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A South African perspective on *MYD88* and *CXCR4* variants in lymphoplasmacytic lymphoma or Waldenström macroglobulinaemia

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Background: Lymphoplasmacytic lymphoma (LPL) is a small mature B-cell neoplasm that primarily involves the bone marrow. When associated with an IgM paraprotein, it is referred to as Waldenström macroglobulinaemia (WM). Variants in the *MYD88* and *CXCR4* genes are among the most common genetic alterations in LPL.

Aim: To determine *MYD88* and *CXCR4* variant prevalence in South African LPL cases.

Setting: National Health Laboratory Service, Charlotte Maxeke Johannesburg Academic Hospital.

Methods: Samples were obtained from newly diagnosed, treatment-naïve patients with LPL or WM. $MYD88^{L256P}$ variants were detected using allele-specific oligonucleotide polymerase chain reaction (ASO-PCR), and *CXCR4* variants through Sanger sequencing. Clinical and laboratory data were compiled into a database, with statistical analyses performed using GraphPad Prism.

Results: Twenty cases of LPL (90% WM) were diagnosed over a 7-year period. The median age of the cohort was 69 years (interquartile range [IQR]: 63–75) with a male predominance. Allele-specific oligonucleotide polymerase chain reaction confirmed $MYD88^{L265P}$ in 80%, and Sanger sequencing confirmed *CXCR4* variants in 45%. The group with concurrent *MYD88* and *CXCR4* variants showed the highest risk stratification scores and the most severe cytopenias. The median overall survival for the cohort was 11.5 months (IQR: 1.9–20.8), with no clear survival difference between *CXCR4*-mutated and wild-type cases. Survivors tended to be younger and to have lower baseline serum paraprotein levels at presentation.

Conclusion: In the South African public healthcare setting, *CXCR4* variants are frequent in LPL or WM with high-risk profiles, most common among patients harbouring both *MYD88* and *CXCR4* variants.

Contribution: This study presents the first South African description of LPL genetics.

Keywords: lymphoplasmacytic lymphoma; Waldenström macroglobulinaemia; South Africa; *MYD88*; *CXCR4*; polymerase chain reaction; genetics.

Introduction

Background

Lymphoplasmacytic lymphoma (LPL) is a small mature B-cell lymphoproliferative neoplasm that is composed of small B-lymphocytes with a variable admixture of plasmacytoid lymphocytes and plasma cells. Bone marrow involvement is typical with infiltration of extramedullary sites, such as lymph nodes or extranodal tissue, also possible. Lymphoplasmacytic lymphoma involving the bone marrow with an IgM serum paraprotein (of any concentration) is referred to as Waldenström macroglobulinaemia (WM). Waldenström macroglobulinaemia accounts for 95% of LPL cases, while LPL with IgG and IgA paraproteins comprises the remainder.[1]

Lymphoplasmacytic lymphoma or Waldenström macroglobulinaemia is a neoplasm of the elderly, and, while incurable, it typically shows an indolent clinical course, with a 10-year relative survival of 66%, which is higher in those younger than 50 years of age (10-year overall survival [OS] of 86%).[2] Mortality may result from both disease-related and unrelated causes, with non-cancer causes being the most common among patients older than 65 years at diagnosis.[3,4]

While the cause of LPL or WM is unknown, a strong familial association has been observed, with ~18.7% of patients having a family member with a known history of WM or another B-cell neoplasm.[5]

Distinguishing LPL or WM from other low-grade B-cell non-Hodgkin lymphoma (NHL) was originally challenging due to the absence of a specific immunophenotype or genetic aberrations.[6] In 2012, using whole genome sequencing, a recurrent T>C mutation at position 3p.22.2 in exon 5 of the myeloid differentiation factor 88 gene (*MYD88*), causing an amino acid substitution of leucine at position 265 of the coding region into a proline (L265P), was identified in samples from patients with LPL. This somatic mutation was subsequently shown to occur in ~90% of WM cases.[7] While *MYD88*^{L265P} is predominant in LPL or WM, it can also be found at a reduced frequency in other B-cell NHLs, including diffuse large B-cell lymphoma (1%), chronic lymphocytic leukaemia (3.2%), splenic marginal zone lymphoma (7%) and mucosa-associated lymphoid tissue lymphoma (9%). It is notably absent in nodal marginal zone lymphoma and multiple myeloma.[8]

