

Report of Consensus Panel 3 from the 11th International workshop on Waldenström's Macroglobulinemia: Recommendations for molecular diagnosis in Waldenström's Macroglobulinemia

Ramón García-Sanz^{a,*}, Marzia Varettoni^b, Cristina Jiménez^a, Simone Ferrero^c, Stephanie Poulain^d, Jesus F. San-Miguel^e, Maria L. Guerrero^f, Daniela Drandi^c, Tina Bagratuni^g, Mary McMaster^h, Aldo M. Roccaroⁱ, Damien Roos-Weil^j, Merav Leiba^k, Yong Li^l, Luigi Qiu^m, Jian Houⁿ, C. Fernandez De Larrea^o, Jorge J. Castillo^f, M. Dimopoulos^g, R.G. Owen^{p,q}, S.P. Treon^f, Z.R. Hunter^f

^a Hematology Department, University Hospital of Salamanca, Research Biomedical Institute of Salamanca (IBSAL), CIBERONC and Center for Cancer Research-IBMCC (University of Salamanca-CSIC), Salamanca, Spain

^b Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Italy

^c Unit of Hematology, Department of Biotechnology & Health Sciences, University of Torino, Torino, Italy

^d Laboratory of Hematology, Biology and Pathology Center, CHU of Lille, UMR9020 CNRS-UI277 INSERM, University of Lille, and ONCOLILLE Cancer Institute, CANTHER Laboratory, Lille, France

^e Laboratory of Hematology, Biology and Pathology Center, CHU of Lille, Lille, France

^f Hematology department, Clínica Universidad de Navarra, CIMA, IDISNA, CIBERONC, Pamplona, Spain

^g Bing Center for Waldenström's Macroglobulinemia, Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA

^h Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

ⁱ Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD

^j Clinical Trial Center, Translational Research and Phase I Unit, ASST Spedali Civili di Brescia, Brescia, Italy

^k Sorbonne Université, Hematology Unit, Pitié-Salpêtrière Hospital, Assistance Publique des Hôpitaux de Paris (AP-HP), Paris, France

^l Assuta Ashdod University Hospital, Faculty of Health Science, Ben-Gurion University of the Negev, Negev, Israel

^m Department of Medicine, Baylor College of Medicine, Houston, TX

ⁿ National Clinical Research Center for Blood Diseases, Blood Disease Hospital and Institute of Hematology, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China

^o Renji Hospital, Shanghai, China

^p Hospital Clínic de Barcelona, IDIBAPS, Universitat de Barcelona, Barcelona, Spain

^q St James's University Hospital, Leeds Teaching Hospitals NHS Trust, Leeds, UK

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ABSTRACT

Apart from the MYD88^{L265P} mutation, extensive information exists on the molecular mechanisms in Waldenström's Macroglobulinemia and its potential utility in the diagnosis and treatment tailoring. However, no consensus recommendations are yet available. Consensus Panel 3 (CP3) of the 11th International Workshop on Waldenström's Macroglobulinemia (IWWM-11) was tasked with reviewing the current molecular necessities and best way to access the minimum data required for a correct diagnosis and monitoring. Key recommendations from IWWM-11 CP3 included: (1) molecular studies are warranted for patients in whom therapy is going to be started; such studies should also be done in those whose bone marrow (BM) material is sampled based on clinical issues; (2) molecular studies considered essential for these situations are those that clarify the status of 6q and 17p chromosomes, and MYD88, CXCR4, and TP53 genes. These tests in other situations, and/or other tests, are considered optional; (3) independently of the use of more sensitive and/or specific techniques, the minimum requirements are allele specific polymerase chain reaction for MYD88^{L265P} and CXCR4^{S338X} using whole BM, and fluorescence in situ hybridization for 6q and 17p and sequencing for CXCR4 and TP53 using CD19+ enriched BM; (4) these requirements refer to all patients; therefore, sample should be sent to specialized centers.

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* Corresponding author: Ramón García-Sanz, MD, PhD, University Hospital of Salamanca, Avenida de la Transición s/n, Salamanca, 37007 Spain. Tel.: +34 92 329 1100.
E-mail address: rgacias@usal.es (R. García-Sanz).

Introduction

The recently updated International Consensus Classification on Lymphoid Neoplasms [1] has emphasized the value of genetic abnormalities associated with the diagnosis of Waldenström's Macroglobulinemia (WM), a concept that will be extensively evaluated in the Consensus Panel 3. This panel will clarify how such molecular abnormalities can impact WM management even beyond the diagnosis. Accordingly, identifying the mutational landscape of the tumor clone may help to confirm the diagnosis, especially to discriminate WM from other IgM-secreting disorders [2]. However, there is no consensus regarding which molecular abnormalities must be assessed and what molecular methods should be used for that assessment. Consensus Panel 3 (CP3) of the 11th International Workshop on Waldenström's Macroglobulinemia (IWWM-11) reviewed the current molecular abnormalities to recommend which abnormalities must be assessed for diagnosis and what methods could be used for their assessment.

Important advances in understanding the biology of WM have been made in recent years, leading to an increased toolset for differential diagnosis and treatment guidance [3]. From the cytogenetic viewpoint, WM presents with a median of 2 to 3 chromosomal abnormalities per patient [4]. Deletion of 6q (–6q or del6q) is the most frequent chromosomal abnormality (30%–50% of patients) [2,4–12] and it is directly related to the progression from asymptomatic to symptomatic WM [13]. Other frequent abnormalities are trisomy 4 (tri4), tri18, del13q, tri12, and del17p, although none are present in more than 15% of patients [2,4–12,14,15]. Deletion of 17p/TP53 is present in 7% of WM patients and has been associated with poor prognosis [12,16–18]. Unlike multiple myeloma (MM) and other B-cell lymphoproliferative disorders (B-LPD), no translocations have been consistently described in WM.

