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MYD88 wild-type Waldenstrom Macroglobulinaemia: differential diagnosis, risk of histological transformation, and overall survival

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Summary

MYD88 mutations are present in 95% of Waldenstrom Macroglobulinaemia (WM) patients, and support diagnostic discrimination from other IgM-secreting B-cell malignancies. Diagnostic discrimination can be difficult among suspected wild-type *MYD88* (*MYD88*^{WT}) WM cases. We systematically reviewed the clinical, pathological and laboratory studies for 64 suspected *MYD88*^{WT} WM patients. World Health Organization and WM consensus guidelines were used to establish clinicopathological diagnosis. Up to 30% of suspected *MYD88*^{WT} WM cases had an alternative clinicopathological diagnosis, including IgM multiple myeloma. The estimated 10-year survival was 73% (95% confidence interval [CI] 52–86%) for *MYD88*^{WT} *versus* 90% (95% CI 82–95%) for mutated (*MYD88*^{MUT}) WM patients (Log-rank $P < 0{\cdot}001$). Multivariate analysis only showed *MYD88* mutation status ($P < 0{\cdot}001$) as a significant determinant for overall survival. Diffuse large B-cell lymphoma (DLBCL) was diagnosed in 7 (15·2%) and 2 (0·76%) of *MYD88*^{WT} and *MYD88*^{MUT} patients, respectively (Odds ratio 23·3; 95% CI 4·2–233·8; $P < 0{\cdot}001$). Overall survival was shorter among *MYD88*^{WT} patients with an associated DLBCL event (Log-rank $P = 0{\cdot}08$). The findings show that among suspected *MYD88*^{WT} WM cases, an alternative clinicopathological diagnosis is common and can impact clinical care. WM patients with *MYD88*^{WT} disease have a high incidence of associated DLBCL events and significantly shorter survival *versus* those with *MYD88*^{MUT} disease.

Keywords: Waldenström Macroglobulinaemia, IgM myeloma, transformation, MYD88, overall survival.

Next generation sequencing has identified somatic *MYD88* mutations in Waldenstrom Macroglobulinaemia (WM) that include L265P and non-L265P variants (Treon *et al*, 2012, 2015). Allele-specific polymerase chain reaction (AS-PCR) assays and Sanger sequencing methods have determined that *MYD88* mutations are present in 93–97% of WM patients (Jiménez *et al*, 2013; Poulain *et al*, 2013; Varettoni *et al*, 2013; Xu *et al*, 2013). *MYD88* mutations are activating, and provide growth and survival support through Bruton tyrosine kinase (BTK), Interleukin 1 receptor associated kinases 1 and 4 (IRAK1/IRAK4) and haematopoietic cell kinase (HCK) in WM cells (Yang *et al*, 2013, 2016). In contrast, little is known about the genomics and survival signalling in *MYD88* wild-type (*MYD88*^{WT}) WM. Transcriptome profiling shows heterogeneous gene expression in *MYD88*^{WT} cases that differ from *MYD88* mutated (*MYD88*^{Mut}) WM cases (Hunter *et al*, 2016). MYD88^{WT} patients show no major responses to ibrutinib, and increased risk of death *versus MYD88*^{MUT} patients (Treon *et al*, 2014, 2015). *CXCR4* mutations are also uncommon in *MYD88*^{WT} patients, but may be found in up to 40% of *MYD88*^{MUT} cases (Xu *et al*, 2016).