MYD88 is comprised of three domains: a death domain (DD) at the N-terminal, an intermediate domain (INT) and a Toll or interleukin-1 receptor (TIR) domain at the C-terminal.[9] *MYD88* is a cytosolic adaptor protein involved in Toll-like receptor (TLR) as well as interleukin (IL) 1 and IL18 signalling.[10,11] Stimulation of *MYD88* by TLRs occurs through homophilic binding of the TIR domains of both the adaptor protein and the TLR. Activated *MYD88* recruits IL1 receptor-associated kinase 1 (IRAK1) and IRAK4, as well as Bruton's tyrosine kinase (BTK), which leads to activation of nuclear factor kappa B (NF-ĸB).[12] Inhibition of the *MYD88* signalling pathway blocks the normal translocation of NF-ĸB into the nucleus in cells harbouring *MYD88*^{L265P}.[7,13] The L265P mutation increases the activity of the TIR domain relative to the unaffected protein, with enhanced growth and survival of lymphoma cells via increased NF-ĸB activity, Janus kinase (JAK) or signal transducer and activator of transcription (STAT) signalling, and the production of pro-inflammatory cytokines.[12,14] The detection of the L265P mutation in approximately half of cases of IgM monoclonal gammopathy of undetermined significance (MGUS) indicates that this alteration occurs early in the oncogenic process and that additional genetic events are necessary for progression to LPL or WM.[15,16]

In 2014, a second gene, C-X-C chemokine receptor type 4 (*CXCR4*), was found to be recurrently mutated in LPL or WM.[17] Previously reported in the congenital autosomal dominant immunodeficiency disorder, WHIM (warts, hypogammaglobulinaemia, infection and myelokathexis), as a germline mutation, this was the first report of *CXCR4* variants in a neoplasm.[17,18] Present in about 30% to 40% of WM cases, *CXCR4* variants occur almost exclusively with *MYD88* variants, and are predominantly nonsense and frameshift mutations.[19,20] They are located in the C-terminal regulatory cytosolic domain (amino acids 308 to 352) and, unlike *MYD88*, multiple *CXCR4* variants can be present, either in separate clones or in a compound heterozygote state.[21] Nonsense mutations typically involve a C>G substitution at nucleotide 1013, resulting in a stop codon at amino acid 338, whereas frameshift mutations exhibit greater variability.[22] *CXCR4* is a G-protein-coupled receptor that interacts with its ligand, the stromal cell–derived factor-1 (CXCL12/SDF-1), to promote lymphopoiesis, cell migration and adhesion.[23] C-terminal mutations in the *CXCR4* gene lead to the loss of key regulatory serine residues, while the structural domains required for ligand binding and downstream G-protein signalling are preserved. This dysregulation impairs receptor internalisation and desensitisation, leading to sustained *CXCR4* signalling, ultimately altering cellular migration and adhesion.[22,23,24]

The 5th edition of the World Health Organization (WHO) classification system underscores the importance of laboratory testing for *MYD88*^{L265P} and somatic *CXCR4* mutations in LPL or WM. While not mandated, testing is considered desirable, as it may aid in differentiating B-cell NHL subtypes and further inform prognosis and therapeutic decisions, including the selection of targeted therapies.[1] Specifically, *CXCR4* mutations are associated with resistance to BTK inhibitors (BTKis) and a more aggressive clinical phenotype.[19,20,25]

In the South African public sector, *MYD88*^{L256P} testing is available for the work-up of suspected LPL or WM cases; however, *CXCR4* variant analysis is not currently included in routine screening. This study aimed to assess the clinical characteristics and molecular profile of *MYD88* and *CXCR4* variants in a cohort of LPL or WM patients at a single laboratory testing centre in South Africa.

Methods

Study design

This study evaluated patient samples referred for *MYD88* exon 5 variant testing between February 2019 and December 2024 to the Somatic Cell Genetics Unit (SCGU) in the Department of Molecular Medicine and Haematology, National Health Laboratory Service (NHLS), and Charlotte Maxeke Johannesburg Academic Hospital (CMJAH), Gauteng, South Africa.

