang G, Xi L, Deng Q, et al. MYD88(L265P) mutation in lymphoid malignancies. Cancer Res. 2018;78(10):2457-2462.
16. Lu L, Zhu F, Zhang M, et al. Gene regulation by STAT3 in diffuse large B cell lymphoma. Proc Natl Acad Sci U S A. 2011;108(11):4695-4699.
17. Yang G, Buhrlage SJ, Tan L, et al. HCK is a critical player in innate immune signaling. F1000Prime Rep. 2014;6:97.
18. Yu X, Li W, Deng Q, et al. MYD88 L265P mutation in lymphoid malignancies. Cancer Res. 2018;78(10):2457-2462.
19. Wang JC, Jeeall VS, Hurnburg P, et al. Targeting myddosome signaling in Waldenstrom's macroglobulinemia with the interleukin-1 receptor-associated kinase 1/4 inhibitor R191. Clin Cancer Res. 2018;24(24):6408-6420.
20. Lu L, Zhu F, Zhan M, et al. Gene regulation and suppression of type I interferon signaling by STAT3 in diffuse large B cell lymphoma. Proc Natl Acad Sci U S A. 2011;108(11):4695-4699.
21. Yang G, Buhrlage SJ, Tan L, et al. HCK is a
survival determinant transactivated by MYD88 and a direct target of Brutinib. Blood. 2016;127(25):3237-3252.

22. Lee JH, Jeong H, Choi JW, Oh H, Kim YS. Clinicopathologic significance of MYD88 L265P in diffuse large B-cell lymphoma: a meta-analysis. Sci Rep. 2017;7(1):1785.

23. Onda-Rivas V, Patel KP. Clinical utility of recently identified diagnostic, prognostic, and therapeutic molecular biomarkers in mature B-cell neoplasms. Mod Pathol. 2017;30(10):1388-1386.

24. Baer C, Dicker F, Kern W, Haferlach T, Haferlach C. Genetic characterization of MYD88-mutated lymphoplasmacytic lymphoma in comparison with MYD88-mutated chronic lymphocytic leukemia. Leukemia. 2017;31(9):1355-1362.

25. Balestrieri IY, Loghavi S, Kanagal-Shamanna R, et al. Clinical validation of a CXC4R4 mutation screening assay for Waldenstrom macroglobulinemia. Clin Lymphoma Myeloma Leuk. 2016;16(7):395-403.

26. Cilla N, Vercruysse M, Ameye L, et al. [Diagnostic approach of an IgM monoclonal gammapathy and clinical importance of gene set MYD88 L265P]. Rev Med. Brux. 2018 May 30. [Epub ahead of print].

27. Fang H, Kapoor P, Consalves WJ, et al. Defining lymphoplasmacytic lymphoma: does MYD88L265P define a pathologically distinct entity among patients with an IgM paraprotein and bone marrow involvement of low-grade B-cell lymphomas with plasmacytic differentiation? Am J Clin Pathol. 2016;145(5):1657-1671.

28. Insuasti-Beltran G, Gale JM, Wilson CS, et al. MYD88 L265P mutation status in patients with chronic lymphocytic leukemia. J Clin Pathol. 2019;72(1):34-41.

29. Jallades L, Bassegiov L, Sujotent F, et al. Exome sequencing identifies recurrent NR4A1 alterations and the absence of KLF2, TNFAIP3 and MYD88 mutations in splenic diffuse red pulp small B-cell lymphoma. Haematologica. 2017;102(10):1738-1766.

30. Martinez-Lopez A, Curiel-Olmo S, Mollejo M, et al. MYD88 (L265P) somatic mutation in marginal zone B-cell lymphoma. J Surg Pathol. 2015;39(5):644-651.

31. Ondrzelka SL, Lin JH, Warden DW, Durkin L, Cook JR, Hsi ED. MYD88 L265P somatic mutation in detection of the differential diagnosis of bone marrow involvement by B-cell lymphoproliferative disorders. Am J Clin Pathol. 2013;140(3):387-394.

32. Drudi E, Droghetti I, et al. Highly sensitive MYD88(L265F) mutation detection by droplet digital polymerase chain reaction in Waldenstrom macroglobulinemia. Haematologica. 2016;101(6):1029-1037.

33. Poulan S, Roumier C, Decambron A, et al. MYD88 L265P mutation in Waldenstrom macroglobulinemia. Blood. 2013;121(22):4504-4511.

