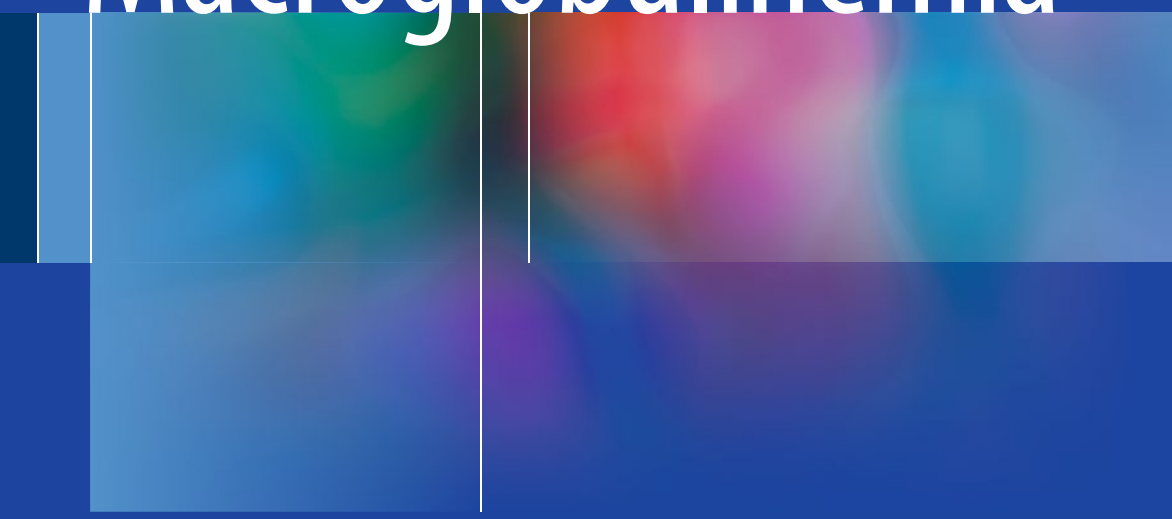
Waldenström's Macroglobulinemia



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ISBN 978-3-319-22583-8 ISBN 978-3-319-22584-5 (eBook)
DOI 10.1007/978-3-319-22584-5

Library of Congress Control Number: 2016953044

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

Foreword

This book is dedicated to Jan Gosta Waldenström who described, more than 70 years ago, two patients who had a disease with features of both multiple myeloma and lymphoma but distinct from both. It was characterized by the presence of a large IgM heavy chain and either kappa or lambda light chains (monoclonal). The molecular weight of the protein approached 1 million and was produced by lymphoplasmacytic cells in the bone marrow. It is now known as Waldenström's macroglobulinemia.

Advances not only in diagnosis but especially the introduction of new, novel agents for this disease in the past 15 years justify a book because of the marked changes in therapy.

A collection of more than 20 experts in the field of Waldenström's macroglobulinemia have collaborated in producing this multiauthored book.

We hope this book will help to promote further interest and update the readers on the diagnosis and management of this uncommon hematologic disorder. We are grateful to the multiple authors from around the world.

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Contents

Part I

Tumor Cells and Microenvironment

Waldenström Macroglobulinaemia: Pathological Features and Diagnostic Assessment

1

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1.1 Morphology and Immunophenotype

Waldenström macroglobulinaemia (WM) is a distinct B-cell lymphoproliferative disorder (LPD) characterised by IgM monoclonal gammopathy and bone marrow (BM) infiltration by lymphoplasmacytic lymphoma (Fig. 1.1) LPL) [1]. The latter is defined as a small B-cell neoplasm composed of lymphocytes, plasma cells and plasmacytoid lymphocytes which does not meet the criteria of other small B-cell disorders [2]. Unfortunately LPL was shown to be one of the least reproducible lymphoma categories by the Non-Hodgkin Lymphoma Classification Project [3]. Similarly, an IgM monoclonal (M) protein, regardless of concentration, cannot be considered indicative of a diagnosis of WM as they may be demonstrable in a proportion of patients with all B-cell LPD, with considerable overlap in serum concentrations [4–6].

Detailed morphologic and immunophenotypic assessment of the bone marrow along with close clinical correlation is therefore required if a definitive diagnosis of WM is to be made. It is good practice that a trephine biopsy be examined in addition to bone marrow aspirate cytology as the pattern of infiltration is important to assess, and it will also provide a better overall assessment of the degree of infiltration [7, 8]. LPL is a LPD comprised of small lymphocytes in which there is morphological evidence of plasma cell differentiation (Fig. 1.1) [2]. This phenomenon is most readily appreciated on trephine biopsy sections and can be further accentuated by staining sections with Giemsa as well as haematoxylin and eosin or more definitively by immunohistochemistry using plasma cell-specific antibodies such as CD138, CD319 and IRF4 (Fig. 1.1c, e, f). The pattern of infiltration is typically interstitial, nodular or diffuse, while a purely paratrabecular pattern is unusual and raises the possibility of follicular lymphoma [7]. Additional morphological clues

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_1

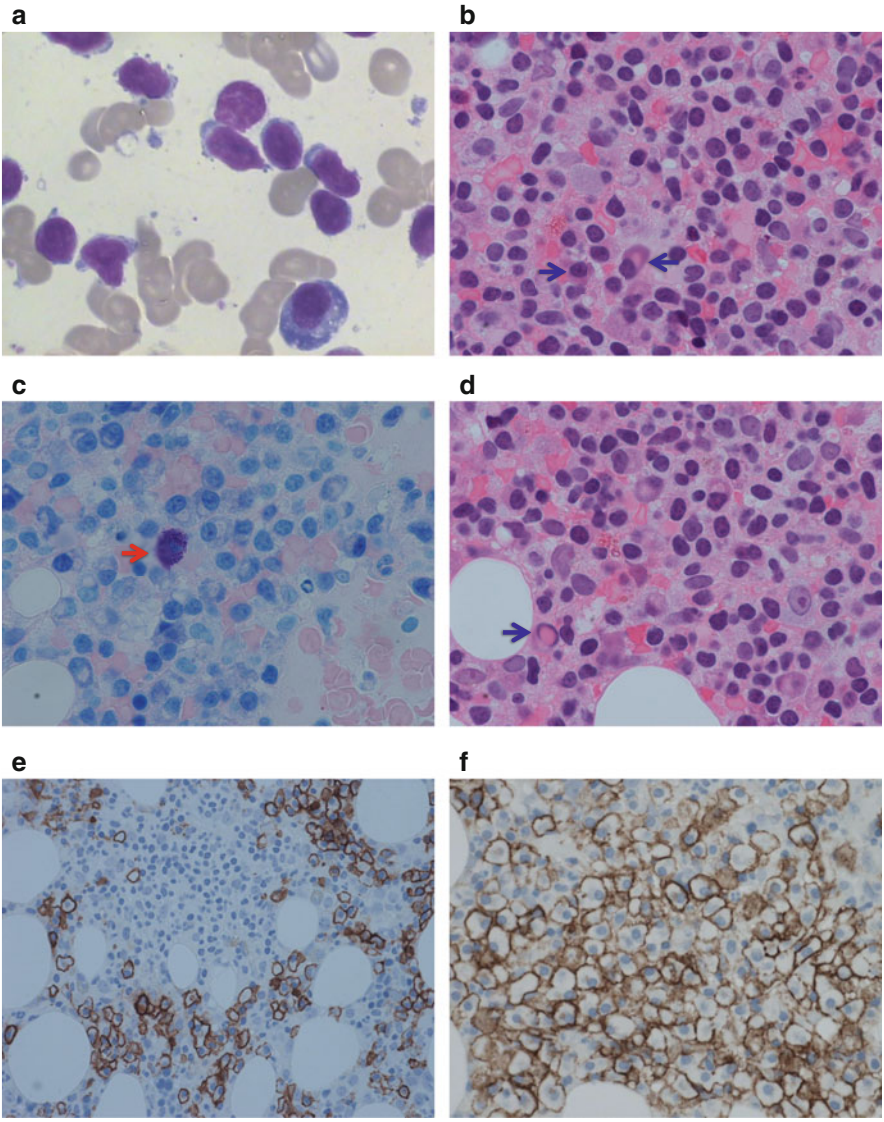

Fig. 1.1 Morphological features of WM. Bone marrow aspirate smears typically show an excess of lymphocytes and plasmacytoid forms with a minority population of plasma cells (image **a**). Trephine biopsy sections show similar appearances with a predominant lymphoid population and variable numbers of plasma cells (highlighted on image **b**). Plasma cell differentiation may be more readily appreciated on Giemsa-stained sections, which also highlight the increase in reactive mast cells typical of WM (image **c**). Immunoglobulin inclusions such as Dutcher bodies are also considered indicative of plasma cell differentiation (image **d**). Plasma cell differentiation may also be highlighted using CD138 immunostaining (image **e**), and this may be particularly prominent in a minority of cases such that IgM myeloma becomes part of the differential diagnosis (image **f**)

can be obtained from the trephine sections, and these include the presence of increased mast cells (readily seen on Giemsa-stained sections or highlighted with CD117 or mast cell tryptase staining) (Fig. 1.1c). These mast cells appear to provide growth and survival signals via CD40-ligand (CD154), APRIL and BLYS, the expression of which is in turn stimulated by soluble CD27 secreted by the LPL cells [9, 10]. Cytoplasmic immunoglobulin inclusions are also characteristically seen in WM and are considered further morphological evidence of plasma cell differentiation. Russell bodies are single or multiple ovoid cytoplasmic inclusions, while Dutcher bodies are also cytoplasmic in nature but occur as single large inclusions, which overlie or invaginate the nucleus (Fig. 1.1d) [11].