Samples included presentation samples from untreated patients with a suspected diagnosis of LPL or WM, or where molecular testing was used to differentiate LPL or WM from other small mature B-cell NHLs. Clinical details (age, sex, clinical presentation) and laboratory results (full blood and differential count, serum protein electrophoresis, serum free light chains, haemolytic work-up, lactate dehydrogenase [LDH], flow cytometry, bone marrow aspirate and trephine biopsy, cytogenetics, fluorescence in-situ hybridisation [FISH]) were obtained from LIS (TrakCare, InterSystems, Cambridge, MA, United States). These results were collated in a database, and cases of LPL or WM were identified.

Risk was assessed using the simplified stratification model by Zanwar et al.,[26] which allocates points for albumin <3.5 g/dL (1 point), age 66–75 years (1 point), age >75 years (2 points) and LDH above the upper limit of normal (2 points). The composite score categorises patients into four risk groups: low, low-intermediate, intermediate and high, with corresponding 5-year OS estimates of 93%, 90%, 75% and 57%, respectively.[26]

Nucleic acid extraction and quality assessment

Peripheral blood, bone marrow aspirate samples or trephine biopsies were referred for *MYD88* genetic testing from several provinces across South Africa, including Mpumalanga, the Western Cape, Free State, Eastern Cape and Gauteng, in either BD Vacutainer® Ethylenediaminetetraacetic acid (EDTA) tubes or in PAXgene® blood RNA tubes (BD Sciences, Franklin Lakes, NJ, United States). Total nucleic acid (RNA and DNA) was extracted from PAXgene® tubes and purified using the Chemagic™ 360 instrument (PerkinElmer, Waltham, MA, United States). Blood samples in EDTA tubes and bone marrow aspirates were extracted using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany), while bone marrow smears were re-suspended in phosphate buffer saline (200 µL) before extraction. Trephine biopsy specimens were extracted using the DNeasy Blood and Tissue Kit (Qiagen, Venlo, Netherlands). Residual DNA samples were used for the *CXCR4* assay.

Polymerase chain reaction and sequencing

MYD88^{L256P} variants were detected using real-time allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) on a real-time PCR system (Thermo Fisher Scientific, Waltham, MA, United States) with wild-type and mutant-specific reverse primers, and a shared forward primer, generating a 142-base pair (bp) amplicon.[27] Results were analysed using the real-time PCR systems software (Thermo Fisher Scientific, Waltham, MA, United States).

CXCR4 variants were detected using conventional PCR with exon 2-specific primers, under standard cycling parameters. Amplicons were visualised on 2% agarose gels, purified (BioSpin Kit, Bioflux, Gentech Biosciences, Colombia), quantified (Nanodrop one, Thermo Fisher Scientific, Waltham, MA, United States) and diluted to 20 ng/µL. Sanger sequencing was performed using the same primers and Big Dye Terminator v3.1, with products purified in 70% isopropanol (Merck, Darmstadt, Germany), re-suspended in Hi-Di™ Formamide and run on an ABI 3730 DNA Analyser (Thermo Fisher Scientific, Waltham, MA, United States). Variant calling used the *CXCR4* reference transcript (NM_003467). A detailed description of the methodology is available in Appendix 1.

Statistical analysis

Statistical analyses were conducted using Prism software, version 5 (GraphPad Software, San Diego, CA, United States). Skewed numerical data were presented as medians with interquartile ranges (IQR), while categorical variables were expressed as frequencies and percentages. The two-sample Wilcoxon rank-sum (Mann–Whitney) and Kruskal–Wallis tests were employed to compare skewed numerical data, and Chi-squared and Fisher's exact tests were used to analyse categorical variables.Crude survival analysis was based on the duration for which patients had laboratory investigations recorded in the LIS at a tertiary referral centre, which was used here as a proxy for survival. Patients with markedly abnormal microbiological, biochemical or haematological results who subsequently had no further laboratory data were presumed to have died. In contrast, those with normal or mildly abnormal results who had no further testing were considered lost to follow-up. Laboratory records were reviewed through 29 May 2025 to determine crude OS.