Molecular diagnostic testing for mutated *MYD88* is increasingly being used to distinguish WM from other IgM secreting malignancies. IgM myeloma (IgM MM) shows no *MYD88* mutations, while marginal zone lymphoma (MZL) and chronic lymphocytic leukaemia (CLL) express *MYD88* ^{MUT} but at very low frequencies (<10%). Despite this, diagnostic ambiguity often exists between *MYD88*^{WT} WM and

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other *MYD88*^{WT} IgM secreting entities. We therefore performed a systematic review of patients with suspected *MYD88*^{WT} WM and examined their clinical, pathological and laboratory studies to establish their underlying clinicopathological diagnosis using World Health Organization (WHO) and WM consensus guidelines (Owen *et al*, 2003; Swerdlow *et al*, 2008). As part of these efforts, we also examined the impact of *MYD88* mutation status on overall survival in WM patients. The findings reveal the importance of clarifying the underlying diagnosis in WM patients suspected of having *MYD88*^{WT} disease, and the recognition that WM patients with *bona fide MYD88*^{WT} disease have a higher risk of disease transformation and shorter overall survival *versus* those with *MYD88*^{MUT} disease.

Patients and methods

Sixty-four patients with suspected *MYD88*^{WT} WM were identified. All patients had a monoclonal IgM protein, and morphological and histopathological findings on a bone marrow (BM) biopsy that were suspicious for lymphoplasmacytic lymphoma. AS-PCR testing for *MYD88*^{L265P} mutation, and investigation for non-L265P *MYD88* mutations in negative cases by Sanger sequencing was performed in all these cases to confirm *MYD88*^{WT} status utilizing CD19-selected and unselected BM mononuclear cells (Xu *et al*, 2013). A systematic review of clinical, pathological, and laboratory studies was undertaken for all *MYD88*^{WT} cases, and further studies were obtained as warranted, which included additional immunohistochemistry, skeletal and computed tomography imaging, and *CXCR4* mutation determination (Xu *et al*, 2016). WHO and WM consensus guidelines (Owen *et al*, 2003; Swerdlow *et al*, 2008) were used to establish definitive diagnosis.

Data sets were evaluated by analysis of variance and non-parametric comparisons made by the Fisher's exact probability test. The survival from WM diagnosis, defined as the time between WM diagnosis to last follow-up or death, and the time from WM diagnosis to transformation to an aggressive lymphoma were estimated using the Kaplan–Meier method. Survival curves were compared using the log-rank test. For the survival estimates in WM patients who did or did not transform to an aggressive lymphoma, we used the Simon-Makuch method, and the Mantel–Byar test for comparison (stsplit in STATA). Follow-up time was estimated by reverse censoring. Univariate and multivariate survival analyses were performed using Cox proportional-hazard regression models. The outcome of interest is reported as hazard ratio (HR) with 95% confidence interval (CI). A P value $<0{\cdot}05$ was deemed to be significant. All calculations and graphs were obtained using STATA/SE 13.1 (StataCorp, College Station, TX).

Results

Table I shows the clinical characteristics at time of diagnosis for patients with suspected *MYD88*^{WT} WM who presented to our clinic. Forty-six (71·8%) patients, 48% of whom were male, fulfilled the WHO and WM consensus criteria for WM. Their median BM involvement was 37·5% (range 2·5–95%) with a predominately interstitial pattern of diffuse B-cells, lymphoplasmacytic cells and plasma cells. Flow cytometric analysis showed monotypic B-cells ($CD19^+CD20^+CD5^{+/-}CD10^-CD23^{+/-}sIgM^+s\kappa/\lambda^+$) and plasma cells ($CD38^+CD138^+CD56^-cIgM^+c\kappa/\lambda^+$). The median serum IgM for these patients was 29·8 (range 1·6–90 g/l), and haemoglobin was 110 (range 40–1·5 g/l). Sixteen (35%) and 13 (28%) had adenopathy and splenomegaly, respectively. Principal reasons for their diagnosis included anaemia (n = 23; 50%), and/or other cytopenias (n = 6; 13%), peripheral neuropathy (n = 8; 13%), adenopathy (n = 5; 8%), hyperviscosity (n = 3; 5%) and renal failure (n = 3; 5%). Twelve (26%) patients had a familial history for B-cell malignancies. Four (8·7%) patients had frameshift *CXCR4* mutations. At a median follow-up of 5·0 (range 0·8–17·9 years), 31 (67%) patients required therapy. Notably, 7 (15%) *MYD88*^{WT} patients were also diagnosed with diffuse

Table I. Baseline characteristics and diagnosis determined by WHO and WM consensus criteria for 64 patients who presented with suspected *MYD88*^{WT} WM.