34. Varettoni M, Boveri E, Zibilini S, et al. Clinical and molecular characteristics of lymphoplasmacytic lymphoma not associated with an IgM monoclonal protein: a monocytic testiculately of the retic ematologica lombardia (REL) network. Am J Hematol. 2019 Aug 4. [Epub ahead of print].

35. Xu L, Hunter ZR, Tsaknaklis N, et al. Clonal architecture of CXC4R4 WHIM-like mutations in Waldenstrom macroglobulinemia. Br J Haematol. 2016;172(5):735-744.

36. Xu L, Hunter ZR, Yang G, et al. Detection of MYD88(L265P) in peripheral blood of patients with Waldenstrom's macroglobulinemia and IgM monoclonal gammapathy of undetermined significance. Leukemia. 2014;28(5):1004-1004.

37. Treon SP, Cao Y, Xu L, Yang G, Liu X, Hunter ZR. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. Blood. 2014;123(12):2791-2796.

38. Abeykoon LR, Phan TL, King RL, et al. MYD88 mutation status does not impact overall survival in Waldenstrom macroglobulinemia. Am J Hematol. 2018;93(2):187-194.

39. Ali YB, Youd RM, Abdel-Wahed E. Lack of associations between TLR9 and MYD88 gene polymorphisms and risk of chronic lymphocytic leukemia. Asian Pac J Cancer Prev. 2017;18(12):5245-5246.

40. Impingo MR, Tesar B, Kutzbard JL, et al. MYD88 L265F mutations identify a prognosis gene expression signature and a pathway for targeted inhibition in CLL. Br J Haematol. 2019;184(6):925-936.

41. Jang M, Li J, Zhou J, Xing C, Xu JJ, Guo F. High-resolution melting analysis for rapid and sensitive MYD88 screening in chronic lymphocytic leukemia. Oncol Lett. 2019;18(1):814-819.

42. Leek JM, Wilson CS, et al. MYD88 L265P mutation status does not impact overall survival in Waldenstrom macroglobulinemia. Blood. 2014;123(12):2791-2796.

43. Patkar N, Subramanian PG, Deshpande P, et al. MYD88 mutant lymphoplasmacytic lymphoma-Waldenstrom macroglobulinemia has distinct clinical and pathological features as compared to its mutation negative counterpart. Leukemia. 2015;29(2):402-403.

44. Putrovski M, Podgornik M, Furlong M, et al. Prognostic impact of NOTCH1, MYD88, and SF3B1 mutations in Polish patients with chronic lymphocytic leukemia. J Clin Pathol. 2019;72(4):401-402.

45. Quijada-Alamo M, Deshpande P, et al. MYD88 mutant lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia has distinct clinical and pathological features as compared to its mutation negative counterpart. Leukemia. 2015;29(2):402-403.

46. Rigolin GM, Saccenti E, Bassi C, et al. MYD88 somatic mutation status does not impact overall survival in Waldenstrom macroglobulinemia. Blood. 2014;123(18):2791-2796.

47. Staiger AM, Ott MA, Vettauer S, et al. Allele-specific PCR is a powerful tool for the detection of the MYD88 L265F mutation in diffuse large B-cell lymphoma and deciliated bone marrow samples. Br J Haematol. 2015;171(1):145-148.

48. Harmaledh F, MacNamara SP, Aguilera NS, Svardblow SH, Cook JR. MYD88 L265F mutation analysis helps distinguish lymphoplasmacytic lymphoma. Mod Pathol. 2015;28(4):564-574.

49. King RL, Consalves WI, Amsil SM, et al. Lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia with/without MYD88 paraprotein shows clinical and pathologic heterogeneity and may harbor MYD88 L265F mutations. Am J Clin Pathol. 2016;145(6):845-851.

50. Varettoni M, Zibilini S, Boveri E, et al. A risk-stratification model based on the initial concentration of the serum monoclonal protein and MYD88 mutation status identifies a subset of patients with IgM monoclonal gammapathy of undetermined significance at high risk of progression to Waldenstrom macroglobulinemia or other lymphoproliferative disorders. Br J Haematol. 2019 Jul 5. [Epub ahead of print].

51. Angelova EA, Li S, Wang W, et al. IgM plasma cell myeloma in the era of novel therapy: a clinicopathological study of 17 cases. Hum Pathol. 2019;84:231-334.