Ethical considerations

Ethics clearance was obtained from the University of the Witwatersrand Human Ethics Committee (protocol number: M1911201). Patient consent was not required. Patient and laboratory information were obtained from the laboratory information system (LIS) (Trakcare, Intersystems, Cambridge, MA, United States). Patient identifying information was stored in a password-protected Excel spreadsheet, with unique study numbers linking to patient results.

Results

The cohort included 20 cases of newly diagnosed LPL for which *MYD88* (exon 5) was confirmed via ASO-PCR, and sequencing of *CXCR4* C-terminal regulatory cytosolic domain (exon 2) was performed by Sanger sequencing. The median age of the cohort was 69 years (IQR: 63–75) with a 70% male predominance (n = 14).

Serum protein electrophoresis and immune-fixation were performed in 19 cases. The criteria for WM were met in 90% (17 or 19), with a median baseline IgM paraprotein level of 27.5 g/L (IQR: 11.25–44.75), and kappa light chain restriction in 82% (n=14). The complication of autoimmune haemolytic anaemia was biochemically confirmed in two of the eight patients tested. The remaining two cases that did not meet the criteria for WM showed an isolated free kappa light chain in one, while the second showed no evidence of a paraprotein.

Allele-specific oligonucleotide polymerase chain reaction confirmed the *MYD88*^{L265P} point mutation in 80% of cases (16 or 20). *CXCR4* variants were detected in 45% (n = 9) and were consistently present in conjunction with a *MYD88* variant. There were nearly equal numbers of frameshift and nonsense *CXCR4* variants, with Table 1 providing a detailed breakdown of each pathogenic variant. A diffuse pattern of tumour infiltration was observed in all histological specimens harbouring *CXCR4* nonsense mutations (n = 4), whereas this pattern was present in only two-thirds of cases with *CXCR4* frameshift mutations (n = 3). The sample size was too small to reliably assess correlations among mutation status, mutation type and clinical outcomes.

The pertinent demographic, laboratory and survival data for the different mutation subcategories are summarised in Table 2. The majority of cases were *MYD88* and *CXCR4* mutated (*MYD88*^{MUT} *CXCR4*^{MUT}) (45%), with around one-third *MYD88*^{MUT} *CXCR4* wild-type$^{(WT)}$ and the minority *MYD88*^{WT} *CXCR4*^{WT} (20%). The lowest peripheral blood counts were evident in the *MYD88*^{MUT} *CXCR4*^{MUT} group, which also showed the highest male predominance. This subgroup was characterised by a predominantly diffuse pattern of infiltration in nearly 80% of the histological specimens. Serum paraprotein levels were higher in the *MYD88*^{MUT} group as compared with the *MYD88*^{WT} (p = 0.26), and lower in those who were alive at the time of the study as compared to those who demised; median 21 g/L (IQR: 3–28) versus 39.5 g/L (IQR: 26.75 to 46.25) (p = 0.66). Beta-2 microglobulin (B2M) testing was not routinely performed at diagnosis (it was available for six patients). Applying the simplified risk stratification score proposed by Zanwar et al.,[26] high and intermediate-risk scores were predominant among the *MYD88*^{MUT} groups, with the *MYD88*^{MUT} *CXCR4*^{MUT} subgroup showing the highest proportion of high-risk scores.

TABLE 1: Pathogenic variants identified in the C-terminal regulatory cytosolic domain region of the *CXCR4* gene.

n	*CXCR4* mutation type	Nucleotide change	Amino acid
4	Nonsense	c.1013C > G	p.S338*
1	Frameshift	c.953_954insA	p.T318Tfs*26
2	Frameshift	c.977_978insC	p.L326Lfs*18
1	Frameshift	c.1021_1022insT	p.S341Ffs*2
1	Frameshift	c.1032_1033insC	p.S346Lfs*10

n, number of patients with the same variant.