WM is a heterogeneous disorder in which there are significant variations in serum IgM levels and BM cellular content. Some studies have failed to demonstrate a correlation between serum IgM levels and overall BM cellular burden [12, 13]. Similarly, prognostic factor analyses have failed to demonstrate a reproducible effect of IgM concentration on overall outcome except in the context of very high levels when additional factors such as cardiovascular and cerebrovascular complications may also contribute to poor survival [14, 15]. However, when flow cytometry is used to quantify bone marrow B-cells and plasma cells, it is noted that while monotypic B-cells are demonstrable in all patients, they do not appear to correlate with IgM concentration. In contrast the percentage of plasma cells, despite representing a minority population in many patients, does correlate with IgM [16]. This is further supported by studies in which the extent of bone marrow B-cell and plasma cell infiltration has been estimated using CD20 and CD138 immunostaining on trephine biopsy sections [17, 18].

Immunophenotypic studies are necessary for a definitive diagnosis of WM, and this may be achieved using either flow cytometry or immunohistochemistry although the former allows for a more extensive assessment of antigenic determinants [1, 7, 19]. In WM it is generally possible to demonstrate both monotypic B-cells and monotypic plasma cells but extended phenotyping is usually only performed on the B-cell component. A recent study has shown that the majority of WM patients have a characteristic immunophenotype. In addition to the almost universal expression of the pan B-cell antigens CD19, CD20 and CD79, approximately 90 % of cases have a $CD22^{weak}$ $CD25^{+}$ $CD27^{+}$ IgM^{+} phenotype but lack expression of CD5, CD10, CD11c, CD23 and CD103 [19]. This provides a robust basis on which to make a diagnosis and in particular allows distinction from marginal zone lymphoma, which frequently has a $CD22^{+}CD25^{-}CD103^{+}$ immunophenotype.

Flow cytometric studies may also provide useful prognostic information. The presence of 100 % light chain restriction within the bone marrow B-cells predicts, in multivariate models, for progression in patients with asymptomatic WM and overall survival in patients with symptomatic disease [19]. This criterion may also be used in conjunction with the International Prognostic Scoring System for WM (IPSSWM). Patients defined as high risk by IPSSWM who also have 100 % light chain restricted B-cells, a combination, which defines 20 % of symptomatic patients, have a particularly poor outcome (median survival 16 months) [14, 19].

It is also recognised that a proportion of patients with LPL have IgG or IgA rather than IgM M proteins. Recent data would suggest that these patients are also characterised by a $CD22^{weak}$ $CD25^{+}$ $CD27^{+}$ immunophenotype as well as the MYD88 L265P mutation [20]. This has clear therapeutic implications in the era of targeted therapies but also suggests that such cases could also be included in WM clinical trials.

1.2 Extramedullary Involvement

Symptomatic extramedullary involvement is uncommon at diagnosis in WM. Lymphadenopathy is present in approximately 15 % of patients at presentation but may be more common at disease progression but is infrequently the primary reason for treatment [13, 14]. Lymph node biopsies in addition to showing a lymphoplasmacytic infiltrate may show extracellular immunoglobulin deposition (both amyloid and non-congophilic) along with a giant cell reaction [21]. In some instances, this histiocytic response is particularly prominent resulting in a so-called pseudo-Gaucher appearance. Splenic involvement is relatively uncommon and splenectomy/splenic biopsy is rarely performed for diagnosis but red pulp infiltration is typical [22].

Examination of the peripheral blood can also be informative in WM. Red cell agglutination and rouleaux formation may be seen although an overt lymphocytosis is rare. Recent studies, using allele-specific PCR for the MYD88 L265P mutation (see below), have demonstrated the presence of low-level peripheral blood involvement in the majority of patients with untreated WM but also a significant proportion of patients with IgM MGUS [23]. Further studies are warranted as non-invasive methods are clearly desirable for both diagnosis and disease response assessment.

In a small minority (<5 %) of patients, involvement of extramedullary sites other than the lymph node and spleen is documented although this does not appear to confer a poor survival outcome. Sites include the lung, central nervous system (CNS), soft tissue, bone and kidney [24]. CNS involvement has been best characterised in WM, the so-called Bing-Neel syndrome. This can be the presenting feature in WM but more commonly occurs as a progression event in patients with an established diagnosis although not necessarily in the context of systemic relapse [24–26]. Diagnosis can be made in the majority of patients through a combination of MRI imaging and CSF examination with flow cytometry showing monotypic B-cells and/or allele-specific PCR demonstrating the MYD88 L265P mutation [27, 28]. In some patients meningeal or parenchymal brain biopsies are however required for definitive diagnosis.

Renal dysfunction is a well-recognised feature of plasma cell myeloma but it is increasingly recognised that a range of renal syndromes can occur in the context monoclonal gammopathies regardless of heavy chain isotype, underlying pathologic diagnosis and disease burden [29, 30]. The concept of monoclonal gammopathy of renal significance (MGRS) has emerged, and a range of pathologies including light chain deposition disease (amyloid and non-amyloid types),

membranoproliferative glomerulonephritis, cast nephropathy, acquired Fanconi syndrome as well as direct tumour infiltration have all been described in WM [31, 32]. Renal biopsy should therefore be considered in WM patients with unexplained renal impairment and/or significant proteinuria.

1.3 Cytogenetics and Molecular Genetics

Conventional karyotyping has limited applicability in WM as it is difficult to obtain tumour metaphases because of the low rate of cell proliferation. There are no disease defining cytogenetic abnormalities but translocations involving the immunoglobulin heavy chain (*IGH*) locus at 14q32 are characteristically rare, and this can be helpful in distinguishing WM from IgM myeloma which is characterised by a high incidence of *IGH* translocations and the t(11;14) in particular (see below) [33–37]. Further data has been obtained with the use of high-resolution methodologies such as array-based comparative genomic hybridisation and single nucleotide polymorphism arrays. These studies have shown a wide range of copy number abnormalities in up to 80 % of patients with a median of three abnormalities per patient. Deletion of chromosome 6q appears to be the commonest cytogenetic abnormality occurring in up to 50 % but other recurrent abnormalities include losses at 11q, 13q and 17p as well as partial or whole gains of chromosomes 3, 4, 18 and X [38–40]. These abnormalities have limited applicability in the diagnostic context but del 6q, del 11q and trisomy 4 appear to be associated with adverse clinical and laboratory parameters, while deletion of *TP53* appears to predict for a short progression-free interval and duration of response [41, 42].

In 2012 whole genome sequencing (WGS) demonstrated the presence of a single T-to-C point mutation in the myeloid differentiation factor 88 (*MYD88*) gene at chromosome 3p22.2. This results in a leucine-to-proline amino acid change at position 265 and has been confirmed to be present in approximately 90 % of patients with WM and results in activation of both interleukin-1 receptor-associated kinase (IRAK) and Bruton tyrosine kinase (BTK) which ultimately results in downstream translocation of nuclear factor kappa B (NFkB) and malignant cell growth (43, 44]. Further studies have confirmed the very high incidence of the MYD88 L265P mutation in WM and its relative rarity in other B-cell LPD and myeloma [45–48]. This has obvious diagnostic utility, and allele-specific PCR strategies have been developed and are in routine use in many laboratories [45, 46, 48, 49]. Allele-specific PCR strategies have reported sensitivities of 0.1–0.25 % but it is recommended that each laboratory determines their own sensitivity thresholds through dilution experiments. There is a clear rationale for the use of allele-specific PCR in the routine assessment of patients with suspected WM but a small minority of patients would be missed with this approach. These patients may have alternative MYD88 mutations such as the S243N or an L265P occurring as a consequence TG-to-CT substitution rather than the single T-to-C point mutation [50]. WGS studies have also demonstrated a number of additional recurrent mutations occurring in >5 % of patients. These involve in decreasing

frequency, the following genes: CXCR4, ARID1A, CD79b, TP53 and MYBBP1A [51].

