To select or not to select? The role of B-cell selection in determining the *MYD88* mutation status in Waldenström Macroglobulinaemia

Waldenström Macroglobulinaemia (WM) is a B-cell malignancy characterized by bone marrow (BM) infiltration with lymphoplasmacytic lymphoma and production of a monoclonal immunoglobulin (Ig) M protein (Owen *et al*, 2003). The tumour cell population in WM is markedly heterogeneous, reflecting mature B-lymphocytes that retain the ability to differentiate into plasmacytoid lymphocytes and plasma cells, though the distribution of the malignant clone within individual patients can vary. Two subsets of malignant WM cells are readily identifiable by flow cytometry in BM aspirates: monotypic B lymphocytes that are $CD19^{+}$, $CD20^{+}$, $CD22^{+}$, $CD38^{-}$, and monotypic plasma cells that are $CD19^{-}$, $CD20^{+/-}$, $CD22^{-}$, $CD38^{+}$ (Paiva *et al*, 2014).

Given the wide spectrum of B-cell differentiation exhibited in WM, there can be great overlap with other IgM secreting B-cell malignancies thereby posing a diagnostic challenge. *MYD88* L265P is a highly prevalent (93–95%) somatic mutation in WM that was first revealed by whole genome sequencing (Treon *et al*, 2012). *MYD88* L265P is absent or rarely expressed in patients with other types of B-cell malignancies, which share many clinical and pathological features with WM, such as marginal zone lymphoma, chronic lymphocytic leukaemia and IgM myeloma (Varettoni *et al*, 2013; Xu *et al*, 2013). The ability to use *MYD88* L265P to support the diagnosis of WM has led to its adoption in World Health Organization, National Comprehensive Cancer Network and WM Workshop Consensus guidelines. Rare non-L265P *MYD88* mutations may also be present in WM patients (Treon *et al*, 2015). The presence of *MYD88* mutations is also associated with longer overall survival, as well as higher overall and major response rates to ibrutinib, the only US Food and Drug Administration and European Medicines Agency approved therapy for WM (Treon *et al*, 2014, 2015). As such, determination of *MYD88* mutational status can convey important diagnostic, prognostic and predictive data for treatment response in WM.

Allele-specific polymerase chain reaction (AS-PCR) is widely used for the detection of *MYD88* L265P, and offers a higher level of sensitivity over Sanger sequencing. However, some series have used sorted B-cells to detect *MYD88* L265P, whereas others used unselected BM mononuclear cells. The use of sorted B-cells versus unsorted BM mononuclear cells for the optimal detection of *MYD88* L265P by AS-PCR remains unclear, though in peripheral blood (PB) the use of sorted B-cells greatly improved detection of *MYD88* L265P over unselected PB mononuclear cells (Xu *et al*, 2014). In clinical laboratories, the ability to pre-sort B-cells poses a time and economic challenge, hence testing is almost always

Table I. Waldenström Macroglobulinaemia patient characteristics at time of bone marrow biopsy.

Characteristic	Untreated (N = 46)	Previously treated (N = 66)
Median age (range), years	66·5 (49–84)	66 (31–83)
Median age at diagnosis (range), years	63 (47–84)	60·5 (30–76)
Sex, *n* (%)		
Male	34 (52)	37 (56)
Female	12 (26)	29 (44)
Median serum antibody levels (range), g/l		
IgM	33·80 (0·74–70·0)	22·71 (0·37–76·3)
IgG	6·0 (0·40–15·63)	4·14 (0·85–14·84)
IgA	0·52 (0·08–13·0)	0·29 (0·05–1·75)
Median absolute neutrophil count (range),$\times 10^9$/l	4·24 (0·82–9·16)	3·92 (0·04–14·99)
Median haemoglobin level (range), g/l	119 (85–161)	116 (74–167)
Median haematocrit (range), %	35·8 (24·8–49·2)	34·5 (22·8–50·7)
Median platelet count (range), $\times 10^9$/l	279 (70–555)	222 (18–630)
Median bone marrow disease involvement* (range), %	32·5 (0–90)	20 (0–95)

*N = 65; No available bone marrow involvement for one previously treated patient due to subcortical biopsy.

performed with unselected samples. We therefore assessed whether the use of B-cell selection improved the accuracy of *MYD88* L265P detection in 112 patients who met the consensus criteria for the diagnosis of WM (Owen *et al*, 2003). The clinical characteristics for these patients at the time of BM aspirations are shown in Table I. *MYD88* L265P was detected by AS-PCR using CD19-sorted cells or unselected mononuclear cells from BM aspirates as previously reported (Xu *et al*, 2013, 2014).