The crude median OS for the entire cohort was 11.5 months (IQR: 1.9–20.8). Among patients who survived, the median OS was 15 months (IQR: 13–33.9), compared with 3.1 months (IQR: 1.1–13.3) among those who died (p = 0.06). The terminal blood results from patients who died showed a range of abnormalities, including severe pancytopenia or bicytopenia, coagulopathy, elevated markers of infection and renal and/or liver dysfunction. Survivors tended to be younger, with a median age of 63 years (IQR: 55–75) compared with 70 years (IQR: 68–73) among non-survivors, but this difference was not statistically significant (p = 0.52). These findings suggest a trend towards improved survival in younger patients, although the sample size limited the power to detect statistically significant differences. A total of five patients were lost to follow-up at a median of 13.5 months (IQR: 6.5–18.3). This group had a median age of 72 years (IQR: 62–82) and was predominantly male (n = 4, 80%).

Discussion

The United States Surveillance, Epidemiology, and End Results (SEER) Programme, which has been conducted over 20 years, reported that WM accounted for 1.9% of all NHL cases.[28] These statistics are unknown in the South African setting due to both a lack of data and the likely under-recognition of this entity. The SEER data further showed that WM was a disease that primarily affects those of advancing age, with a median age at diagnosis of 73 years and a male predominance of 60%.[28] The age of our South African cohort was comparable to SEER, although it included a higher proportion of male patients. The basis for the observed male

TABLE 2: Patient demographic, laboratory and survival data.

Variable	*MYD88*^{WT} *CXCR4*^{WT} (n = 4)				*MYD88*^{MUT} *CXCR4*^{WT} (n = 7)				*MYD88*^{MUT} *CXCR4*^{MUT} (n = 9)				p-value*
	Median	IQR	*n*	%	Median	IQR	*n*	%	Median	IQR	*n*	%	
Age (years)	64	56–69	-	-	70	69–74	-	-	66	63–77	-	-	0.44
Sex	-	-	-	-	-	-	-	-	-	-	-	-	0.65
Male	-	-	3	75	-	-	4	57	-	-	7	78	-
Female	-	-	1	25	-	-	3	43	-	-	2	22	-
WCC (× 10^9/L)	11.49	9.51–14.33	-	-	6.96	5.87–9.85	-	-	5.74	4.82–8.99	-	-	0.19
ANC (× 10^9/L)	4.22	3.15–5.34	-	-	3.14	3.00–3.50	-	-	1.86	1.68–3.47	-	-	0.13
Haemoglobin (g/L)	8.6	6.9–10.5	-	-	8.6	5.7–9.3	-	-	6.3	5.4–7.6	-	-	0.30
Platelet count (× 10^9/L)	247	187–425	-	-	162	120–325	-	-	119	80–225	-	-	0.26
Paraprotein level (g/L)	21	2.5–50	-	-	27	23.5–46.5	-	-	26.5	2.75–40.25	-	-	0.82
Histological pattern of infiltration	-	-	3	-	-	-	7	-	-	-	9	-	0.05†
Nodular	-	-	3	100	-	-	4	57	-	-	2	22	-
Diffuse	-	-	0	-	-	-	3	43	-	-	7	78	-
Risk scoring	-	-	3	-	-	-	7	-	-	-	8	-	0.42
Low	-	-	0	67	-	-	0	-	-	-	1	13	-
Low or Intermediate	-	-	2	3	-	-	1	14	-	-	1	13	-
Intermediate	-	-	0	-	-	-	3	43	-	-	2	25	-
High	-	-	1	3	-	-	3	43	-	-	4	50	-
Outcomes	-	-	-	-	-	-	-	-	-	-	-	-	0.94
Alive	-	-	1	25	-	-	1	14	-	-	3	33	-
Demised	-	-	2	50	-	-	4	57	-	-	4	44	-
LTF	-	-	1	25	-	-	2	29	-	-	2	22	-

ANC, absolute neutrophil count; *CXCR4*^{WT}, *CXCR4* wild-type; *CXCR4*^{MUT}, *CXCR4* mutated; IQR, interquartile range; LFT, lost to follow-up; *MYD88*^{MUT}, *MYD88* mutated; *MYD88*^{WT}, *MYD88* wild-type; WCC, white cell count.

*, Kruskal–Wallis test applied for skewed numerical data and Chi-squared for categorical data.