CXCR4 mutations occur in approximately 30 % of patients and involve the carboxyl terminal of the gene which is responsible for regulating signalling following ligation with SDF-1a (CXCL12). A number of truncating nonsense and frameshift mutations have been described, and these are similar to those described in a known immunodeficiency disorder the warts, hypogammaglobulinaemia, infection and myelokathexis (WHIM) syndrome [51]. These mutations were initially evaluated by Sanger sequencing but recent studies with sensitive allele-specific PCR have suggested a higher incidence of mutation [52]. In addition these studies have demonstrated that CXCR4 mutations are almost exclusively seen in patients with the MYD88 L265P and appear to be subclonal in nature and that multiple mutations may occur in some patients [51, 52]. CXCR4 mutations are also demonstrable in approximately one third of IgM MGUS [52].

Recent studies have also demonstrated that tumour genotype has a profound influence on clinical phenotype, response to targeted therapy and overall survival. Patients who are wild type for MYD88 L265P are characterised by lower levels of IgM and marrow involvement, poor quality categorical responses to ibrutinib and an inferior overall survival outcome [53, 54]. Patients with CXCR4 mutations have a lower incidence of lymphadenopathy, and in particular those with nonsense mutations have higher levels of BM involvement and serum IgM and as a consequence more frequent hyperviscosity syndrome [53].

Immunoglobulin heavy chain variable region (*IGHV*) sequence analysis has been performed by a number of investigators, and this shows evidence of somatic hypermutation without intraclonal diversity in virtually all patients which is consistent with an origin in a post-germinal centre B-cell. Furthermore these studies have also demonstrated preferential use of *VH3* segments and the *VH3-23* in particular with no evidence of canonical motifs indicative of antigen selection [55–59]. This is in contrast to splenic marginal zone lymphoma which is characterised by both mutated and unmutated *IGHV* with a significant proportion of cases showing preferential use of *VH1-2* with canonical motifs [59].

1.4 IgM MGUS

It is important that IgG and IgA MGUS be distinguished from IgM MGUS as the former is a precursor of myeloma and the latter a precursor of WM or other B-cell LPD. IgM MGUS is defined by IgM monoclonal gammopathy without morphological evidence of BM infiltration or other features indicative of an underlying LPD such as lymphadenopathy [1]. It is however recognised that clonal B-cells are demonstrable in a significant proportion of patients without morphological evidence of BM disease. It has been demonstrated that B-cells comprise approximately 2 % of total BM cells in IgM MGUS and that a median of 75 % are monotypic, while only a small minority (~1 %) of patients show complete light chain restriction and >10 % B-cells [19]. This latter observation may provide a more meaningful

and reproducible definition of IgM MGUS. Similarly the MYD88 L265P mutation is demonstrable in approximately 50 % of cases, while CXCR4 mutations and specific copy number abnormalities (+4, del 6q, +12 and +18q) are demonstrable in a significant minority of patients [40, 45, 46, 48, 52, 60].

The role of marrow assessment in asymptomatic individuals is not established but an arbitrary cut-off of 10 g/l has been proposed in some guidelines [61]. Bone marrow examination should however be considered at lower IgM concentrations particularly if the patient is suspected of having an IgM-related syndrome (see below). If marrow examination is performed, it is important that a trephine biopsy be obtained in addition to a marrow aspirate and that flow cytometry and molecular studies for MYD88 L265P be performed as they provide prognostic information. Some asymptomatic patients will be reclassified as asymptomatic WM following marrow examination and by definition have a greater risk of progression to symptomatic disease requiring therapy [62]. Similarly IgM MGUS patients with the MYD88 L265P mutation show a greater rate of progression to symptomatic disease [63]. It is unclear whether this prognostic impact relates to the presence or absence of the mutation per se or reflects disease burden above and below the sensitivity threshold of allele-specific PCR.

1.5 IgM-Related Syndromes

In a proportion of patients with IgM monoclonal gammopathy, clinical features may occur as a consequence of the physico-chemical and immunological properties of the M protein rather than disease burden. A number of syndromes are recognised, and these include anti-myelin-associated-glycoprotein (MAG) peripheral neuropathy, cold agglutinin disease (CAD), cryoglobulinaemia, Schnitzler syndrome and acquired von Willebrand disease [13]. There is limited specific pathological data in these disorders but overall marrow burdens are typically low, and many patients would be formally classified as IgM MGUS.

In CAD the monoclonal IgM (typically kappa) has binding specificity for I/i red cell antigens causing complement activation and phagocytosis by the reticuloendothelial system. The pathological features underlying CAD have been recently described [64]. Morphological evidence of marrow infiltration is demonstrable in the majority of patients but the level of infiltration is low (median 10 %) and a nodular pattern is typical. Monotypic plasma cells are seen but these surround the lymphoid nodules but also show a dispersed interstitial pattern. Monoclonal B-cells are usually demonstrable by flow cytometry in those patients lacking morphological evidence of disease [64, 65]. There is some evidence to suggest that there may be significant immunophenotypic and genotypic differences between WM and CAD. Flow cytometry has shown that CAD B-cells show higher levels of expression of CD5, CD11c, CD23, CD39 and CD200 along with lower levels of CD19, CD79 and IgM compared to WM B-cells [65]. Similarly, *IGHV* sequencing has demonstrated almost universal usage of the *IGHV4-34* in CAD while *IGHV3* genes predominate in WM. The *IGHV4-34* framework region 1 (FR1) is mainly responsible for

I-antigen binding [55–59, 66]. Two studies have also suggested a lower incidence of the MYD88 L265P mutation in CAD [64, 65].

1.6 IgM Plasma Cell Myeloma

In a proportion of patients with WM (up to 20 % in some series), the degree plasma cell differentiation may be such that plasma cells are the predominant cell type and IgM myeloma becomes part of the differential diagnosis (Fig. 1.1f) [67]. This phenomenon may also be seen at disease progression and may be promoted by rituximab-based therapies [68–70] (see below). Although this is a rare scenario, it is essential that a correct diagnosis be made given the availability of targeted therapies in WM and the poor clinical outcome in IgM myeloma [54, 71]. For a diagnosis of WM to be made, a $CD20^+$ IgM^+ B-cell component needs to be demonstrated, and this is most readily done by flow cytometry, which may also demonstrate the WM-specific immunophenotype [19]. Similarly WM plasma cells lack the phenotypic characteristics of myeloma plasma cells such as the absence of CD19 and the expression of CD56 and cyclin D1 [19, 33, 67].

IgM myeloma is characterised by translocations involving the *IGH* locus at 14q32 and the *CCND1-IGH* translocation in particular, and while only limited numbers of cases have been assessed, the MYD88 L265P appears to be lacking [33, 37, 72].

1.7 Assessment of Bone Marrow Response

Criteria for the assessment of post-treatment response were first proposed in 2003 and have been further revised in 2006 and 2013 [73–75]. These have been developed to promote uniform reporting of clinical trial data, and the response categories are predominantly based upon percentage reduction in IgM as this appears to predict progression-free survival in patients treated with rituximab-containing regimens [76, 77]. It has however become clear that there may be discrepancies between serum IgM and bone marrow responses. IgM responses are typically slow with purine analogue and monoclonal antibody-based therapy as these agents selectively deplete the $CD20^+$ B-cell component while sparing the $CD138^+$ plasma cell component of the disease [68, 69]. In this context it is possible to demonstrate significant B-cell depletion in the marrow but suboptimal IgM responses. Satisfactory IgM responses may subsequently be documented at a median of 6 months following the completion of therapy in fludarabine-treated patients for instance [68]. Conversely bortezomib-containing regimens may demonstrate excellent IgM responses but suboptimal BM responses [78, 79].

Previous studies in myeloma and CLL have shown the value of quantitative assessment of residual BM disease as there are demonstrable improvements in outcome with each log depletion [80–83]. Furthermore, it is noted that conventional complete response (CR; immunofixation-negative CR in myeloma and iWCLL

defined CR in CLL) fails to retain prognostic significance in multivariate models for both progression and overall survival when considered along with quantitative residual BM disease assessment by flow cytometry [82, 83]. Accurate and reproducible quantitative methods are clearly desirable in WM, and planned sequential BM assessments are encouraged in clinical trials. The most appropriate methodologies have not been established in WM. Flow cytometry can be used to quantitate residual B-cells and is applicable to most patients on the basis of the WM-specific $CD22^{weak}$ $CD25^{+}$ immunophenotype [19]. In this setting a single study has demonstrated that a residual B-cell burden of >5 % is associated with an inferior outcome [84]. Given the heterogeneity of cellular responses in the BM, it is important to assess residual plasma cells as well as B-cells and to correlate this with quantitative changes in IgM. It is also possible to quantitatively assess residual disease with molecular methods based either on the presence of the disease defining MYD88 L265P mutation or unique immunoglobulin sequence. Molecular methods are likely to offer greater sensitivity compared to flow cytometry but they will not be able to demonstrate any heterogeneity within B-cell and plasma cell responses [81]. Ideally studies should evaluate the peripheral blood in parallel with the BM as non-invasive methods are clearly desirable.