The landscape of point mutations is completely different from the cytogenetic landscape described above. Using whole genome sequencing, Treon et al. [19] identified a somatic variant (T→C) WM cells at position 38182641 of chromosome 3p22.2 that predicted an amino acid change (L265P) in the MYD88 gene and was highly recurrent in patients with WM. Several additional studies using different techniques, such as Sanger sequencing, allele-specific quantitative polymerase chain reaction PCR (AS-PCR) [20–22], droplet digital PCR (ddPCR) [23–25], and Competitive Allele-Specific TaqMan PCR (Cast-PCR) [26], confirmed that this MYD88^{L265P} mutation is present in around 95% of WM. Interestingly, MYD88^{L265P} mutation is completely absent in patients with MM (including IgM isotype) [22,27], and it is rare in marginal zone lymphoma (MZL) with plasmacytic differentiation or chronic lymphocytic leukemia (CLL) [22,28–30]. Therefore, MYD88^{L265P} assessment is considered crucial to discriminate between WM and other B-LPDs with overlapping clinical features. Moreover, MYD88 mutations other than the classical L265P can be found in WM [18,31]. Presented at the last 11th IWWM held in Madrid, the international FIL-BIOWM study using a specific next generation sequencing (NGS) panel found 4 different MYD88 mutations (M232T, S243N, V217F, & R209C) in 9 out of 244 tested patients, which means that around 4% of WM patients harbor an MYD88 mutation different from L265P [32]. This is very relevant, since these patients can be highly sensitive to Bruton Tyrosine Kinase inhibitors (BTKi) despite lacking the classical MYD88^{L265P} mutation [31].

Mutations in the CXCR4 gene have been identified as the second most common alterations in WM (30%–40% patients) [33–35], and they play an important role in WM pathogenesis and disease progression [36–38]. CXCR4 mutations are essentially unique to WM, as they have not been described so far in other diseases, with the exception of a few MZL cases [3]. Germline mutations present in patients with WHIM (warts, hypogammaglobulinemia, infection, and myelokathexis) syndrome are very similar to those found as

somatic mutations in WM [39], so they are called CXCR4^{WHIM} mutations. These mutations might also impact the clinical presentation and outcome of WM patients. Thus, MYD88^{L265P}/CXCR4^{WHIM} patients may present with more aggressive clinical behavior, and inferior response to BTKi [40].

Almost 50% of WM present mutations in other genes [34,41,42]. ARID1A was mutated in 17% of patients with WM in the initial study, including nonsense and frameshift variants, including nonsense and frameshift variants, and are thought to be associated with more advanced disease. Interestingly, the homolog of this gene, ARID1B, is sited at chromosome 6q and is commonly deleted in WM [33]. TP53 mutations are rare in WM (8%), but they have been associated with poor survival [3,12,16], and their frequency increases in patients beyond the first-line therapy [35]. Mutations in CD79A and CD79B can be found in 8–12% of patients with WM [3,34,42]. Both are components of the BCR pathway and can form heterodimers with each other, so activating mutations of these components could contribute to the chronic BCR signaling observed in WM cells [3], and have an independent role in facilitating mutated MYD88-directed progression in WM [43]. In addition, CD79B mutations have also been associated with disease transformation to diffuse large B-cell lymphoma (DLBCL) in some WM patients [44]. The number of detectable genetic abnormalities in IgM monoclonal gammopathies is increased as the disease's aggressiveness evolves [34].

Since WM is a rare disease and procedures may vary across different laboratories, CP3 of the IWWM-11 has reviewed all these molecular abnormalities and the methods that should be used in clinical practice for better assistance in the final diagnosis of all IgM monoclonal gammopathies. The findings and recommendations from CP3 on revising the current molecular evaluation in WM are reported herein.

Samples for molecular studies

Can we avoid bone biopsy during the workup of WM? Could bone marrow aspiration be enough for diagnostic purposes?

The panel reinforces the view that no precise data ensures that WM diagnosis can be made without bone marrow (BM) biopsy to distinguish from other disorders. In addition, there is no complete agreement on the influence of MYD88 status on the final diagnosis. In addition, the discrimination between WM and monoclonal gammopathy of uncertain significance (MGUS) discrimination still requires a BM biopsy. The recent update of the classification of mature lymphoid neoplasms [1] confirmed that the diagnosis of WM requires the demonstration of abnormal lymphoplasmacytic aggregates and evidence of clonal B cells and plasma cells on trephine biopsy. The diagnosis does not require a minimum level of infiltration nor a minimum level of IgM paraprotein in the serum, in keeping with the diagnostic criteria proposed by the IWWM in 2002 [45]. Though the MYD88^{L265P} mutation is a molecular marker highly recurrent in WM patients, the presence of MYD88^{L265P} mutation *per se* is not pathognomonic of WM, as it is also detectable in a significant proportion of patients with IgM-MGUS. It can also be detected, albeit rarely, in other B-LPD such as MZL [22,46], DLBCL [22,47], or CLL [30]. During the 11th IWWM the first data of the FIL-BIOWM study were presented including an extensive immunophenotypic evaluation of BM aspirations that combined with a ddPCR study in BM and peripheral blood which raised the possibility of avoiding the BM biopsy [32]. However, no definitive consensus was yet achieved.

Although BM biopsy is still needed for a complete final diagnosis, it is of worth mentioning that BM aspiration can provide representative samples for a sufficient molecular testing. Even after possible hemodilution, this sample could be enough for highly

sensitive molecular studies, providing a median frequency of monoclonal B-cells of 2.2%, 8.7%, and 12.2% in BM samples from MGUS, asymptomatic, and symptomatic WM patients, respectively.[48] However, with these numbers, it is easily understandable that many samples fail to provide accurate results with low sensitivity techniques (Sanger and even NGS) [49,50].