†, statistically significant.

predominance in LPL or WM remains unclear. Still, it may be attributable to intrinsic sex-specific differences in the immune response, hormone levels, occupational and environmental exposures, or genetic susceptibility.[29]

The principal genetic alteration in LPL or WM is a somatic mutation in the *MYD88* gene, which is almost exclusively the *MYD88*^{L625P} variant.[1] Our study revealed a slightly lower proportion of *MYD88*^{L625P} mutations as compared to the international literature, which may be attributed to samples with haemodilution or low tumour infiltration, leading to false negative results, or cases misclassified as LPL or WM.[27] In *MYD88*^{MUT} LPL or WM, secondary co-operating genetic events are necessary for the progression to overt clinical disease. Among the most common are the mutually exclusive recurrent cytogenetic abnormality, del(6q), which is reported in around 40–50% of cases[30], and sub-clonal C-terminal *CXCR4* mutations, reported in 30–40% of cases.[19,20] While data on the cytogenetic profile of our cohort were limited, the proportion of *CXCR4* mutations was slightly higher than reported in the literature.[19,20]

The *MYD88* and *CXCR4* variant status is linked with differences in clinical presentation, therapeutic response and disease outcomes.[19,31] *MYD88*^{WT} occurs in older patient groups and is more commonly accompanied by lymphadenopathy. While it is associated with a lower burden of bone marrow disease and serum IgM levels, it is linked to higher levels of poor prognostic markers (such as B2M), inferior OS, increased risk of death and inferior response to ibrutinib monotherapy (no major responses [MR] and shorter progression-free survival [PFS]).[19,32] In contrast, patients with *MYD88*^{MUT} *CXCR4*^{WT} demonstrated deeper responses, shorter time to MR and improved PFS with ibrutinib therapy.[31] More than 40 distinct *CXCR4* variants have been identified in LPL or WM, with both the mutation type and burden of *CXCR4* clone shaping the clinical phenotype and behaviour.[19,33] In C-terminal *CXCR4* nonsense$^{(NS)}$ versus *CXCR4*^{WT}, enhanced and prolonged AKT (protein kinase B) and BTK signalling follows activation of the *CXCR4* receptor by SDF-1α.[22,34] This enhanced signalling is explained by the loss of essential regulatory regions in the distal region of the *CXCR4* C-terminus, including key serine residues required for the attenuation of SDF-1α mediated *CXCR4* signalling. This attenuation is typically mediated by β1 and β2 arrestin proteins, which assemble only when the serine residues are phosphorylated.[35,36] As demonstrated in *CXCR4*^{S338*} (c.1013C>G), this enhanced signalling leads to upregulation of genes associated with invasiveness, anti-apoptosis and drug resistance, thereby driving tumour proliferation, organ infiltration and drug resistance. Although translating into a shorter time to treatment, resistance to certain small molecule inhibitors, such as ibrutinib, OS remains unaffected by *CXCR4*^{MUT}.[31,34,37,38] Further, differences exist between *MYD88*^{L265P} with *CXCR4*^{NS} versus frameshift$^{(FS)}$ C-terminal mutations, the former (when present in ≥25% of tumour cells)[25] associated with a higher bone marrow disease burden, elevated serum IgM levels, resulting in a higher incidence of symptomatic hyperviscosity requiring therapeutic intervention, and inferior response to ibrutinib therapy (worse MR, shorter PFS compared with *CXCR4*^{WT}).[19,20,25] The association of mutation status with extent of bone marrow infiltration was demonstrated in our cohort, where all cases with *CXCR4* nonsense mutations demonstrated a diffuse infiltration pattern on histology. The reason for this difference in behaviour, based on mutation type, has been explained in cellular-level studies. Frameshift mutations were shown to alter the terminal amino acid sequence, resulting in a truncated *CXCR4* protein with altered functional properties. In contrast, nonsense mutations triggered the messenger ribonucleic acid (mRNA) decay pathway, leading to markedly reduced levels of the truncated *CXCR4* protein, thereby impairing *CXCR4* receptor internalisation and signalling termination.[39]

Risk stratification in WM is most commonly performed using the International Prognostic Scoring System for WM (IPSSWM), introduced by Morel et al.[40] In our South African public healthcare setting, however, the applicability is limited by inconsistent B2M testing. To address this, a simplified risk stratification model proposed by Zanwar and colleagues[26] was applied to better identify high-risk patients using more accessible clinical and laboratory parameters.[26] In our cohort, intermediate to high risk scores predominated among patients with *MYD88* variants, with nearly half of those in the *MYD88*^{MUT} *CXCR4*^{MUT} subgroup classified as high risk. This group also exhibited other adverse features, including high IgM levels, diffuse bone marrow infiltration and the most pronounced cytopenias. Despite these high-risk features, no significant difference in OS was observed compared with other mutational subgroups, likely due to the cohort's limited size.