Diagnosis	*N*	Age, years	Gender, % male	BM, %	sIgM, g/l	Hb, g/l	Adenopathy, %	Splenomegaly, %
WM	46	58·5 (29–85)	48	37·5 (2·5–95)	29·80 (1·6–90)	110 (40–155)	35	28
IgM MM	7	59 (55–75)	71	60 (10–80)	83·75 (25·3–120)	90 (84–121)	14	14
MZL	6	64·5 (51–74)	0	10 (5–25)	16·42 (0·95–28)	113 (86–123)	67	33
IgM PC MGUS	3	62 (61–76)	33	5 (5–10)	18·46 (18·46–23·9)	139 (131–147)	0	0
CLL	1	83	0	5	18·22	132	0	0
DLBCL	1	76	0	5	3·55	95	0	100

BM, bone marrow; CLL, Chronic lymphocytic leukaemia; DLBCL, Diffuse large B-cell lymphoma; Hb, haemoglobin; WHO, World Health Organization; IgM MM, IgM secreting multiple myeloma; IgM PC MGUS, IgM secreting plasma cell monoclonal gammopathy of unknown significance; MZL, Marginal zone lymphoma; sIgM, serum IgM; WM, Waldenstrom macroglobulinaemia.
All patients had a monoclonal IgM paraprotein, and were *MYD88*^{WT}. Median values and corresponding ranges are provided for WM, IgM MM, MZL, and IgM PC MGUS patients.

large B-cell lymphoma (DLBCL), three synchronous and four metachronous to their WM diagnosis. Eleven (24%) patients succumbed during the follow-up period; 7 deaths were due to WM, and 4 because of DLBCL.

Among the other *MYD88*^{WT} patients, 7 (10%) had findings consistent with IgM MM. Their median BM disease involvement was 60% (range 10–80%), with a predominant plasma cell infiltrate. In 3 (43%) patients, monotypic B-cells ($CD19^+CD20^+CD5^-CD10^-CD23^{+/-}sIgM^+s\kappa/\lambda^+$) and plasma cells ($CD38^+CD138^+CD56^-cIgM^+c\kappa/\lambda^+$) were detected, while only clonal plasma cells ($CD38^+CD138^+CD56^-cIgM^+c\kappa/\lambda^+$) were observed in 4 other patients. Fluorescence *in-situ* hybridization (FISH) testing of BM mononuclear cells showed t(11;14) in 5 patients; t(14;16) in one patient; and normal cytogenetics in one patient. Lytic lesions were detected in three (43%) patients. The median serum IgM level was significantly higher at 83·75 (range 25·3–120 g/l) *versus* *MYD88*^{WT} WM patients ($P = 0{\cdot}016$). One (14%) patient had both adenopathy and splenomegaly. Two (28%) had a familial history. No *CXCR4* mutations were observed. Principal reasons that led to diagnosis included anaemia ($n = 7$; 100%) and symptomatic hyperviscosity ($n = 3$; 43%). With a median follow-up of 2·4 (range 0·4–7·4 years), all patients required treatment and one died of myeloma. No DLBCL events occurred.

Findings consistent with marginal zone lymphoma (MZL) were observed in 6 (9%) patients, all female. Their median BM involvement was 10% (range 5–25%), with paratrabecular and nodular predominant infiltrate of mainly small to medium sized lymphocytes and few mature plasma cells. Three (50%) patients had monotypic B-cells ($CD19^+CD20^+CD5^{+/-}CD10^-CD23^+CD45^+s\kappa/\lambda^+$) by flow cytometric analysis. Their median serum IgM level was 16·42 (0·95–28 g/l), and was not significantly different *versus* *MYD88*^{WT} WM patients. Adenopathy and splenomegaly were present in 4 (67%) and 2 (33%) patients. None had a familial history, and no *CXCR4* mutations were observed. Only one patient had noteworthy cytogenetics that showed a t(3;6)(q13.3;p25) translocation. Hepatitis C virus testing was unremarkable. Principal reasons that led to diagnosis included anaemia ($n = 4$; 67%) and/or other cytopenias ($n = 1$; 17%) and peripheral neuropathy ($N = 1$; 17%). With a median follow-up of 3·8 (range 1·3–5·2 years), four (67%) required therapy, and are alive. No DLBCL events occurred in these patients.