Accordingly, the panel agreed that CD19+ sorting by immunomagnetic approaches or flow cytometry is required to increase the sensitivity of molecular diagnostics, and ensure a reliable genetic or molecular analysis, especially when using techniques with intrinsic limits of sensitivity (eg, NGS or fluorescent in situ hybridization -FISH) or when analyzing samples with low tumor infiltration. With the number of clonal cells that can be found in the BM (see above), many patients will not have a percentage of clonal cells sufficient for a reliable genetic or molecular analysis. Accordingly, CD19+ cell enrichment of BM aspirations by immunomagnetic approaches or flow cytometry sorting is strongly recommended [2,13]. Although the access to this methodology can be difficult in community centers, sample referral to specialized laboratories can solve this problem; this will give the opportunity of a complete diagnosis to all patients, and facilitate the research in academic centers.

CD19+ enrichment is dispensable to assess the *MYD88*^{L265P} mutation status on BM or cell free DNA (cfDNA) from plasma samples, as the results obtained on unsorted samples were superimposable to those obtained on enriched samples [25]. The panel also acknowledged that CD19 sorting is costly and time-consuming limiting its widespread use in laboratories. Nevertheless, it has an additional reason to be recommended as it may reduce the possibilities of detecting gene mutations present in clonal hematopoiesis of indeterminate potential (CHIP) [51]. This has special relevance for *TP53* and *TERT2* mutations, which are very frequent in elderly populations and hematological malignancies, as WM is. The use of CD19+ cell enrichment would help to minimize the detection of these abnormalities, mainly present in myeloid cells [52].

Chromosomal abnormalities

Should 6q copy number alterations be examined in WM during diagnostic evaluation?

The panel agreed that it is worth assessing the presence of 6q deletion using FISH analysis, or other reliable method if available (ie, single nucleotide polymorphism -SNP- array, whole genome sequencing, etc.). This test should be performed in BM samples with CD19+ cell enrichment. In asymptomatic cases of IgM monoclonal gammopathies, the presence of del6q is associated with a high probability of progression to symptomatic disease [12,13]. Accordingly, asymptomatic patients in whom a del6q is identified could be considered for an individualized follow-up to facilitate early detection of progression. In addition, del6q is a prognostic factor in symptomatic cases [12,13,53–55]. It is debatable whether BM examination should be recommended for all patients with IgM monoclonal gammopathies (ie, low risk IgM-MGUS) [56], but if the attending physician decides to perform a BM aspiration or biopsy, at least a FISH study to detect del6q should be performed.

Should 17p assessment be assessed by FISH during the WM diagnostic evaluation?

As in other related B-LPDs and monoclonal gammopathies, in WM, *TP53* can be altered by 2 main mechanisms: (1) via 17p deletion, which results in the loss of 1 complete allele, and (2) via *TP53* gene mutation, which usually results in a loss of function of the involved allele. The presence of a double lesion, deletion plus mutation or double mutation, will result in a complete loss of the tumor

suppressor activity of the *TP53* protein, and thus in a more aggressive disease [57,58]. There is not so much information on WM, although some reports have demonstrated that del17p and *TP53* mutations confer a poor prognosis [11,12,16–18,35]. With these data, the panel recommends, together with the del6q, FISH studies to assess del17p (*TP53*), a test that should be done with appropriate probes in BM samples after CD19+ cell enrichment [2]. Alternative methods, such as SNP arrays [10] or whole genome sequencing [33] in samples with CD19+ enrichment may also be used, but they cannot be considered for daily laboratory practice.

For *TP53* mutation assessment, see below.

MYD88 gene mutations

Should MYD88 mutation testing be performed in all cases of suspected WM?

The detection of mutated *MYD88*^{L265P} alone is insufficient to diagnose WM. However, the panel affirms that the presence of such mutation can support the diagnosis of WM in the appropriate clinicopathological context. The absence of *MYD88* mutation does not exclude the diagnosis of WM [59], but recent data on WM with high-sensitivity techniques and whole *MYD88* gene sequencing [41], including those presented at the IWWM-11 [32,60], reveal that these cases are extremely rare. Thus, 96% of WM cases harbor the *MYD88*^{L265P} mutation [60], and around 4% harbor another *MYD88* mutation [32]. In addition, the presence of *MYD88*^{L265P} mutation is rarer in other B-LPDs [22,28–30], with the exception of lymphomas presenting at immune-privilege sites [61]. Accordingly, the absence of this mutation should at least raise questions regarding the validity of a WM diagnosis, while the presence of the mutation would support review the pathological findings and to search for an IgM monoclonal protein for considering the potential diagnosis of WM. With these data, the panel recommends that the *MYD88* mutation must be investigated when a WM diagnosis is considered.

The recommendation for *MYD88* gene mutation testing should be followed especially when the patients are being considered for BTK inhibitor therapy due to their impact that they have on the quality and duration of the response to this class of drugs.

What platform should be recommended for MYD88 mutation testing?

MYD88^{L265P} testing is required using molecular techniques with a detection limit of at least 1×10^{-3} (capable to detect 1 mutated allele among 1000 normal alleles). AS-PCR and droplet digital PCR (ddPCR) can provide appropriate reproducibility and sensitivity on unselected BM samples [20–22,24,62]. Sanger sequencing and/or NGS can also provide reliable results, but they require the use of BM CD19+ selected cells [19]. Cast-PCR is also another useful technique when only low amounts of DNA are available, and can be used in unselected BM samples [26]. BM trephines may also be used for DNA extraction and mutational screening, especially in samples with high percentage of infiltrating tumor cells [63]. Although no well-designed direct comparative studies have been provided, current data suggest that ddPCR is the most sensitive technique for *MYD88*^{L265P} testing in BM samples, providing a sensitivity of 0.035% (10 mutated copies in 30,000 wild-type copies) [24] and a detection capacity of 96% in WM cases [25,60].