Repeat BM assessments can provide significant value in the management of individual patients particularly if there are uncertainties surrounding IgM responses or persisting cytopenia. In order to make a detailed assessment of residual infiltrates, it is recognised that both BM aspirate and trephine biopsies should be obtained and that these should be routinely supplemented by flow cytometric and immunohistochemistry studies. Attempts should be made to characterise residual infiltrates with respect to their B-cell and plasma cell content, and immunohistochemical assessment of trephine biopsy sections provides the optimal method at present. CD138 and/or IRF4 may be used to demonstrate residual plasma cells, while CD20 may be used to define residual B-cell infiltration although additional markers such as PAX5 may be necessary in rituximab-treated patients due to the loss of CD20 expression which can be seen in post-treatment specimens.

1.8 Disease Progression and Histologic Transformation

Histologic transformation, primarily to diffuse large B-cell (DLBCL), is a well-recognised phenomenon in all forms of indolent B-cell LPD. It has been reported to occur in 5–10 % of patients with WM which is similar to the incidence reported in CLL [7, 85–92]. Histologic transformation events have been thought traditionally to occur within a single B-cell clone through the sequential acquisition of additional genetic events. Analysis of paired samples has demonstrated this linear pattern of disease evolution in CLL. It is estimated that approximately 20 additional/novel events are acquired within the predominant CLL clone [93]. A more complex pattern has been demonstrated in follicular lymphoma with transformed and non-transformed clones appearing to arise by divergent evolution from a common precursor through the acquisition of distinct genetic events [94]. This so-called

branching pattern of disease progression and transformation is clearly more complex than the traditional linear model of evolution. It is unclear whether disease transformation and progression in WM occurs through branching or linear patterns and detailed assessment of sequential biopsies will be required to clarify.

It is also noted that Epstein-Barr virus (EBV) may have a role in the aetiology of some transformation and progression events in patients with B-cell LPD. These may occur within EBV-infected but clonally unrelated B-cell populations as a consequence of both disease-related and treatment-induced immunosuppression. EBV-associated DLBCL is well recognised in CLL typically occurring in the context of highly immunosuppressive therapy such as purine analogues and alemtuzumab. Immunoglobulin sequencing has shown independent B-cell clones and a poor clinical outcome is typical [95–98]. EBV-associated DLBCL has been reported in WM [99].

EBV-associated mucocutaneous ulcer is also a well-recognised consequence of immunosuppression and is characterised by ulcerative lesions affecting the skin, oropharynx and GI tract. Histologically these lesions consist of a population of large "Hodgkin-like" mononuclear cells, which show expression of CD30 and IRF4 within a polymorphous inflammatory background. The large mononuclear cells, similar to Hodgkin Reed-Sternberg cells, show variable expression of B-cell antigens (CD20, CD79 and PAX5) and the transcription factors OCT2 and BOB1 [100, 101]. EBV-associated mucocutaneous ulcer has been described in WM in the context of fludarabine-based therapy and spontaneous regression can occur [102]. Rare cases of peripheral T cell lymphoma have also been described in WM, and these may also be associated with minority populations of $CD30^+$ B-cells showing EBV incorporation [102].

Progression events in which plasma cells dominate have also been described [70, 102]. These can occur in the previously asymptomatic patients but also in heavily pretreated patients and may mimic plasmacytoma/myeloma. Rituximab-based conventional chemotherapy regimens may have a role in this pattern of disease progression given that they have been shown to selectively deplete B-cells in the post-treatment setting [68, 69].

Oncogenic MYD88 mutations, predominantly the L265P, are well described in de novo DLBCL and were originally described in approximately one third of patients with an activated B-cell (ABC) gene expression profile [103]. Subsequent studies have shown a particularly high incidence of the mutation in primary extranodal DLBCL at immune-privileged sites such as the brain, testis and breast [104–114]. It is unclear at this stage whether there is a unifying etiological link between these lymphomas and WM but it is intriguing to note that a recent study has demonstrated the MYD88 mutation in the peripheral blood of patients with primary CNS lymphoma [112].

The complex and diverse nature of disease progression events highlights the importance of detailed pathological evaluation that should include the assessment of EBV by immunohistochemistry and/or in situ hybridisation. The assessment of sequential samples using next-generation sequencing approaches is likely to provide insights into the nature of transformation and disease progression. Ideally

surgical biopsy specimen should be obtained if histologic transformation is suspected and PET imaging can guide biopsy procedures [115, 116].

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Waldenström's Macroglobulinemia Immunophenotype

2

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2.1 Introduction

According to World Health Organization (WHO), in Waldenström Macroglobulinemia (WM), the bone marrow (BM) is involved by a lymphoplasmacytic lymphoma (LPL) in which the infiltrating cells produce an IgM monoclonal component [1]. LPL is composed by a clonal population with small B lymphocytes, plasmacytoid lymphocytes, and plasma cells. Thus, the definition of the pathological cell population is rather wide among the spectrum of the B-cell differentiation, and the addition of a negative requirement in the histopathologic definition of WM has been necessary: the absence of diagnostic criteria for any of the other small B-cell lymphoid neoplasms that may also have plasmacytic differentiation. In fact, the WHO states that the differentiation between LPL and other lymphomas, especially some marginal zone lymphomas (MZL) [2], is not always clear-cut, and some cases are diagnosed only as small B-cell lymphomas with plasmacytic differentiation. This can be more complicated considering that an IgM paraprotein can also be observed in other lymphoma subtypes, as well as in chronic lymphocytic leukemia (CLL) [3]. In addition, the variability is also present at the clinical level, with a behavior ranging from asymptomatic indolent forms to highly symptomatic patients that can show hyperviscosity, organomegaly, or cytopenias, among other symptoms. Thus, differential diagnosis of WM requires the distinction between IgM monoclonal gammopathy of undetermined significance (MGUS),

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_2

asymptomatic WM (aWM) and symptomatic WM (sWM), as well as IgM related disorders (IgMRD) and the above mentioned pure LPL (without WM), MZL, CLL, and even IgM Multiple Myeloma. In summary, WM requires an accurate and comprehensive diagnosis based on well-defined histological, immunophenotypic, cytogenetic, and molecular criteria.

In recent years, multiparameter flow cytometry (MFC) immunophenotyping has become a very useful tool for the diagnosis and monitoring of hematological malignancies, with a particular role as an adjuvant technique for the differential diagnosis of monoclonal gammopathies (MG) [4]. We and others have previously reported that the immunophenotypic profile of tumor cells in WM could be very helpful for the differential diagnosis of WM vs. other lymphomas and IgM monoclonal gammopathies [5–9]. In addition, MFC assessment of residual tumor cells after therapy can be directly correlated with treatment response and may help with therapy selection and prognostic evaluation [10].

Here, we describe the main immunophenotypic features of WM tumor cells, as well as on the role of MFC as an adjuvant technique for the differential diagnosis with other entities, patient' prognostication, and monitoring of treatment response.

2.2 Immunophenotype of WM Lymphocytes

Clonal B-cells from WM patients are typically characterized by the constant expression of pan-B-cell markers (CD19, CD20, CD22) together with monoclonal expression of IgM surface immunoglobulins (sIg) and restricted κ or λ light chain expression (Table 2.1). Interestingly, the predominance of κ+ over λ+ cases is higher in WM than in other B-cell neoplasms (κ:λ ratio of 5:1) [6]. Compared to mature normal B lymphocytes, CD22 expression in WM is consistently lower, while lower staining for CD20 and sIg ($CD20^{strong+}$&sIg^{+}) is only found in selected cases. In a high proportion of cases, clonal B-cells are FMC7 and CD25 positive (>20 % of the cells) for 70 % and 66 % of the cases, respectively. FMC7 is usually heterogeneous, while CD25, when present, is homogenously expressed by virtually all tumor B-cells. Albeit not fully specific (e.g., MZL or CLL), the $CD22^{low}CD25^{+}$ aberrant phenotypic expression profile is a hallmark of WM. Informative markers for the differential diagnosis of hairy cell leukemia and follicular lymphoma (CD103 and CD10, respectively) are constantly negative in WM. Adhesion molecules such as the β_2 integrins are rarely present in the clonal WM B-cells. CD11b is absent and CD11c is rarely positive and, if expressed, at a low intensity [5, 6]. Two antigens are very helpful in the differential diagnosis between WM and CLL: CD5 and CD23 [6, 7, 11, 12]. These two markers are typically expressed in CLL, but they are present in less than one-fifth of WM patients, and when present, they show a heterogeneous pattern of expression. CD38 is present in half of WM cases, and its expression is dimmer than that of progenitor B-cells as well as plasma cells (PCs). CD45 is homogeneously positive in all cases, though lower reactivity can be found in a small group of cases. CD45RA is consistently positive, whereas CD45RO is negative in virtually all patients. WM B-lymphocytes are also typically