For 3 patients, the findings favoured a diagnosis of IgM monoclonal gammopathy of undetermined significance (MGUS) with plasma cell infiltration. The median BM involvement for these patients was 5% (range 5–8%) with flow cytometric analysis demonstrating a clonal plasma cell population. The median serum IgM level for these patients was 18·46 (range 12·62–23·9 g/l), and did not differ significantly *versus* *MYD88*^{WT} WM patients. None had a familial history, and none had *CXCR4* mutations. In two patients, the diagnosis was incidental to elevated total protein. In one patient, IgM MGUS was diagnosed for work-up of fatigue. With a median follow-up of 2·5 (range 2·5–3·2 years), none have progressed or received treatment, and all remain alive. No DLBCL events were observed. Lastly, in one patient, the findings favoured the diagnosis of CLL while another patient had intravascular DLBCL, with respective serum IgM levels of 18·22 and 3·55 g/l. The BM disease burden was 5% in both patients. None had a familial history or *CXCR4* mutations. The CLL patient remains alive without treatment and a DLBCL event, while the patient with DLBCL required therapy and remains alive.

We next sought to address the impact of *MYD88* mutation status on overall survival in WM patients. Survival outcome was compared to 262 patients with *MYD88*^{MUT} disease who were diagnosed over the same time-period. The median follow-up for all patients was 74·7 (0·5–324·9 months), and was similar for *MYD88*^{WT} and *MYD88*^{MUT} WM patients (64·1 vs. 73·7 months, respectively; $P = 0{\cdot}71$). Patient characteristics are shown in Table II. Among 262 *MYD88*^{MUT} patients, 261 had L265P and 1 had S243N *MYD88* mutation; 101 (38·5%) were also *CXCR4* mutated. During the follow-up period, there were 11 (23·9%) deaths for the *MYD88*^{WT} *versus* 15 (5·7%) deaths among *MYD88*^{MUT} patients ($P = 0{\cdot}003$). Figure 1A shows overall survival based on *MYD88* and *CXCR4* mutation status from time of diagnosis for all patients. The estimated 10-year survival was 73% (95% CI 52–86%) and 90% (95% CI 82–95%) for *MYD88*^{WT} and *MYD88*^{MUT} patients, respectively (Log-rank $P < 0{\cdot}001$), and did not differ within *MYD88*^{MUT} patients by *CXCR4* mutation status (Log-rank $P = 0{\cdot}69$). Multivariate analyses that included age, gender, serum IgM, haemoglobin, BM disease involvement, serum β_2-microglobulin, *MYD88* and *CXCR4* mutation status showed that *MYD88* mutation status alone was a significant determinant for overall survival. The

Table II. Baseline characteristics for *MYD88*^{WT} and *MYD88*^{MUT} patients with WM by WHO and WM consensus criteria in overall survival analysis.

WM patients	*N*	Age, years	Gender Male/Female	IgM, g/l	BM, %	Hb, g/l	B_2M, mg/l
MYD88^{WT}	46	59 (29–85)	47·8%/52·2%	29·8 (1·6–90)	37·5 (2·5–95)	110 (40–155)	3·4 (1·5–19·2)
MYD88^{MUT}	262	61 (31–91)	62·9%/37·1%	26·5 (0·94–124)	35 (5–95	120 (6·0–16·3)	2·8 (1·4–11·8)
P-value		0·29	0·07	0·77	0·88	0·002	0·09

B_2M, β_2-microglobulin; BM, bone marrow; WHO, World Health Organization; WM, Waldenstrom macroglobulinaemia.
Median values and corresponding ranges are provided.