MYD88^{L265P} test by AS-PCR in unseparated BM samples is sufficient for a correct assessment in BM and it is covered in many countries in Academic laboratories and reference hospitals [2]. For the remaining centers in which it would not be available, it should be covered by sending samples to specialized laboratories, which is likely to be reimbursed. More sensitive techniques (ie, ddPCR)

should be used before accepting a definitive negative result if the initial test does not identify the *MYD88*^{L265P}. Such techniques are limited to some countries and some centers and probably not reimbursed. In this case, they should be covered via centralizing networks.

Should all PCR-negative MYD88^{L265P} patients be referred for NGS?

The panel agreed that for patients who are negative for *MYD88*^{L265P} by ddPCR, the complete sequence of the *MYD88* gene should be evaluated, if available. Non-L265P *MYD88* mutations have been identified in 3% to 5% of patients with WM, including R209C, V217F, S219C, M232T, and S243N [32,33]. During the IWWM-11, the international effort FIL_BIOWM provided a frequency of 3.7% of non-L265P *MYD88* mutations among 244 analyzed WM patients [32]. Non-L265P *MYD88* mutation detection can be very relevant when BTK inhibitors are being planned for the therapy since ibrutinib seems to work optimally in *MYD88* mutated patients independently of the type of mutation [31]. WM cases confirmed as lacking on *MYD88* mutations are difficult to be categorized [59,64], although the CP3 consider that the diagnosis of WM cannot be excluded in the absence of a *MYD88* mutation if the clinicopathologic features are consistent with this diagnosis [1]. NGS and Sanger sequencing of BM samples can be used to detect the *MYD88* mutations outside the L265P site, but Sanger sequencing usually does not have optimal sensitivity, especially for samples not enriched in CD19+ cells [2]. Accordingly, the panel recommends including probes covering the entire *MYD88* gene in all NGS-targeted panels designed to be used in the diagnosis B-LPD and monoclonal gammopathies.

CXCR4 gene mutations

Should CXCR4 mutation testing be performed in all cases of suspected WM?

A *CXCR4* mutation is associated with symptomatic hyperviscosity and, in asymptomatic patients, is associated with a shorter time to first treatment and can aid in diagnosing some cases since it is rarely observed outside of WM [18]. In addition, *CXCR4* mutation subtypes (nonsense, NS, and frameshift, FS) may impact response and survival outcomes in WM patients treated with ibrutinib, with *CXCR4* NS having lower odds of major response and worse progression-free survival (PFS) than *CXCR4*^{FS} [65]. These findings must be confirmed and biologically explained, but they could be very relevant in clinical management, so they are should be investigated. Therefore, *CXCR4* mutational testing is highly recommended to unveil treatment resistance mechanisms and anticipate slow responses.

All these recommendations for testing mutations of the *MYD88* and *CXCR4* genes should be followed when the patients are considering therapy with BTKis due to their impact on the quality and duration of the response to such drugs.

What platform should be recommended for CXCR4 testing?

The most extended technique for *CXCR4* mutation testing is Sanger sequencing in DNA from BM samples with CD19+ cell enrichment. However, *CXCR4* mutations are frequently subclonal, which may necessitate more sensitive techniques [66]. Accordingly, the panel resolved to recommend the use of NGS targeted panels to test the presence *CXCR4*^{WHIM} mutations in CD19+ selected cells, when feasible. The panel recognizes this methodology is not available in many centers and cannot be considered mandatory. However, due to the clinical informativeness of these mutations, the

panel advocates for development of network or other approaches to improve feasibility.

Considering the difficulties of the *CXCR4* mutational testing with Sanger and NGS in routine practice, there may be an alternative scenario in which testing only for *CXCR4*^{WHIM} NS mutations is prioritized. *CXCR4*^{WHIM} NS mutations seem to be associated with increased risk of progression and/or death compared to *CXCR4*^{WT} and *CXCR4*^{WHIM} FS mutations [65]. Almost 90% of *CXCR4*^{WHIM} NS mutations are located in S338, either as a stop codon (C→G in 54% or C→A in 25%) or a FS mutation (21%) [38]. Thus, the result of an AS-PCR assay targeting *CXCR4*^{S338X} might be sufficient to decide on the use of ibrutinib in WM patients [67]. The panel agreed that this approach would help to identify the 25% of WM patients who are less sensitive to ibrutinib [68], and could be addressed by AS-PCR [67] or ddPCR [23], especially when NGS is not available.

Other genes and situations

Should TP53 mutations be assessed during the WM diagnostic evaluation?

TP53 mutations should be tested in all WM patients in whom therapy is going to be started and should be repeated before each new line of therapy. The method for mutation assessment of the *TP53* gene should be at least Sanger sequencing in genomic DNA extracted from BM CD19+ selected cells. Considering that *TP53* mutations may be present in clonal subpopulations only, the use of an NGS panel is preferred, as for *CXCR4*^{WHIM} mutations. With the availability of highly effective new drugs for WM, the assessment of *TP53* abnormalities could be very useful for the design of the treatment and monitoring strategy. In asymptomatic WM patients, we do not have enough evidence to make this assessment mandatory, but it is recommended if available.

Should FISH evaluation of any other genetic abnormality be scheduled in the diagnostic evaluation?

Given the position of WM between B-LPDs and MM, detection of trisomy 4, del13q, 11q abnormalities and 14q32 translocations might be of interest, but the current evidence and clinical applicability make these tests not to be considered at this time.

Should TP53, MYD88, or CXCR4 assessment be repeated in relapsing or refractory patients?

Considering previous results and data presented in the Madrid workshop [35], *TP53* abnormalities can be relevant for prognostic predictions and for adjusting therapy in our patients. Such abnormalities may not be detectable or present at diagnosis, but they can be acquired along the disease course, so its assessment may be useful in relapsing or refractory patients. *MYD88* and *CXCR4* mutations are usually present at diagnosis, and their evaluation at other time points may be helpful to assess changes in BM tumor burden following therapy, but this should be considered only in research contexts.