Table 2.1 Phenotypic profile of the B-cell and Plasma cell compartment in the Bone Marrow from patients with WM

	B-lymphocytes	Plasma cells		B-lymphocytes	Plasma cells
CD19	100	73	CD38	21 (0–100)	100
–	0 %	0 %	–	70 %	0 %
∓	16 %	69 %	∓	15 %	6 %
+	84 %	31 %	+	15 %	94 %
CD5	5 (0–100)	0 (0–50)	CD45	100	41 (0–100)
–	94 %	92 %	–	0 %	53 %
∓	2 %	8 %	∓	0 %	12 %
+	4 %	0 %	+	100 %	35 %
CD10	1 (0–100)	0	CD45RA	100	41 (0–100)
–	98 %	100 %	–	0 %	53 %
∓	1 %	0	∓	0 %	12 %
+	1 %	0	+	100 %	35 %
CD11b	1 (0–47)	0	CD45RO	6 (0–64)	0
–	98 %	98 %	–	87 %	100 %
∓	2 %	2 %	∓	13 %	0 %
+	0 %	0 %	+	0 %	0 %
CD11c	7 (0–100)	0 %	CD56	0 (0–2)	4 (0–50)
–	89 %	100 %	–	99 %	68 %
∓	8 %	0 %	∓	1 %	32 %
+	3 %	0 %	+	0 %	0 %
CD20	100	48 (0–100)	CD103	0	0
–	0 %	19 %	–	100 %	100 %
∓	6 %	54 %	∓	0 %	0 %
+	94 %	27 %	+	0 %	0 %
CD22	88 (2–100)	4 (0–100)	CD138	0	60 (0–100)
–	11 %	96 %	–	100 %	37 %
∓	72 %	4 %	∓	0 %	6 %
+	17 %	0 %	+	0 %	57 %
CD23	5 (0–100)	6 (0–100)	BCL2	100	96 % (46–100)
–	89 %	84 %	–	0 %	0 %
∓	9 %	13 %	∓	2 %	12 %
+	2 %	3 %	+	98 %	88 %
CD24	90 (0–100)	7 (0–100)	FMC7	55 (0–100)	15 (0–100)
–	2 %	91 %	–	14 %	77 %
∓	17 %	3 %	∓	62 %	14 %
+	81 %	6 %	+	34 %	9 %
CD25	90 (0–100)	3 (0–100)	HLA-DR	84 (30–100)	35 (0–100)
–	1 %	91 %	–	0 %	32 %
∓	33 %	7 %	∓	31 %	50 %
+	66 %	2 %	+	69 %	18 %
CD27	58 (0–100)	100	sIgM	97 (40–100)	83 (0–100)
–	9 %	0 %	–	0 %	13 %
∓	66 %	2 %	∓	16 %	13 %
+	25 %	98 %	+	84 %	74 %

Results from 244 patients evaluated at the University Hospital of Salamanca. Results are expressed as median percentage of positive cells for a given antigen, whereas for each specific pattern of antigen expression the percentage of cases is shown (taken from [5])

positive for CD24, HLA-DR, and BCL2, whereas the memory-associated antigen CD27 is heterogeneously expressed (from negative to dim-positive) in more than half of WM patients [5, 6]. BCL2 and PAX5 usually show nuclear immunoreactivity, which requires to evaluate them by immunohistochemistry in tissue sections [13]. CD79b and CD81 are constantly positive though lower expression levels can be seen in selected cases; CD200 is positive in 60 % of patients, and CD305 (LAIR1) is usually negative [9]. This last marker is important, because normal cells are usually CD305 positive. Accordingly, the Waldenström's clone most common immunophenotypic expression profile could be summarized as: $CD5^{-}$ $CD10^{-}CD11c^{-}CD19^{+}CD20^{+}CD22^{low}CD23^{-}CD25^{+}CD27^{het}CD38^{het}CD79b^{+}CD81^{+}$ $CD103^{-}CD200^{het}CD305^{-}$. The clonal nature of B-lymphocytes harboring such immunophenotypes must be demonstrated by intracytoplasmic (K or L) light-chain restriction.

Importantly, the above-mentioned phenotype is very useful to distinguish WM from other specific mature lymphoid malignancies, including CLL [9], MZL [14], and MM [5]. However, when different antibodies are used in combination with different conjugates in a multi-tube panel, the criteria that we use for evaluating them (fluorescence intensity, stain index, etc.) are variable. Therefore, the unique staining pattern of each reagent needs to be evaluated in the different cell populations in a multidimensional space defined by all parameters included in a multicolor tube. Thus, the evaluation of immunophenotypic profiles of the WM cell populations must be based on a detailed comparison of the phenotypes of individual cells for several markers together, rather than on subjective interpretation of arbitrary mean fluorescence levels of a list of single markers [15]. Visualization of multidimensional flow data (based on principal component analysis (PCA) combining more than 10 parameters) and selection of the most relevant parameters for optimal discrimination between the relevant cell populations are now possible through new software tools that have proven to be essential for a critical evaluation and interpretation of the immunophenotypic profile of a B-cell lymphoproliferative disorders [16–18]. Accordingly, immunophenotypic protein expression profiles (iPEP) can be generated by merging data from different studies (tubes) with up to 8-color MoAb combinations (or more) for the same sample. The resulting fusion into a single data file contains all information measured for each cell in the sample [19]. Then, the calculation function of the specific software can be used to fill in the values of those antigens not directly evaluated ("missing values"), based on the "nearest neighbor" statistical principle, defined by its unique position in a multidimensional space created by the common parameters (backbone markers). Ultimately, the iPEP, generated for every single clonal B-cell, includes many phenotypic markers plus for ward light scatter (FSC)/side light scatter (SSC) [20]. Then, the iPEP of clonal WM B-cells can be compared with the iPEP of cells from other related diseases or situations. The EuroFlow group has tested this methodology proving to be a very useful approach to evaluate WM iPEP against other B-cell LPD, although it failed to distinguish related disorders such as LPL (including both IgM and non-IgM) and MZL [5]. In a very recent work, we used this methodology, but we have added up to 17 different phenotypic markers to generate

a new specific iPEPof clonal B-cells in 31 cases of WM, and the same approach was conducted to generate the iPEP from patients with MZL and B-cell chronic lymphocytic leukemia (B-CLL) (Fig. 2.1). The most significant markers to discriminate the Waldenström's clone vs. MZL were sIgM, CD79b, and CD305, the first two markers being overexpressed in IgM MGUS/WM while CD305 was upregulated in MZL clonal B-cells [9]. PCA results showed a good separation of the respective iPEPs of these three disorders, which could be a very useful tool for a better differential diagnosis of WM, especially when CLL and MZL present with IgM monoclonal component.

Accordingly, we are currently recommending a single 8-color tube for screening purposes in cases with the suspected diagnosis of WM including the following MoAbs: surface immunoglobulin-M (sIgM), CD25, CD22, CD19, intracytoplasmic kappa (cyIgκ), intracytoplasmic lambda (cyIgλ), CD38, and CD27. While the former combination may suffice for detection, quantification, and identification of WM-resembling clonal B-cells, more comprehensive panels may be informative for an accurate characterization of the WM's clone and particularly for PCA-based discrimination between WM and other B-cell disorders. We currently recommend 4&8-color combinations in a clinical research goal:

(1) CD38/CD45/sIgM/CD27/CD79b/CD19/cyIgκ/cyIgλ;
(2) CD38/CD45/CD20/CD25/CD22/CD19/cyIgκ/cyIgλ;

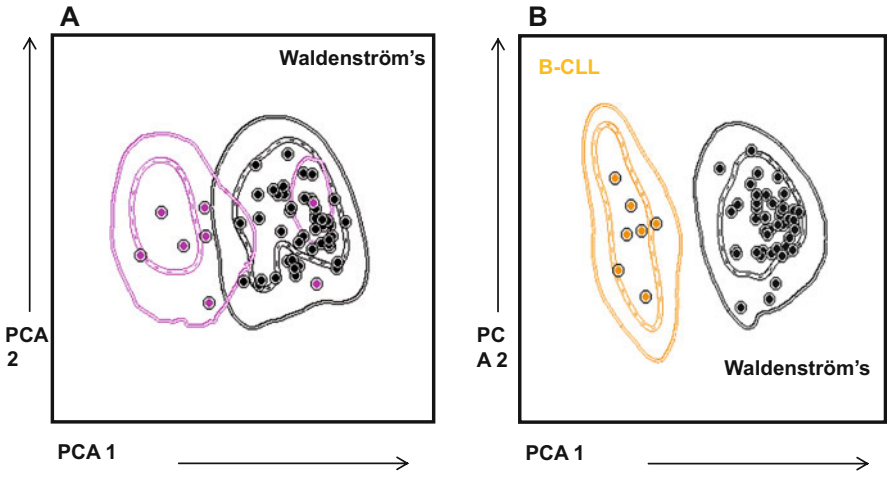

Fig. 2.1 Principal component analysis (PCA)-based classification model for the differential diagnosis between the Waldenström's clone (pooled data from IgM MGUS, smoldering and symptomatic WM patients) versus clonal B-cells from patients with marginal zone lymphoma (MZL, $n = 8$; Panel **A**) and chronic lymphocytic leukemia (B-CLL, $n = 7$; Panel **B**). This figure was originally published in Blood. (Paiva et al., The cellular origin and malignant transformation of Waldenström's Macroglobulinemia. Blood 2015;125:2370-80. © the American Society of Hematology)

(3) CD38/CD45/CD103/CD305/CD11c/CD19/cyIgκ/cyIgλ; &
(4) CD38/CD45/CD10/CD200/CD5/CD19/cyIgκ/cyIgλ [9].