52. Li ZM, Rinaldi A, Cavalli A, et al. MYD88 somatic mutations in MALT lymphomas. Br J Haematol. 2012;159(5):662-664.

53. Moody S, Escudero-Ballester J, Wei M, et al. Significant association between TNFαF3 inactivation and biased immunoglobulin heavy chain variable region 4-3-4 use in mucosa-associated lymphoid tissue lymphoma. J Pathol. 2017;243(1):3-8.
Clinical relevance of MYD88 mutations in B-NHL

66. Gurth M, Bernard V, Bernd HW, Schemoene J, Thiriet C. Clinical and molecular findings in B-cell lymphomas with MYD88 mutation status analyses of CD79A, CD79B, and MYD88 reveal no specific recurrent lesions. Leuk Lymphoma. 2017;58(9):167-177.

67. Hsu SS, Meissner A, Chavez EA, et al. Assessment of capture and amplicon-based approaches for the development of a targeted next-generation sequencing pipeline to personalize lymphoma management. J Mol Diagn. 2018;20(2):203-214.

68. Okosun J, Bodor C, Wang J, et al. Expression of MYD88, CARD11, and CD79B mutations in B-cell lymphomas: a retrospective cohort study. Eur J Immunol. 2017;47(11):3445-3455.

69. Setsuo H, Takahashi T, Yoshinaga K, et al. Clinical relevance of protein expression, clinical factors, and GCB in extranodal DLBCL is not reflected in mutation patterns. Am J Hematol. 2018;93(2):235-241.

70. Hallas C, Freulekshas M, Tiernan M. Immunohistochemical distinction of ABC and GCB in extranodal DLBCL is not reflected in mutation patterns. Leuk Res. 2017;65:19-25.

71. Rahbari A, Tanaka Y, Kim J, et al. Genetic landscape of hepatitis B virus-associated diffuse large B-cell lymphoma. Blood. 2018;132(7):999-1008.

72. Nyangeri N, White MD, Gill CM, et al. MYD88 L265P mutation and CDKN2A loss are early mutational events in primary central nervous system diffuse large B-cell lymphoma. Blood. 2019;133(11):2759-2769.

73. Sethi TK, Kovach AE, Grover NS, et al. Frequent structural variations involving protein expression, clinical factors, and GCB in extranodal DLBCL is not reflected in mutation patterns. Leuk Res. 2018;65:19-25.
113. Yu S, Luo H, Fan M, et al. High frequency and prognostic value of MYD88 L265P mutation in diffuse large B-cell lymphoma with R-CHOP treatment. Oncol Lett. 2018;15(2):1707-1715.

114. Lee YS, Liu J, Frisano KA, et al. Lack of a Prognostic impact of the MYD88 L265P mutation for diffuse large B cell lymphoma patients undergoing autologous stem cell transplantation. Blood Marrow Transplant. 2017;23(12):2199-2204.

115. Parry M, Rose-Zerilli MJ, Lungstrom V, et al. Genetics and prognostication in splenic marginal zone lymphoma: revelations from deep sequencing. Clin Cancer Res. 2015;21(18):4174-4183.

116. Gertz MA. Waldenstrom macroglobulinaemia: 2019 update on diagnosis, risk stratification, and management. Am J Hematol. 2019;94(2):266-276.

117. Treon SP, Gustine J, Xu L, et al. MYD88 wild-type Waldenstrom macroglobulinemia: differential diagnosis, risk of histological transformation, and overall survival. Br J Haematol. 2018;180(3):374-380.

118. Hunter ZR, Xu L, Tsakmaklis N, et al. Insights into the genomic landscape of MYD88 wild-type Waldenstrom macroglobulinemia. Blood Adv. 2018;2(21):2937-2946.

119. Weber ANR, Cardona Gloria Y, Cinar O, Reinhardt HC, Pezzutto A, Wolz OO. Oncogenic MYD88 mutations in lymphoma: novel insights and therapeutic possibilities. Cancer Immunol Immunother. 2019;68(7):2073-2089.

120. Hattori K, Sakata-Yanagimoto M, Kubakabe M, et al. Genetic evidence implies that primary and relapsed tumors arise from common precursor cells in primary central nervous system lymphoma. Cancer Sci. 2019;110(1):401-407.

121. Camus V, Sarafan-Vasseur N, Boher S, et al. Digital PCR for quantification of recurrent and potentially actionable somatic mutations in circulating free DNA from patients with diffuse large B-cell lymphoma. Leuk Lymphoma. 2016;57(9):2171-2179.