Other abnormalities: point mutations of less frequently mutated genes and gross in/dels can be assessed by NGS, FISH, or SNP analyses in CD19+ BM samples, but their value is not well established and therefore should be restricted to research studies.

Should TERT be evaluated as part of the WM workup (see also for CP4)?

In the ASPEN trial, a *TERT* mutation was found in 9% of patients [35]. In the pooled analysis of the arms with *MYD88* mutated patients, those with mutations in the *CXCR4*, *TP53*, and *TERT* genes

had a trend toward to lower deep response rate (very good partial response plus complete response), as well as a less favorable progression-free survival (PFS) compared to patients with the respective wild-type alleles (HR=1.32, 2.15, and 1.79, respectively). The median time to the deep response also appeared longer in patients with mutant alleles. Based on these findings, *TERT* mutations could be a prognostic factor for patients that are candidate to 1st and 2nd generation BTKis. However, these results are still isolated (1 single trial) and should be validated in other series or real-life studies. We also lack on a biological background justifying a more aggressive disease when *TERT* is mutated in WM cells. In addition, the extent to which *TERT* mutations do or do not belong to CHIP in WM patients still needs to be clarified, since these mutations are frequently observed in the general age-matched population [51]. The panel proposes to include this gene in the future genetic research studies that are planned to be done in prospective and retrospective series of WM patients (as well as in other studies of other indolent lymphomas and monoclonal gammopathies).

Final considerations

While formulating these recommendations, the panel tried to include consideration of economical aspects and access issues that could affect their implementation while also balancing the need for accurate diagnosis that is required in WM. The panel acknowledges regional and international differences in circumstances, accessibility and reimbursement policies. However, weighing these constraints against the potential expense of current widely-approved drug therapies, the panel believes it is worthwhile to pursue the most complete molecular diagnosis in all IgM monoclonal gammopathies (Fig. 1). With these thoughts in mind, the tests that should be considered essential for patients initiating a certain therapy are the evaluation of 6q, 17p, *MYD88*, *CXCR4* and *TP53*. These tests in other situations, and/or other molecular/cytogenetic tests, should be considered as optional.

Due to the low incidence of WM and related disorders, the general recommendation is to include these patients in clinical trials to improve the knowledge of this disease and of the patients who suffer from it. The panel considers a similar recommendation for the molecular diagnosis of this disease: whenever pos-

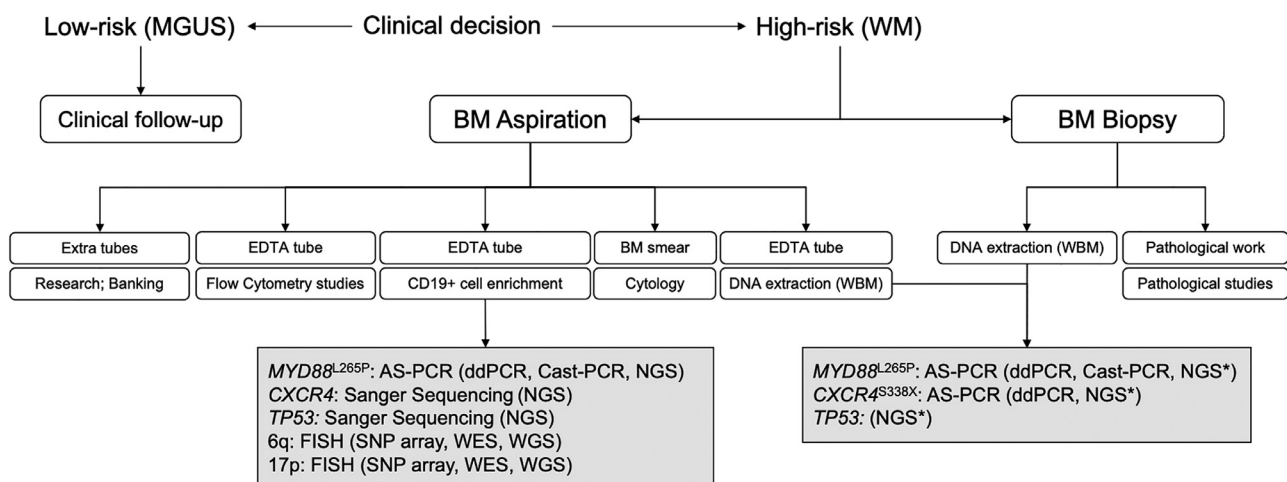
sible, all WM patients should be included in research studies investigating known and yet-to-be-discovered molecular abnormalities. More importantly, all data from such studies should be shared between scientists via national and international registries, data repositories, and cooperative scientific collaborations. Only through these efforts we can effectively evaluate the real diagnostic impact of molecular abnormalities associated with WM and establish their real prognostic value, especially when compared with other clinical and biological prognostic factors.

Author contributions

RGS, MV, ZRH, JSM, MD and SPT prepared, reviewed, and submitted key questions for Consensus Panel 3 (CP3). Questions were reviewed in an open general assembly by attendants of IWWM-11, and additional questions for CP3 deliberations were formulated and submitted. RGS wrote the first draft of CP3 responses, and draft was reviewed and modified by MV, ZH, SPT, and JSM. Final draft was submitted to CP3 general panel for review and commentary. CP3 general panel was composed of individuals with experience in the care of WM patients who attended IWWM-11 and volunteered to be on CP3 panel.