Therapeutic intervention is not mandatory at the time of diagnosis of LPL or WM; the decision to initiate treatment remains a clinical judgement guided by disease-related symptoms.[3] Recommended first-line therapeutic agents include rituximab (an anti-CD20 monoclonal human antibody), bendamustine (an alkylating chemotherapeutic agent) and BTKis. The class of drug and combination of agents depend, among other factors, on the specific disease-related complication; mutation status (*MYD88*, *CXCR4*, and *TP53*); and the availability of treatment options.[3] Rituximab monotherapy yields lower response rates in LPL or WM compared to combination regimens, except when used to manage IgM-related complications. However, it should be avoided in the setting of elevated IgM levels or symptomatic hyperviscosity owing to the risk of rituximab-induced IgM flare.[3,41,42] For other disease-related complications, either chemo-immunotherapy (i.e., bendamustine–rituximab), continuous BTKi monotherapy (e.g., zanubrutinib or ibrutinib) or proteasome inhibitor-based regimens are utilised.[3,41,43,44]

Regarding BTKi monotherapy, zanubrutinib has shown superior outcomes compared with ibrutinib in *MYD88*^{NS}

CXCR4^{MUT} (improved MR and PFS) and *TP53*^{MUT} (improved MR, complete response, PFS).[31,45] For patients who develop resistance to first- and second-generation BTKi, newer alternatives, such as pirtobrutinib (a reversible BTKi) or venetoclax (a BCL-2 inhibitor), offer additional therapeutic options.[43] It is clear that routine evaluation of *MYD88*, *CXCR4* mutation status, in patients requiring treatment is essential for guiding the selection of first-line therapy and to support efforts to expand access to BTKi and other targeted therapies in the South African public healthcare sector, where treatment options are largely restricted to rituximab (monotherapy or in combination with cyclophosphamide based regimens), with bendamustine availability inconsistent across the country.[43]

Testing methods have advanced since the *MYD88*^{L265P} was first identified, with low-sensitivity Sanger sequencing (15%–20%)[46] surpassed by ASO-PCR (limit of detection 1%), albeit limited in detecting non-L265P variants.[27] The move is now towards droplet digital PCR, which shows deeper sensitivity (able to detect VAF as low as 0.1%), overcoming the limitation of low-infiltrated samples.[47,48] While next-generation sequencing offers the advantage of multigene analysis and the detection of mutations of prognostic importance in other genes, its diagnostic utility is limited by relatively low sensitivity (≥5%), leading to false negative *MYD88* results in up to a third of bone marrow cases.[46,49] Importantly, the limitations of each testing method should be carefully considered when selecting a testing platform and interpreting the results.

Conclusion

In the South African public healthcare setting, *CXCR4* variants are frequent in LPL or WM with high risk profiles most common among patients harbouring both *MYD88* and *CXCR4* variants.

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Competing interests

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CRediT authorship contribution

Katherine Hodkinson: Conceptualisation, Data curation, Formal analysis, Methodology, Writing – original draft. Irene Ketseoglou: Methodology, Writing – original draft, Writing – review & editing. Hanri van Zijl: Formal analysis, Writing – review & editing. Jenifer Vaughan: Writing – review & editing. Ashleigh Walton: Conceptualisation, Methodology, Writing – original draft. All authors reviewed the article, contributed to the discussion of results, approved the final version for submission and publication, and take responsibility for the integrity of its findings.

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Data availability

The data that support the findings of this study are available from the corresponding author, Katherine Hodkinson, upon reasonable request.