122. Shen J, Liu X, Munshi M, et al. BTK(Cys481Ser) drives ibrutinib resistance via ERK1/2 and protects BTK(wild-type) MYD88-mutated cells by a paracrine mechanism. Blood. 2018;131(18):2047-2059.

123. Woyach JA, Furman RR, Liu TM, et al. Resistance mechanisms for the Bruton tyrosine kinase inhibitor ibrutinib. N Engl J Med. 2014;370(24):2286-2294.

124. Cao Y, Hunter ZR, Liu X, et al. CXCR4 WHIM-like frameshift and nonsense mutations promote ibrutinib resistance but do not supplant MYD88(L265P)-directed survival signaling in Waldenstrom macroglobulinemia cells. Br J Haematol. 2015;168(5):701-707.

125. Byrd JC, Harrington B, O’Brien S, et al. Acalabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. N Engl J Med. 2016;374(4):323-332.

126. Wang M, Rule S, Zinzani PL, et al. Acalabrutinib in relapsed or refractory mantle cell lymphoma (ACE-LY-004): a single-arm, multicentre, phase 2 trial. Lancet. 2018;391(10121):659-667.

127. Trotman J, Opat S, Marlton P, et al. Bruton’s tyrosine kinase (Btk) inhibitor Bgb-3111 demonstrates high very good partial response (VGPR) rate in patients with Waldenstrom macroglobulinemia (Wm). Hematol Oncol. 2017;35(5):70-71.

128. Ghobrial IM, Witzig TE, Gertz M, et al. Long-term results of the phase II trial of the oral mTOR inhibitor everolimus (RAD001) in relapsed or refractory Waldenstrom macroglobulinemia. Am J Hematol. 2014;89(3):237-242.

129. Forero-Torres A, Ramchandren R, Yacoub A, et al. Acalabrutinib in relapsed or refractory B-cell malignancies. Cancer Discov. 2017;7(9):1018-1029.

130. He Y, Li J, Ding N, et al. Combination of enzastaurin and ibrutinib synergistically induces anti-tumor effects in diffuse large B cell lymphoma. J Exp Clin Cancer Res. 2019;38(1):86.

131. Liu X, Hunter ZR, Xu L, et al. Targeting myddosome assembly in Waldenstrom macroglobulinemia. Br J Haematol. 2017;177(5):808-815.

132. Nelde A, Walz JS, Kowalewski DJ, et al. MYD88 wild-type Waldenstrom macroglobulinemia cells: novel insights and therapeutic possibilities. Expert Rev Mol Med. 2017;6(7):e132184.

133. Ciu H, Tong S, Xu L, et al. MYD88 L265P mutation promoted malignant B cell resistance against T cell-mediated cytotoxicity via upregulating the IL-10/STAT3 cascade. Int Immunopharmacol. 2018;64:394-400.

134. Camus V, Jardin F, Tilly H. The value of liquid biopsy in diagnosis and monitoring of diffuse large B-cell lymphoma: recent developments and future potential. Expert Rev Mol Diagn. 2017;17(6):557-566.

135. Scherer F, Kurtz DM, Newman AM, et al. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. Sci Transl Med. 2015;7(304):304ra155.

136. Khatron K, Sakata-Yazagimoto M, Kusakabe M, et al. Genetic evidence implies that primary and relapsed tumors arise from common precursor cells in primary central nervous system lymphoma. Cancer Sci. 2019;110(1):401-407.

137. Camus V, Sarafan-Vasseur N, Boher E, et al. Digital PCR for quantification of recurrent and potentially actionable somatic mutations in circulating free DNA from patients with diffuse large B-cell lymphoma. Leuk Lymphoma. 2016;57(9):2171-2179.

138. Boher E, Vially FJ, Becker S, et al. Non-invasive monitoring of diffuse large B-cell lymphoma by cell-free DNA high-throughput targeted sequencing: analysis of a prospective cohort. Blood Cancer J. 2018;8(8):74.

139. Kurtz DM, Scherer F, Jin MC, et al. Circulating tumor DNA measurements as early outcome predictors in diffuse large B-cell lymphoma. J Clin Oncol. 2018;36(28):2845-2853.

140. Witzig TE, Reeder CB, LaPlant BR, et al. A phase II trial of the oral mTOR inhibitor everolimus in relapsed aggressive lymphoma. Leukemia. 2011;25(2):341-347.