Conflicts of interest

RGS received research funding and/or consulting fees from Novartis, Gilead, Astellas, Janssen, Amgen, Takeda, Janssen, Incyte, Astellas, BeiGene, AstraZeneca, Pfizer, and Pharmacyclics. MV received honoraria from Abbvie, AstraZeneca, Beigene and Janssen-Cilag. SF declares honoraria from Janssen, EUSA Pharma, Morphosys, Incyte, Gilead, Beigene, Abbvie, Servier, Gentili, Italfarmaco, Sandoz. JSM declares participation on advisory boards and consulting services, on behalf of my Institution, for Abbvie, Amgen, BMS, Celgene, GSK, Haemalogix, Janssen-Cilag, Karyopharm, MSD, Novartis, Pfizer, Takeda, Regeneron, Roche, Sanofi, and SecuraBio. AMR received research funding from AstraZeneca, European Hematology Association, Transcan2-ERANET, Italian Association for Cancer Research (Fondazione AIRC); and honoraria from Amgen, Celgene, Janssen, Takeda. LQ declares participation on advisory boards and consulting services for Beigene, Xi'an Janssen, Pfizer, Sanofi,



MGUS: monoclonal gammopathy of uncertain significance; WM: Waldenström's Macroglobulinemia; BM: Bone Marrow; WBM: Whole Bone Marrow; FISH, fluorescence in situ hybridization; AS-PCR: Allele Polymerase chain reaction; ddPCR: droplet digital PCR; Cast-PCR: Competitive Allele-Specific TaqMan PCR; NGS, next-generation sequencing (either panels or whole sequencing); SNP: single nucleotide Polymorphism; WES: Whole exome sequencing; WGS: Whole Exome Sequencing; NGS*: results could be questionable in case of low infiltration or BM hemodilution

Fig. 1. Proposed workup for molecular assessment in IgM monoclonal gammopathies. The minimum required results are sited in the first place of each line, while the alternatives are in parenthesis, although they can be more sensitive than the technique in the first place.