2.3 Phenotypic Comparison with Lymphoplasmocytic Lymphoma Without IgM Component

The Immunophenotypic profile of clonal B lymphocytes in LPL without an IgM component is not well known. Most studies do not distinguish between this form and the classical WM (with BM involvement and IgM component), although it is likely that clonal cells could be different taking into account the differences in the clinical behavior of the disease [6]. Based on relatively small patient series, FMC7 is less frequently expressed (40 %) in LPL cells with no IgM component, and CD5 and CD23 are more frequently positive (in 80 % and 40 % of cases, respectively). Interestingly, CD22 expression, constantly positive in WM, is usually negative in cells without IgM secretion, while CD11c is present in 80 % of cases (it is positive in minor populations of only 15 % of WM patients). No information is available for other markers such as CD19, CD103, CD25, CD10, or sIg expression. Ultimately, based on limited experience, it is not currently possible to discriminate clonal cells from LPL cases fitting or not WM criteria merely based on phenotypic grounds.

2.4 Phenotypical Heterogeneity Among the WM Clone

Clonal heterogeneity has become highly relevant in hemato-oncology with many potential implications in the biological pathogenesis of hematological malignancies [21], including disorders that are quite close to WM, such as CLL [22] and MM [23]. In WM, this variability is already recognized in the pathological definition of LPL, where at least three subpopulations are thought to belong to the clone: small B lymphocytes, lymphoplasmocytes, and PCs. Such heterogeneity has not been well established yet from the molecular point of view in WM, but some data reveal that this entity could also be molecularly heterogeneous, since the genetic landscape is very variable [24, 25] with some data pointing out that some mutations such as C1013G/CXCR4 could be present only in a subpopulation of total tumor cells [26].

In fact, high sensitive multiparameter FCM can also accurately quantify and characterize the PC compartment in WM. The number of BM PCs in WM patients (median: 0.3 %, range: 0.07–2.8 %) is usually within normal range (median: 0.36 %, range: 0.15–0.58 %) [5]. Actually, only in 10 % of WM patients, the percentage of BM PCs is beyond the normal limit. In addition, the PC antigenic profile does not differ from normal BMPCs ($CD38^{+++}CD19^{++/-}CD56^{-}CD45^{++/+}$) although they show the same cytoplasmic immunoglobulin light chain restriction as the IgM component shows. Thus, less than 10 % of WM cases show antigenic aberrancies in PCs, although it is frequent that WM PCs show a relatively high CD20 expression compared to normal PCs. Interestingly, the BMPC rather than B-cell compartment seems to be the main responsible for the production of serum IgM monoclonal

component, since the total number of BMPCs (and not B-cells) is directly correlated with the size of the IgM component [5].

Mast cells (MC) are usually increased in the BM of WM patients [27]. This elevated number is confirmed by MFC, since the number of MC (0.058 % ± 0.13 %, range: 0.01–0.47 %) is significantly higher than in BM from healthy individuals (0.019 % ± 0.02 %; range: 0.002–0.08 %) [6]. Interestingly, this is not applicable to all patients, since only half of all WM cases display MC above the maximum normal limit. In addition, MC from WM patients show a normal morphological appearance (round cells, well granulated with round central nuclei) and immunophenotypic features (CD117+++, CD2−, CD25−, CD69+dim, CD63 +dim, CD35−, FcεRI++) in all patients [6], and no molecular abnormalities have been found in them (unpublished results). Overall, these results indicate that, although MC are increased in the WM microenvironment, they do not belong to the tumor clone.

2.5 Correlation Between Immunophenotype and Disease Characteristics

2.5.1 Immunophenotype to Differentiate Between IgM MGUS and WM

The evaluation of the BM aspirate by MFC reveals a progressive increase of monoclonal B-cells from IgM MGUS (~2 %) to aWM (~9 %) and sWM (~12 %), in such a way that only 1 % of IgM MGUS patients show more than 10 % B-lymphocytes at baseline. By contrast, one-third of smoldering WM and half of symptomatic WM patients show less than 10 % BM B-cells. In addition, there is also a progressive increment of light-chain restricted B-cells from IgM MGUS to WM. Of note, absence of residual normal B-cells is never observed in IgM MGUS patients, whereas we cannot detect normal B-cells in one-fifth of asymptomatic WM patients and in half of symptomatic WM patients [5]. Thus, although the presence of higher tumor burden and clonality virtually excludes the diagnosis of IgM MGUS, no MFC-based criteria can completely discriminate between IgM MGUS and WM. This reflects the thin lines that separate the three clinical stages of the disease [28–30].

As far as the value of individual antigens is concerned to distinguish between the different stages of the disease, some associations have been shown with the level of M-component and/or BM infiltration, but their relevance is low for daily clinical practice [6]. Something similar can be said for profiles, and virtually the same iPEPs can be found in MGUS, aWM, and sWM (Fig. 2.2a) [9]. Accordingly, we cannot use immunophenotyping information to directly distinguish between different aspects of the clinical spectrum in IgM monoclonal disorders, but we will be able to use such information to identify the tumor clone, and then to quantify the tumor load, which at the end will be of great help in the diagnostic discrimination.

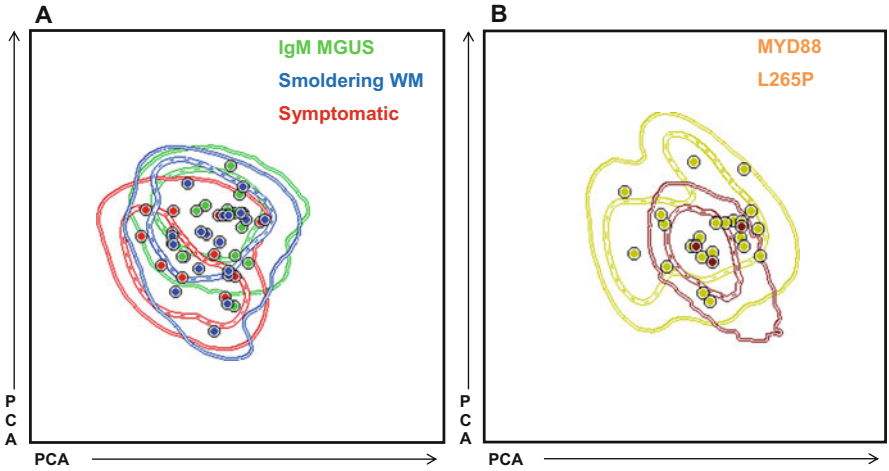

Fig. 2.2 PCA analysis graphical view of IgM MGUS, aWM, and sWM according to disease stage (**A**) and *MYD88* mutational status (**B**). Every patient is represented by a single dot; disease reference groups are represented by *dashed color lines*; *solid lines* represent the standard deviation curve for each group. No differences can be observed between the above mentioned groups. This figure was originally published in Blood. (Paiva et al., The cellular origin and malignant transformation of Waldenström's Macroglobulinemia. Blood 2015;125:2370-80. © the American Society of Hematology)

2.5.2 Immunophenotype to Distinguish Molecular Subgroups

After the initial findings resulting in the identification of a recurrent somatic mutation (L265P) involving the *MYD88* gene in WM [31], other studies confirmed and extended such observations to IgM MGUS (with >50 % mutated cases) [32–34], thereby confirming its role as an early oncogenic event. This mutation promotes NF-kB signaling, JAK kinase activation of STAT3, and secretion of IL-6 [35], which could induce a disease-specific WM signature. However, MYD88 mutated and unmutated patients do not display very different immunophenotyping features. The only consistent difference could correspond to the FMC7 expression, since MYD88 wild-type cells are more frequently positive than mutated cases. CD23 antigen could also be different, because it is more frequently present in WM mutated cases that are also usually positive or strongly positive for CD27 [32]. However, based on PCA of multidimensional flow data, no differences can be found in the phenotype of MYD88 wild-type vs. mutated cases. Accordingly, the typical Waldenström's-related phenotypic aberrancies ($CD22^{+low}$ $CD25^{+}$) [9] were equally noted in both groups.