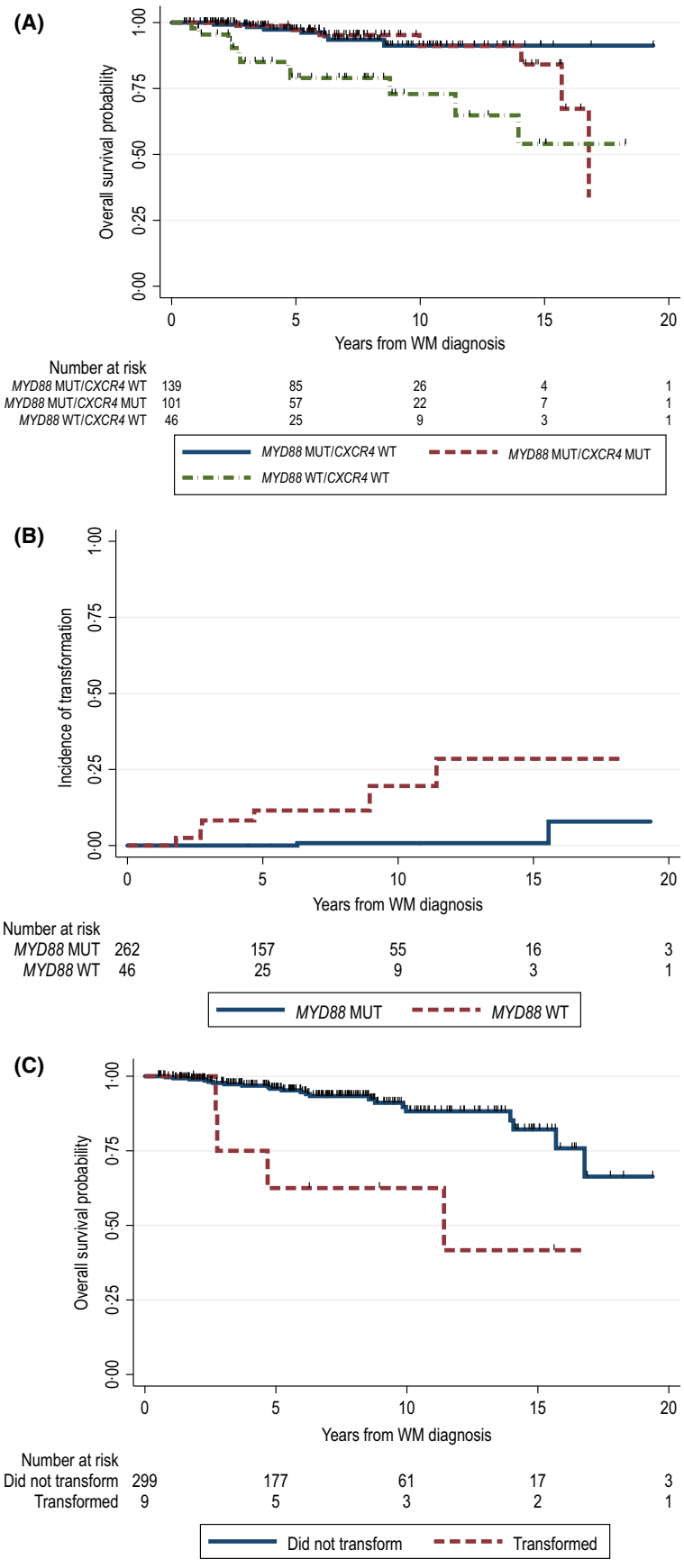

Fig 1. Overall survival estimates for *MYD88* and *CXCR4* genotyped patients with Waldenstrom macroglobulinaemia (WM) determined by World Health Organization and WM consensus criteria. (A) Overall survival for all patients shown by *MYD88* mutation status, and *CXCR4* mutation status for *MYD88* mutated patients (Kaplan–Meier). (B) Incidence of transformation according to *MYD88* mutational status (Kaplan–Meier, failure), and (C) Overall survival for $MYD88^{WT}$ patients with and without an associated diffuse large B-cell lymphoma event (Simon–Makuch).