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Appendix 1 starts on the next page →

Appendix 1

Detailed methodology

Nucleic acid extraction and quality assessment

Peripheral blood, bone marrow aspirate samples or trephine biopsies were referred in either BD Vacutainer® ethylenediaminetetraacetic acid (EDTA) tubes or in PAXgene® blood ribonucleic acid (RNA) tubes (BD Sciences, Franklin Lakes, NJ, United States). Total nucleic acid (RNA and DNA) was extracted from PAXgene® tubes and purified using the Chemagic™ 360 Instrument (PerkinElmer, Waltham, MA, United States) according to the manufacturer's instructions, with minor modifications. Namely, the PAXgene tubes were centrifuged, the pellets were washed with wash buffer and then lysed.

Bone marrow aspirates and blood samples in EDTA tubes were extracted using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. However, bone marrow smears were re-suspended in phosphate-buffered saline (200 µL), cells were scrapped off the slide and placed into a clean micro-centrifuge tube prior to extraction with the High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany). Trephines with the DNeasy Blood and Tissue Kit (Qiagen, Venlo, Netherlands). Samples were processed according to the manufacturer's recommendations. Residual DNA samples were used for the *CXCR4* mutation assay.

Polymerase chain reaction

MYD88^{L256P} was determined using real-time allele-specific oligonucleotide polymerase chain reaction (ASO-PCR).[27] Briefly, two different PCR reactions are performed, one being for the detection of the mutation using the mutated reverse primer and one for the detection of the wild-type using the reverse wild-type primer. Both reactions had a common forward primer.[27] This resulted in a 142-bp amplicon. Each reaction was carried out in a final volume of 20 µL, containing primers (forward and reverse wild-type or mutated), probe, TaqMan Universal PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, United States) and 20 ng of genomic DNA.

The PCR was performed in a real-time PCR system (Thermo Fisher Scientific, Waltham, MA, United States) and consisted of an initial step to remove any RNA contamination (UNG initiation) for 2 min at 50 °C. This was followed by a denaturation step of 10 min at 95 °C, followed by 50 cycles of 95 °C for 15 s and 60 °C for 60 s. Data were analysed using the real-time PCR systems software (Thermo Fisher Scientific, Waltham, MA, United States).

CXCR4 was detected using conventional PCR with a forward primer (5′-GCTGCCTTACTACATTGGGATCAGC-3′) and a reverse primer (5′-TTGGCCACAGGTCCTGCCTAGACA-3′). The cycling conditions consisted of an initial denaturation step of 94 °C for 7 min, followed by 35 cycles of 94 °C for 30 s, 64 °C for 30 s and 72 °C for 45 s. The final elongation step was performed at 72 °C for 7 min. The resulting amplicons were resolved on a 2% (w/v) agarose gel with an expected amplicon size of approximately 520 bp. Successfully amplified samples were purified using the BioSpin Purification kit (Bioflux, Gentech Biosciences, Colombia) according to the manufacturer's instructions. The purified samples were quantified using a NanoDrop One (Thermo Fisher Scientific, Waltham, MA, United States) and diluted to 20 ng/µL.

Sanger sequencing of *CXCR4*

CXCR4 variant status was determined with Sanger sequencing of a region of exon 2 of the *CXCR4* gene, including the C-terminal regulatory cytosolic domain, using 6.4 µL of forward primer (5′-GCTGCCTTACTACATTGGGATCAGC-3′) and 6.4 µL of reverse primer (5′-TTGGCCACAGGTCC TGCCTAGACA-3′), 3 µL of amplicon, 2 µL of Big Dye Terminator v3.1 Cycle Sequencing Kit, 4 µL of 5 × Sequencing buffer (Thermo Fisher Scientific, Waltham, MA, United States), in a final volume of 25 µL. Cycling conditions were performed as per the manufacturer's instructions.

Post-cycle sequencing, the amplicons were purified according to the manufacturer's instructions using 70% isopropanol (Merck, Darmstadt, Germany), air-dried and then re-suspended in 10 µL of Hi-Di™ Formamide (Thermo Fisher Scientific, Waltham, MA, United States).

An ABI 3730 DNA Analyser (Thermo Fisher Scientific, Waltham, MA, United States) was used to obtain the sequencing data. Sequence analysis was performed by comparing the sequences to the CXCR4 transcript (NM_003467) using Geneious Prime 2023.1.1 software (Biomatters Ltd., Auckland, New Zealand). Available at: https://www.geneious.com.