Nevertheless, PCA analysis of B-lymphocytes from WM patients allows a more accurate comparison of the iPEPs of clonal B-cells from patients with mutated vs. wild-type MYD88 gene. Although clonality is detected in most cases independently of the MYD88 status, comparison of the iPEP of wild-type vs. mutated

MYD88 WM cases showed fully overlapping phenotypes (Fig. 2.2b), although more cases have to be analyzed to reach definitive conclusions. Anyway, these results suggest that the unique phenotypic characteristics of Waldenström's clonal B-cells are of help in the differential diagnosis between WM and other B-cell lymphoproliferative disorders, even in the absence of mutated MYD88. As far as other molecular abnormalities are concerned, such as CXCR4 and ARIC1A mutations, no data are yet available, and more cases have to be incorporated into the immunophenotypic and molecular parallel evaluation.

2.5.3 Prognostic Value of Immunophenotyping

A high degree of BM infiltration by clonal B-cells (i.e., >10 %) is an independent prognostic factor to predict the risk of transformation among a WM patients [36]. Conversely, symptomatic WM patients in whom residual normal B-cells can be detected are characterized by a more indolent disease and superior OS; thus, patients in whom we cannot detect normal B-cells have a median of time to tumor progression of only 2 years, while patients retaining normal B-cells survive without progression more than one decade [5]. This concept is similar to the immuno paresis in smoldering MM patients: those who preserve normal immunoglobulins have a lower risk of progression to symptomatic disease [37]. These results suggest that, similarly to MGUS, MM, or AL amyloidosis [38–44], the degree of clonality (i.e., balance between malignant and residual normal cells) is at least as effective as the evaluation of total tumor burden in predicting outcome. So far, we and others have not reported on the correlation between specific antigen profiles and different patient' outcomes.

2.6 Immunophenotyping for Disease Monitoring After Therapy

Consensus criteria for the assessment of clinical response in patients with WM have been recently updated [45]. Challenges do however remain, based on the variability in kinetics of IgM reduction depending on treatment modalities, especially due to apparent discrepancies between IgM and bone marrow evaluations, which is a key point in clinical trials. IgM responses are typically slow with purine analogues and monoclonal antibody-based therapy, as it appears that these agents selectively deplete the CD20+ B-cell component with sparing of the CD138+ plasma cell component of the disease [10, 11, 46]. In this context, it is possible to demonstrate significant B-cell depletion in the marrow but suboptimal IgM responses. Conversely, treatment with regimens containing bortezomib, everolimus, or ibrutinib may demonstrate excellent IgM responses but discordant bone marrow results [47–49]. Accordingly, the updated response criteria from the VI International Workshop recommended serial bone marrow assessments in all patients enrolled in clinical

trials reinforcing its value as a guide in the routine management of individual patients. Although the value of determining the degree of BM infiltration is not supported by definitive data, it is recognized that both bone marrow aspirate and trephine biopsies should be obtained and that these should be routinely supplemented by flow cytometric and immunohistochemistry studies.

Attempts should be made to characterize the respective B-cell and PC content of residual infiltrates, and immunohistochemical assessment of trephine biopsy sections represents the optimal method [11, 46, 50]. However, the only methodology that has demonstrated a good correlation between the BM assessment after therapy and the final outcome is the evaluation of the residual disease by FCM [10]. Numerous studies in myeloma and CLL have shown that minimal residual disease is detectable by FCM in a significant proportion of patients in conventional CR and that this is highly predictive of outcome, with a reproducible sensitivity of 0.01 % [38, 51]. Similar encouraging but limited results have been observed in WM, by evaluating the presence of residual disease with FCM in the BM of patients actively treated with chemotherapy [10]. The persistence of abnormal (clonal) B-cells after therapy is associated with a poorer conventional response, with very few discordant results. Accordingly, it is possible to observe clearance of the B-cell population despite detectable serum monoclonal IgM protein; such discordance is likely explained by the persistence of residual PC, and this situation is usually translated into a delayed response. On the other side, significant reduction of the M-component can be observed despite persistent residual clonal B-cells, which is frequently associated with early relapses. Overall, these results translate in a better prediction of survival with FCM than with serum determinations of the M protein [10]. Accordingly, the lower the number of BM tumor cells detected the longer the survival, including overall survival, disease free survival, and duration of response. The cutoff point for this evaluation can vary, although 5 % of residual tumor cells would be a minimum desirable limit to be considered as a therapeutic goal. However, in view of the success of the new therapeutic possibilities, it is probable that future investigations will reduce this limit to 10^{-3} or 10^{-4}, as it has occurred for MM and CLL.

2.7 Conclusion

This chapter supports the high value of immunophenotyping in the diagnosis, prognostication, and monitoring of WM since it may help to discriminate WM from other LPD as well as to differentiate WM from IgM-MGUS. Immunophenotyping may also contribute to identify WM patients at higher risk of transformation, as well as WM cases with inferior survival based on accurate estimation of the tumor burden using sensitive immunophenotypic methods. Whether or not MFC can help to distinguish different cytogenetic and molecular subgroups or unique clinical features requires further investigation.

Acknowledgments The authors thank Alicia Antón, Montserrat Ruano, and Rebeca Maldonado for their technical assistance. This work has been partially supported by the grant PI12/02311 from the Spanish "Instituto de Salud Carlos III (ISCIII)" and GRS 847/A/13 from the Consejería de Sanidad de la Junta de Castilla y León and the Cooperative Research Thematic Network (RTICC) RD12/0036/0046-0048-0058 and Solórzano FS/23-2014.

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Predispositions and Origins of Waldenstrom Macroglobulinemia: Implications from Genetic Analysis

3

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3.1 Introduction

Waldenstrom macroglobulinemia (WM), a slowly progressive clonal lymphoid malignancy, involves lymphoplasmacytic BM proliferation accompanied by a monoclonal IgM component. Familial WM is frequent, suggesting that genetic factors predispose to the development of WM [1, 2]. WM is associated with disseminated MALT lymphoma, primary splenic marginal zone lymphoma, nodal monocytoid lymphoma, and B-CLL. Analysis of Ig VDJ regions that identify the WM malignant clone indicates that these cells almost always express somatically mutated variable regions [3–6]. WM is frequently biclonal, with two different clones having differing molecular signatures and anatomic locations in blood or bone marrow [7]. The heterogeneity within the population of monotypic lymphoblastoid B cells in WM was dramatically illustrated by an analysis of CD45 isoform expression [8]. This revealed that the WM clone is dynamic, with the WM clone as a whole undergoing apparently continuous differentiation over time [8], providing early evidence of extensive WM heterogeneity and clonal evolution. These dynamic temporal tides of WM clonal phenotype, in the face of treatment, clearly have potentially profound implications for clinical management of the disease. WM is in many respects "moving target" for therapeutic intervention. That moving target includes cancer stem cells, a heterogeneous hierarchy of

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_3

malignant WM cells in blood and bone marrow, and frequent inter-clonal diversity [4, 5, 7, 9, 10].

3.2 The WM Clone

WM is characterized by extensive morphological heterogeneity that includes lymphocytes, lymphoblastoid cells, and plasmacytoid cells. The extent to which each of these populations contribute to malignant progression is unknown. Informed speculation about originating events that lead to WM requires an accounting of the B cell populations and the clonal expansions that occur in WM patients. Because WM is a B cell malignancy, the IgM rearrangement in the parent B cell that originally gave rise to WM provides a unique molecular signature, termed clonotypic, with which to identify any member of the WM clone regardless or phenotype or morphology. It also provides a means to determine whether or not additional clones exist that are unrelated to the WM clone. As discussed below, nearly all WM clones arise from B cells that have encountered the somatic hypermutation system, with or without antigenic selection and in or out of the germinal center, as previously discussed [5, 10]. Overall, WM appears to arise from a B cell that is programmed to undergo hypermutation in the absence of antigenic selection, to bypass the germinal center and to avoid IgH class switch recombination.

3.2.1 Molecular Analysis of IgM Clonotypic Sequences

Based on clinical and phenotypic evidence, WM cells populate both the blood and bone marrow. Molecular analysis of IgM signatures in individual B cells indicates that during active disease, these share the same clonotypic IgM sequence [5]. However, WM colonization of the blood, confirmed to include B cells that harbor the IgM VDJ signature sequence, is not always reflected by abnormal numbers of B cells [5]. Conversely, when circulating IgM protein and clonotypic IgM transcripts are infrequent in blood, the number of B cells can be abnormally high [5]. This indicates that the number of circulating B cells can be a misleading indicator of malignant clonal expansion but at the same time raises questions about the nature of these expanded B cell populations. In one case, these were clearly polyclonal and unrelated to the WM clone [5], suggesting that one explanation may be a dysregulation of B cell development that increases B lymphopoiesis.