Overall, comparison of *MYD88* L265P detection using CD19-selected and unselected BM cells showed concordance in the findings for most patients (104/112; 93%, Cohen's κ coefficient 0·71). When stratified by previous treatment history, a greater proportion of untreated (46/46; 100%, Cohen's κ coefficient 1·00) *versus* previously treated patients (58/66; 84%, Cohen's κ coefficient 0·62) showed concordance for detection of *MYD88* L265P (Fischer's exact $P = 0{\cdot}02$). Among the 8 previously treated patients with discordant findings, 2 had *MYD88* L265P detected only in CD19-selected BM cells, whereas it was only detected in unselected BM cells in the 6 other discordant patients. Analysis of clinicopathological characteristics showed that discordant patients had more prior therapies (3 vs. 1; Mann–Whitney $P = 0{\cdot}0533$) and lower BM involvement (4% vs. 30%; Mann–Whitney $P = 0{\cdot}0062$) *versus* concordant patients (Table II). For the two discordant patients with *MYD88* L265P detected by AS-PCR using CD19-sorted cells, the BM involvement was 10% and 5%, respectively, and clonal B-cells were only detectable by flow cytometry in the latter patient. The findings in these patients are not unexpected given the low tumour burden, as well as the lower sensitivity for *MYD88* L265P detection by AS-PCR with unselected versus B-cell selected samples (Xu *et al*, 2014). However in the 6 discordant patients for whom *MYD88* L265P was detected only in unselected aspirate samples, the findings were unexpected, particularly as the median BM tumour burden was only 3%, and clonal B-cells were undetectable in 5 of 6 (83%) patients. However, clonal plasma cells were present in 5 of 5 (100%) discordant patients in whom flow cytometric analysis was undertaken to evaluate for clonal plasma cells. We have previously reported the presence of *MYD88* L265P in both clonal B-cells and plasma cells derived from individual WM patients (Treon *et al*, 2012), and the findings herein suggest that residual plasma cells not amenable to CD19-selection probably accounted for the detection of *MYD88* L265P in unselected but not CD19-sorted BM samples. All of these patients had previously undergone treatment with agents known to deplete B-cells, including rituximab. The presence of residual clonal plasma cells following treatment has previously been reported (Varghese *et al*, 2009; Barakat *et al*, 2011). In one of these studies, one quarter of WM patients had monotypic plasma cells that represented

Table II. Clinical characteristics of previously treated WM Patients stratified by BM allele-specific polymerase chain reaction findings.

Characteristic	Concordant ($N = 58$)	Discordant ($N = 8$)	*P*-value
Median age (range), years	66 (31–83)	66·5 (63–74)	0·6462
Median age at diagnosis (range), years	61 (30–76)	59 (51–68)	0·8810
Sex, *n* (%)			
Male	34 (59)	3 (38)	0·2852
Female	24 (41)	5 (63)	
Previous therapy for WM			
Median number of previous treatment regimens (range)	1 (1–8)	3 (1–5)	0·0533
Type of therapy among previously treated, *n* (%)			
Monoclonal antibody	54 (93)	8 (100)	1·0000
Glucocorticoid	33 (57)	5 (63)	1·0000
Proteasome inhibitor	33 (57)	3 (38)	0·4525
Alkylator	27 (47)	6 (75)	0·2576
Nucleoside analogue	13 (22)	1 (13)	1·0000
Other	19 (32)	3 (38)	1·0000
Median serum antibody levels (range), g/l			
IgM	24·64 (0·37–76·3)	13·38 (1·12–51·48)	0·1067
IgG	3·99 (0·885–14·84)	5·13 (0·86–6·96)	0·6349
IgA	0·29 (0·05–1·75)	0·29 (0·05–1·18)	0·6817
Median absolute neutrophil count (range), $\times 10^9$/l	4·12 (0·04–14·99)	2·72 (1·22–6·67)	0·0512
Median haemoglobin level (range), g/l	115 (74–167)	121 (99–158)	0·3108
Median haematocrit (range), %	34·3 (22·8–50·7)	35·7 (29·6–46·4)	0·3108
Median platelet count (range), $\times 10^9$/l	228 (18–630)	182 (115–295)	0·1470
Median BM involvement* (range), %	30 (0–95)	4 (1–10)	0·0062

WM, Waldenström Macroglobulinaemia; BM, bone marrow.

*$N = 57$; No available bone marrow involvement for one concordant patient due to subcortical biopsy.

0·5–46% of the cellularity, and persisted for up to 50 months following the last course of chemotherapy (Barakat *et al*, 2011). These observations combined with our findings highlight the importance of including the plasma cell compartment in any evaluations for *MYD88* mutations in previously treated WM patients.

The use of next generation deep sequencing in unselected BM aspirates may be ideally suited for clinical diagnostic use because time consuming and cost additive cell sorting is not required, and non-L265P *MYD88* mutations may also be evaluated simultaneously. Efforts to establish a uniform testing method for determining *MYD88* mutational status should be pursued given the importance of *MYD88* mutational status in diagnostic, prognostic and treatment considerations in WM patients.

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Authorship

JG, JJC and SPT designed the study. JG, KM, JJC and SPT collected study samples and data. LX and ZRH processed the tumour samples and performed *MYD88* genotyping studies. All authors analysed the data. JG wrote the initial draft, and all authors reviewed and approved the final manuscript.

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