Table III. Univariate and multivariate Cox proportional hazard regression models for overall survival in patients with WM.

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age ≥65 years	2·51 (1·13–5·54)	0·02	1·79 (0·64–4·98)	0·27
Male sex	1·20 (0·53–2·69)	0·67	1·45 (0·49–4·29)	0·50
Haemoglobin <115 g/l	0·66 (0·30–1·44)	0·30	2·92 (0·82–10·4)	0·10
Serum IgM ≥40 g/l	1·32 (0·59–2·96)	0·51	2·34 (0·82–6·67)	0·11
Bone marrow ≥50%	1·22 (0·54–2·76)	0·63	0·88 (0·31–2·45)	0·80
Serum B_2M ≥3 mg/l	1·99 (0·85–4·66)	0·11	2·24 (0·82–6·12)	0·12
MYD88^{MUT}	4·24 (1·94–9·24)	<0·001	7·47 (2·27–24·6)	<0·001
CXCR4^{MUT}	0·78 (0·33–1·81)	0·56	1·07 (0·36–3·12)	0·91

B_2M, β_2-macroglobulin; CI, confidence interval; HR, hazard ratio.

results of the survival analyses are shown in Table III. Transformation to DLBCL occurred during the follow-up period in 7 (15·2%) and 2 (0·76%) *MYD88*^{WT} and *MYD88*^{MUT} patients, respectively. The incidence of transformation to DLBCL at 10 and 20 years was 1% (95% CI 0·1–5%) and 8% (1–39%) in *MYD88*^{MUT} patients *versus* 20% (95% CI 8–45%) and 29% (95% CI 12–58%) in *MYD88*^{WT} patients (HR for DLBCL transformation 19·8, 95% CI 4·08–95·8, $P < 0·001$; Fig 1B). Overall survival estimates at 10 and 20 years for WM patients who did not transform to DLBCL were 88% (95% CI 81–93%) and 66% (95% CI 40–83%), respectively, and for WM patients who transformed to DLBCL were 63% (95% CI 23–86%) and 42% (7–75%), respectively (Mantel-Byar $P = 0·003$; Fig 1C).

Discussion

The above findings suggest that while most patients with suspected *MYD88*^{WT} WM disease fulfil WHO and consensus criteria for the diagnosis of WM, up to 30% had an alternative diagnosis. Surprising was the high number of IgM MM cases, many of which had clonal B-cells and plasma cells. These patients had a predominant plasma cell component by histological examination, and exhibited high serum IgM levels (median 83·75 g/l) that distinguished them from *MYD88*^{WT} WM patients (median 29·8 g/l; $P = 0·016$). FISH studies showed that 86% of IgM MM patients carried chromosome 14 translocations, consistent with earlier reports (Avet-Loiseau *et al*, 2003a; Willenbacher *et al*, 2013). High serum IgM levels and a lymphoplasmacytic-like BM infiltrate in IgM MM was also observed by Avet-Loiseau *et al* (2003b). Therefore in *MYD88*^{WT} patients with high serum IgM levels and/or a predominant plasma cell clone, IgM MM should be suspected and additional work-up undertaken that includes FISH studies for chromosome 14 translocations. Distinguishing IgM MM is particularly important because the care plan is different from WM, and can include the use of immunomodulatory drugs (IMIDs), CD38- and CS1-directed monoclonal antibodies, bisphosphonates, consolidative autologous stem cell transplantation, and/or maintenance therapy with IMIDs. It is noteworthy, that several cases of IgM MGUS with plasmacytic cell infiltrates were also identified among suspected *MYD88*^{WT} WM cases. These cases differed from the "classic" WHO- and consensus-defined IgM MGUS cases that have a predominant B-cell clone and no histologically demonstrable BM infiltrate. In distinction to the "classic" IgM MGUS cases that typically evolve to WM or MZL, IgM MGUS cases with a plasma cell infiltrate may serve as precursors to IgM MM. Further work to subcategorize IgM MGUS (B-cell *versus* plasma cell predominant) and clarify their respective evolution is needed. Not surprising were the few cases of MZL included among *MYD88*^{WT} WM patients. All MZL patients were female, consistent with prior reporting for female prevalence for MZL (Arcaini *et al*, 2011). Subtle differences in BM morphology, including a more predominant paratrabecular and nodular pattern of disease involvement with small to medium size lymphocytes and few plasma cells, helped discriminate these patients (Arcaini *et al*, 2011; Kyrtsonis *et al*, 2011; Bassarova *et al*, 2015). Principal diagnostic complaints, findings from the physical examination, imaging and laboratory studies, including serum IgM levels, flow cytometry and cytogenetics, did not provide much separation between *MYD88*^{WT} WM, and MZL, highlighting the need for investigation of the genomic basis of *MYD88*^{WT} WM, and development of molecular markers to discriminate this entity from MZL and other related diseases.