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References

- [1] Campo E, Jaffe ES, Cook JR, et al. The International Consensus Classification of Mature Lymphoid Neoplasms: a report from the Clinical Advisory Committee. *Blood* 2022;140(11):1129–253.
- [2] Dogliotti I, Jiménez C, Varettoni M, et al. Diagnostics in Waldenström's macroglobulinemia: a consensus statement of the European Consortium for Waldenström's Macroglobulinemia. *Leukemia* 2022 :Online ahead of print.
- [3] Treon SP, Xu L, Guerrero ML, et al. Genomic landscape of Waldenström Macroglobulinemia and its impact on treatment strategies. *J Clin Oncol* 2020;38(11):1198–208.
- [4] Braggio E, Keats JJ, Leleu X, et al. Identification of copy number abnormalities and inactivating mutations in two negative regulators of nuclear factor-kappaB signaling pathways in Waldenström's macroglobulinemia. *Cancer Res* 2009;69(8):3579–88.
- [5] Mansouri L, Medeiros LJ, Weber DM, et al. Cytogenetic findings in lymphoplasmacytic lymphoma/Waldenström macroglobulinemia. Chromosomal abnormalities are associated with the polymorphous subtype and an aggressive clinical course. *Am J Clin Pathol* 2001;116(4):543–9.
- [6] Ocio EM, Hernandez JM, Mateo G, et al. Immunophenotypic and cytogenetic comparison of Waldenström's macroglobulinemia with splenic marginal zone lymphoma. *Clin Lymphoma* 2005;5(4):241–5.
- [7] Terré C, Nguyen-Khac F, Barin C, et al. Trisomy 4, a new chromosomal abnormality in Waldenström's macroglobulinemia: a study of 39 cases. *Leukemia* 2006;20(9):1634–6.
- [8] Braggio E, Keats JJ, Leleu X, et al. High-resolution genomic analysis in Waldenström's macroglobulinemia identifies disease-specific and common abnormalities with marginal zone lymphomas. *Clin Lymphoma Myeloma* 2009;9(1):39–42.
- [9] Poulain S, Braggio E, Roumier C, et al. High-throughput genomic analysis in Waldenström's macroglobulinemia. *Clin Lymphoma Myeloma Leuk* 2011;11(1):106–8.
- [10] Poulain S, Roumier C, Galiègue-Zouitina S, et al. Genome wide SNP array identified multiple mechanisms of genetic changes in Waldenström macroglobulinemia. *Am J Hematol* 2013;88(11):948–54.
- [11] Nguyen-Khac F, Lambert J, Chapiro E, et al. Chromosomal aberrations and their prognostic value in a series of 174 untreated patients with Waldenström's macroglobulinemia. *Haematologica* 2013;98(4):649–54.
- [12] Krzisch D, Guedes N, Boccon-Gibod C, et al. Cytogenetic and molecular abnormalities in Waldenström's macroglobulinemia patients: Correlations and prognostic impact. *Am J Hematol* 2021;96(12):1569–79.
- [13] Garcia-Sanz R, Dogliotti I, Zaccaria G, et al. 6q deletion in Waldenström macroglobulinemia negatively affects time to transformation and survival. *British Journal of Haematology* 2021;192(5):843–52.
- [14] Ocio EM, Mateo G, Vidrales B, et al. Cell cycle analysis of Waldenström's macroglobulinemia. *Clin Lymphoma* 2005;5(4):250–2.
- [15] Schop RF, Van Wier SA, Xu R, et al. 6q deletion discriminates Waldenström macroglobulinemia from IgM monoclonal gammopathy of undetermined significance. *Cancer Genet Cytogenet* 2006;169(2):150–3.
- [16] Poulain S, Roumier C, Bertrand E, et al. TP53 mutation and its prognostic significance in Waldenström's macroglobulinemia. *Clin Cancer Res* 2017;23(20):6325–35.
- [17] Gustine JN, Tsakmaklis N, Demos MG, et al. TP53 mutations are associated with mutated MYD88 and CXCR4, and confer an adverse outcome in Waldenström macroglobulinemia. *Br J Haematol* 2019;184(2):242–5.
- [18] Varettoni M, Zibellini S, Defrancesco I, et al. Pattern of somatic mutations in patients with Waldenström macroglobulinemia or IgM monoclonal gammopathy of undetermined significance. *Haematologica* 2017;102(12):2077–2085.
- [19] Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. *N Engl J Med* 2012;367(9):826–33.
- [20] Xu L, Hunter ZR, Yang G, et al. MYD88 L265P in Waldenström macroglobulinemia, immunoglobulin M monoclonal gammopathy, and other B-cell lymphoproliferative disorders using conventional and quantitative allele-specific polymerase chain reaction. *Blood* 2013;121(11):2051–8.
- [21] Varettoni M, Arcaini L, Zibellini S, et al. Prevalence and clinical significance of the MYD88 (L265P) somatic mutation in Waldenström's macroglobulinemia and related lymphoid neoplasms. *Blood* 2013;121(13):2522–8.
- [22] Jiménez C, Sebastián E, Chillón MC, et al. MYD88 L265P is a marker highly characteristic of, but not restricted to, Waldenström's macroglobulinemia. *Leukemia* 2013;27(8):1722–8.
- [23] Moreno DF, López-Guerra M, Paz S, et al. Prognostic impact of MYD88 and CXCR4 mutations assessed by droplet digital polymerase chain reaction in IgM monoclonal gammopathy of undetermined significance and smoldering Waldenström macroglobulinemia. *Br J Haematol* 2023;200(2):187–96.
- [24] Drandi D, Genuardi E, Dogliotti I, et al. Highly sensitive MYD88(L265P) mutation detection by droplet digital polymerase chain reaction in Waldenström macroglobulinemia. *Haematologica* 2018;103(6):1029–37.
- [25] Ferrante MD, D Zibellini S, Marcheselli, et al. Non-invasive screening of MYD88 L265P mutation by droplet digital PCR in IgM-gammopathies: results of the multicentric "BIOWM" trial of the Fondazione Italiana Linfomi (FIL), 11th International Workshop on Waldenström's Macroglobulinemia. Madrid, Spain; 2022:Abstract #13.
- [26] Bagratuni T, Markou A, Patseas D, et al. Determination of MYD88L265P mutation fraction in IgM monoclonal gammopathies. *Blood Adv* 2022;6(1):189–199.
- [27] Willenbacher W, Willenbacher E, Brunner A, Manzl C. Improved accuracy of discrimination between IgM multiple myeloma and Waldenström macroglobulinemia by testing for MYD88 L265P mutations. *Br J Haematol* 2013;161(6):902–4.
- [28] Martínez-Trillos A, Pinyol M, Navarro A, et al. Mutations in TLR/MYD88 pathway identify a subset of young chronic lymphocytic leukemia patients with favorable outcome. *Blood* 2014;123(24):3790–6.
- [29] Gachard N, Parrens M, Soubeyran I, et al. IGHV gene features and MYD88 L265P mutation separate the three marginal zone lymphoma entities and Waldenström macroglobulinemia/lymphoplasmacytic lymphomas. *Leukemia* 2013;27(1):183–9.
- [30] Mansouri L, Thorvaldsdottir B, Sutton LA, et al. Different prognostic impact of recurrent gene mutations in chronic lymphocytic leukemia depending on IGHV gene somatic hypermutation status: a study by ERIC in HARMONY. *Leukemia* 2023;37(2):339–47.
- [31] Treon SP, Xu L, Hunter Z. MYD88 mutations and response to ibrutinib in Waldenström's Macroglobulinemia. *N Engl J Med* 2015;373(6):584–6.
- [32] Varettoni M, NFerrante MPérez-López, JDrandi DZibellini, et al. Genomic studies in WM: first results from the BIOWM study of Fondazione Italiana Linfomi (FIL). In: IWWM ed. 11th International Workshop on Waldenström's Macroglobulinemia. Madrid, Spain: IWWF; 2022.
- [33] Hunter ZR, Xu L, Yang G, et al. The genomic landscape of Waldenström macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. *Blood* 2014;123(11):1637–46.
- [34] Jiménez C, Prieto-Conde MI, García-Álvarez M, et al. Unraveling the heterogeneity of IgM monoclonal gammopathies: a gene mutational and gene expression study. *Ann Hematol* 2018;97(3):475–84.
- [35] Dimopoulos MO, D'Sa S, Jurczak S, et al. ASPEN biomarker analysis: response to Bruton Tyrosine Kinase Inhibitor (BTKi) treatment in patients with Waldenström Macroglobulinemia (WM) harboring CXCR4, TP53, and TERT mutations. In: 11th International Workshop of Waldenström Macroglobulinemia. Madrid, Spain; 2022.
- [36] Roccaro A, Sacco A, Jimenez C, et al. C1013G/CXCR4 acts as a driver mutation of tumor progression and modulator of drug resistance in lymphoplasmacytic lymphoma. *Blood* 2014;123(26):4120–31.
- [37] Cao Y, Hunter ZR, Liu X, et al. The WHIM-like CXCR4(S338X) somatic mutation activates AKT and ERK, and promotes resistance to ibrutinib and other agents used in the treatment of Waldenström's Macroglobulinemia. *Leukemia* 2015;29(1):169–76.
- [38] Kaiser LM, Hunter ZR, Treon SP, Buske C. CXCR4 in Waldenström's Macroglobulinemia: chances and challenges. *Leukemia* 2021;35(2):333–45.
- [39] Liu Q, Chen H, Ojode T, et al. WHIM syndrome caused by a single amino acid substitution in the carboxy-tail of chemokine receptor CXCR4. *Blood* 2012;120(1):181–9.
- [40] Castillo JJ, Sarosiek SR, Gustine JN, et al. Response and survival predictors in a cohort of 319 patients with Waldenström macroglobulinemia treated with ibrutinib monotherapy. *Blood Adv* 2022;6(3):1015–24.
- [41] Hunter ZR, Yang G, Xu L, Liu X, Castillo JJ, Treon SP. Genomics, signaling, and treatment of Waldenström Macroglobulinemia. *J Clin Oncol* 2017;35(9):994–1001.