An important finding in this study was also the high incidence of associated DLBCL cases in *MYD88*^{WT} WM patients. It is unclear whether these events represented true histological transformation or secondary primary B-cell malignancies, but they contributed to 4 of the 11 (36%) deaths observed in the *MYD88*^{WT} WM patients. In contrast, none of the other *MYD88*^{WT} patient cohorts had an associated DLBCL diagnosis. Transformation is uncommon in WM patients, particularly among those naïve to nucleoside analogues, with an estimated incidence rate of <5% (Leleu *et al*, 2009; Castillo *et al*, 2016). However, *MYD88* mutation status was not addressed in those studies. In our series, all but one of the 7 *MYD88*^{WT} patients was naïve to nucleoside analogues. The findings infer a considerably different biology underlying

many *MYD88*^{WT} WM cases, that may also account for the higher risk of death previously reported by us (Treon *et al*, 2014), and that was also observed in this greatly expanded study. As before, we saw no impact of *CXCR4* mutation status on overall survival in *MYD88*^{MUT} patients. Among *MYD88*^{WT} patients who transformed, overall survival was shorter in this study. Significantly shorter overall survival (9 years vs. 16 years) was also observed previously in transformed WM patients (Castillo *et al*, 2016).

The median overall survival for all patient populations, including *MYD88*^{WT} WM patients, was not reached in this study (Fig 1A). Longer follow-up will invariably be required to better clarify the relative risk of death associated with *MYD88* mutation status in WM patients, and among IgM secreting B-cell malignancies. Lastly, further clarification of the underlying genomic landscape in *MYD88*^{WT} WM may ultimately provide not only the molecular tools that would more definitively distinguish *MYD88*^{WT} WM disease from other overlapping IgM secreting disorders, but also identify those patients at increased risk of associated DLBCL events.

In summary, we report that up to 30% of suspected *MYD88*^{WT} WM cases had an alternative clinicopathological diagnosis that included both malignant and non-malignant entities. IgM MM was frequently present among suspected MYD88^{WT} cases, and presented with high serum IgM levels and chromosome 14translocations. WM patients with *MYD88*^{WT} disease had a high incidence of associated DLBCL events and significantly shorter survival *versus* those with *MYD88*^{MUT} disease. Associated DLBCL events in MYD88^{WT} patients were associated with shortened survival.

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Authorship

SPT, ZRH, and JJC designed the study. JG, KM, PS, TD, JJC, SPT, cared for the patients. RJM, JG, KM, CJP provided clinical data. LX, NT, MD, AK, MLG, MM, JC, GY, XL collected and processed tumour samples, and performed MYD88 and CXCR4 genotyping studies. SPT, ZRH, GC, JJC analyzed the study data. SPT and JJC wrote the manuscript.

Conflicts of interest

No conflicts of interest are identified by the investigators for this work.

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