- [42] Varettoni M, Boveri E, Zibellini S, et al. Clinical and molecular characteristics of lymphoplasmacytic lymphoma not associated with an IgM monoclonal protein: a multicentric study of the Rete Ematologica Lombarda (REL) network. *Am J Hematol* 2019;94(11):1193–9.
- [43] Poulain S, Roumier C, Venet-Caillault A, et al. Genomic landscape of CXCR4 mutations in Waldenström Macroglobulinemia. *Clin Cancer Res* 2016;22(6):1480–8.
- [44] Jiménez C, Alonso-Álvarez S, Alcoceba M, et al. From Waldenström's macroglobulinemia to aggressive diffuse large B-cell lymphoma: a whole-exome analysis of abnormalities leading to transformation. *Blood Cancer J* 2017;7(8):e591.
- [45] Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenström's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenström's Macroglobulinemia. *Semin Oncol* 2003;30(2):110–15.
- [46] Martínez-López A, Curiel-Olmo S, Mollejo M, et al. MYD88 (L265P) somatic mutation in marginal zone B-cell lymphoma. *Am J Surg Pathol* 2015;39(5):644–51.
- [47] Yu X, Li W, Deng Q, et al. L265P mutation in lymphoid malignancies. *Cancer Res* 2018;78(10):2457–62.
- [48] Paiva B, Vídriales MB, Rosiñol L, et al. A multiparameter flow cytometry immunophenotypic algorithm for the identification of newly diagnosed symptomatic myeloma with an MGUS-like signature and long-term disease control. *Leukemia* 2013;27(10):2056–61.
- [49] Kofides A, Hunter ZR, Xu L, et al. Diagnostic next-generation sequencing frequently fails to detect MYD88. *Hemasphere* 2021;5(8):e624.
- [50] Gustine JN, Xu L, Yang G, et al. Bone marrow involvement and sub-clonal diversity impairs detection of mutated CXCR4 by diagnostic next-generation sequencing in Waldenström macroglobulinaemia. *Br J Haematol* 2021;194(4):730–3.
- [51] Kessler MD, Damask A, O'Keeffe S, et al. Common and rare variant associations with clonal haematopoiesis phenotypes. *Nature* 2022;612(7939):301–9.
- [52] Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 2014;371(26):2477–87.
- [53] Ocio E, Schop R, Gonzalez B, et al. 6q deletion in Waldenström macroglobulinemia is associated with features of adverse prognosis. *Br J Haematol* 2007;136(1):80–6.
- [54] Schop RF, Fonseca R. Genetics and cytogenetics of Waldenström's macroglobulinemia. *Semin Oncol* 2003;30(2):142–5.
- [55] Forgeard N, Baron M, Caron J, et al. Inflammation in Waldenström macroglobulinemia is associated with 6q deletion and need for treatment initiation. *Haematologica* 2022;107(11):2720–4.
- [56] Kyle RA, Larson DR, Therneau TM, et al. Long-term follow-up of monoclonal gammopathy of undetermined significance. *N Engl J Med* 2018;378(3):241–9.
- [57] Herrero AB, Rojas EA, Misiewicz-Krzeminska I, Krzeminski P, Gutiérrez NC. Molecular mechanisms of p53 deregulation in cancer: an overview in multiple myeloma. *Int J Mol Sci* 2016;17(12):2003–21.
- [58] Catherwood MA, Gonzalez D, Donaldson D, Clifford R, Mills K, Thornton P. Relevance of TP53 for CLL diagnostics. *J Clin Pathol* 2019;72(5):343–6.
- [59] Hunter ZR, Xu L, Tsakmaklis N, et al. Insights into the genomic landscape of MYD88 wild-type Waldenström macroglobulinemia. *Blood Adv* 2018;2(21):2937–46.
- [60] Ferrante M, Furlan D, Zibellini S, et al. MYD88^{L265P} detection in IgM monoclonal gammopathies: methodological considerations for routine implementation. *Diagnostics (Basel)* 2021;11(5):779–90.
- [61] Kraan W, Horlings HM, van Keimpema M, et al. High prevalence of oncogenic MYD88 and CD79B mutations in diffuse large B-cell lymphomas presenting at immune-privileged sites. *Blood Cancer J* 2013;3(9):e139.
- [62] Jimenez C, Chillon M, Balanzategui A, et al. Detection of MYD88 L265P mutation by real-time allele-specific oligonucleotide polymerase chain reaction. *Appl Immunohistochem Mol Morphol* 2014;22(10):768–73.
- [63] Willenbacher E, Willenbacher W, Wolf DG, et al. Digital PCR in bone marrow trephine biopsies is highly sensitive for MYD88. *Br J Haematol* 2019;186(1):189–91.
- [64] Alcoceba M, García-Álvarez M, Medina A, et al. MYD88 mutations: transforming the landscape of IgM monoclonal gammopathies. *Int J Mol Sci* 2022;23(10):5570–85.
- [65] Castillo JJ, Xu L, Gustine JN, et al. CXCR4 mutation subtypes impact response and survival outcomes in patients with Waldenström macroglobulinaemia treated with ibrutinib. *Br J Haematol* 2019;187(3):356–63.
- [66] Gustine JN, Meid K, Dubeau TE, Treon SP, Castillo JJ. Atrial fibrillation associated with ibrutinib in Waldenström macroglobulinemia. *Am J Hematol* 2016;91(6):E312–13.
- [67] Xu L, Hunter ZR, Tsakmaklis N, et al. Clonal architecture of CXCR4 WHIM-like mutations in Waldenström Macroglobulinaemia. *Br J Haematol* 2016;172(5):735–44.
- [68] Gustine JN, Xu L, Tsakmaklis N, et al. CXCR4^{WHIM} clonality is an important determinant of ibrutinib outcomes in patients with Waldenström macroglobulinemia. *Blood Adv* 2019;3(19):2800–3.