In some cases, however, a highly expanded second clone, unrelated to the WM clone as defined by different IgM CDR3 signatures, resides predominantly in the blood [11]. However, the increased numbers of B lineage cells and partner clones that circulate or are found in bone marrow of WM patients are not necessarily able to undergo the extensive clonal expansion characteristic of the primary WM malignant clone [9]. It seems likely that the clone with cancer stem cell capabilities is most likely to be the clinically relevant clone, based on its ability to undergo

clonal expansion, and thus to regenerate the malignancy post-therapy to mediate relapse. Overall, the clinical impact of inter-clonal diversity remains unknown.

Nearly all IgM CDR3 sequences from WM are somatically mutated [5, 6, 10]. The B cells in WM are unable to undergo class switch recombination [4, 6], perhaps due to an absence of mutations in the switch region of IgH [4]. Together, their mutational profile and inability to engage in class switch recombination suggest an unusual cell of origin [4, 5, 10]. IgM monoclonal gammopathies of undetermined significance (MGUS) are thought to be the most significant risk factor for developing WM [2]. However, since the IgM MGUS analyzed have mutated (normal) IgH switch regions, this means that by definition they could not give rise to a WM clone with unmutated switch regions [4]. This definitively limits the categories of IgM MGUS that can transform to WM.

3.2.2 Clonal Diversity in WM Patients

Although WM is a monoclonal IgM gammopathy, we have found that WM patients also harbor additional unrelated clones that have expanded and become frequent. For 4 of 20 WM patients analyzed, a frequent partner clone harboring a different IgH CDR3 was identified, being either completely unrelated to the nominal WM clone or being distantly related to it, perhaps through a common ancestral progenitor [7]. These second WM partner clones sometimes had anatomically distinct localization as well, with one clone predominant in the BM and a second predominant in blood. For some cases, the partner B cell clones appear to have arisen from separate transformation events. The extent to which each partner clone contributes to pathological symptoms and/or disease progression, whether separately or in synergy, is as yet unknown.

3.2.3 Clonal Dominance

B cell populations are frequently affected by clonal dominance in which one clone exerts inhibitory effects on other clones despite the fact that they may be better fitted to the antigenic challenge in question [12]. Only when dominant clones weaken do they allow subservient clones to expand [13]. The mechanism through which clonal dominance is imposed is as yet unknown but appears to be operative in patients with monoclonal gammopathies [14]. We speculate that in WM patients, multiple transformation events may occur that usually lead to the emergence of one dominant clone but sometimes result in two "dominant" clones. This also occurs in other systemic B cell malignancies [chronic lymphocytic leukemia (CLL) [15] and multiple myeloma (MM)] [11, 14]. Multiple cryptic clones appear to arise and may participate in the disease process as WM progresses, raising questions as to the mechanisms that generate this kind of inter-clonal diversity.

3.2.4 Second Malignancies in WM

The inter-clonal diversity shown to appear in some WM patients may or may not have pathological consequences and may or may not contribute to disease processes. The relative frequency of second hematological malignancies in WM lends weight to the idea that inter-clonal diversity may give rise to new malignancies. There may be a treatment-related increase in incidence of second hematological malignancies in WM [16]. A substantial proportion of WM patients develop diffuse large cell lymphoma (Richter's transformation), acute myeloid leukemia (AML), myelodysplastic syndrome, multiple myeloma, or non-Hodgkin's lymphoma [17, 18], with an incidence of second lymphohematopoietic cancers of 8 % at 15 years [19]. In this context, it is worth noting that a substantial proportion of AML harbor IgH VDJ gene rearrangements [20] and thus may be of otherwise cryptic B cell origin. This prompts the idea that apparent myeloid second malignancies arising in WM patients may mask their origin from a transformed B cell with myeloid morphology, but retain a rearranged IgH VDJ gene that definitively identifies the malignancy as in the B lineage. Some second cancers are likely to reflect inherited genetic predispositions towards lymphoid and/or hematological malignancies and some may arise through acquired mutations that promote transformation events.

3.3 Genetic Predispositions for WM

WM includes familial and sporadic forms of disease. Thus genetic factors certainly play a role in familial forms and likely also contribute to sporadic WM. Genetic influences on WM can be either inherited [e.g., single nucleotide polymorphisms (SNPs)] [21] or acquired (somatic mutations) [22–24]. A series of monoclonal gammopathy-prone families with IgM MGUS and WM as well as other B cell malignancies have been identified in Iceland [25, 26], further supporting the idea that IgM MGUS presents a risk for WM. First degree relatives of WM patients have a high relative risk for WM, with a lower but still increased risk for other lymphoproliferative disorders, including MGUS [1]. Relatives of CLL are at increased risk of CLL but also of WM [27].

Identification of genetic factors that contribute to a given cancer can take two approaches. One frequently used approach is to carry out whole genome or whole exome screening that identifies numerous genes with potential impact, whose biological relevance must be extensively confirmed later. Another approach is to evaluate genetic changes by targeted sequencing within a candidate gene already known to have a functional impact on cancer cells.

3.3.1 Genetic Changes Lead to Aberrant Splicing Events in WM

In this context, we have shown that the hyaluronan synthase 1 (HAS1) gene is aberrantly spliced in WM, with most WM cells expressing the aberrant intronic splicing product [28, 29], making it a promising candidate gene. We have identified a family of five aberrant splice variants of HAS1 expressed in WM and MM [28, 30]. Four of these result from aberrant intronic splicing, characteristic of cancer. Supporting a role in oncogenesis and progression of WM, intronic HAS1 splice variants are transforming [31]. The functional impact of HAS1 spice variants is likely driven through synthesis by these aberrant splice variants of intracellular hyaluronan (HA) and subsequent HA-mediated aberrant regulation of mitotic events [32], as well as via modulation of malignant spread mediated by extracellular HA [29, 30, 33]. Aberrant HAS1 splicing is controlled by genetic alterations in HAS1 introns and exons, including both inherited and acquired mutations affecting splicing modulator sequences [21–23, 34, 35].

3.3.2 Splicing Defects Contribute to Origins of Cancer

It is becoming increasingly apparent that splicing defects play a key role in cancer and that genetic changes in splicing sites or in genes that encode spliceosome components promote aberrant splicing that leads to malignant transformation. In myeloid malignancies, acquired somatic mutations in splicing factors are frequent [36, 37]. Genes encoding splicing factors and/or splicing molecules are also mutated in CLL [38]. The close relationship between WM and CLL [27] and the frequent occurrence of myeloid disorders as second cancers in WM (whatever their origins, see above) are observations consistent with the observed extent of aberrant HAS1 splicing in WM and the influence of inherited and acquired mutations in HAS1 [22, 23].

WM provides a clear-cut example of genetic changes in splicing sites contributing to aberrant splicing events that promote transformation and oncogenesis. In individual WM cells, aberrant splice variants appear to be the dominant species with little or no synthesis of the normally spliced HAS1 gene product [34]. Normally spliced HAS1 and aberrant HAS1 splice variants form dimers and multimers through intermolecular bonds with themselves and with each other. HAS1 family members form heteromeric multimer assemblies that result in aberrant localization of HAS1 and a prolonged half-life inside the cell [31]. One of the intronic HAS1 splice variants found in patients is transforming in vitro and tumorigenic in vivo, when introduced as single oncogene to untransformed cells [31].

Inherited polymorphisms appear to predispose individuals to WM and together with acquired mutations promote aberrant splicing events [22, 23, 35]. Many novel HAS1 mutations found in WM patients are recurrent, defined as present in two or more unrelated patients with WM, and frequently found in both WM and MM [21, 22]. The presence of shared novel mutations among WM and MM patients implies that the early stages of oncogenesis are shared between these two cancers

and that the cell type in which the culminating genetic events occur determines whether an individual develops MM or WM. In turn, the close relationship between WM and CLL suggests that CLL may also arise from early events that are shared with WM and MM. These three systemic B cell malignancies may share at least some genetic predispositions and a common first progenitor with subsequent oncogenic events determining the cancer stem cells from which each malignancy ultimately arises in any given patient. Furthermore, the acquisition of recurrent HAS1 mutations shared by WM and MM but absent from healthy donors may, in the context of other oncogenic events, inexorably lead to malignant disease. As will be discussed below, these observations may provide insight into the originating events that lead to WM.

3.4 Inherited Polymorphisms in HAS1 as Predisposing Factors for WM

Inherited genetic changes, which are present at birth, must by definition have their impact well before transformation events occur. They may predispose to development of a given condition. For cancer, however, predisposing factors must almost certainly act in tandem with later changes acquired after birth and ultimately with further tumor-specific genetic changes in the parent B cells that give rise to WM. Single nucleotide polymorphisms, inherited single nucleotide changes/mutations, alter gene products in a multiplicity of ways that include an enhanced predisposition to malignant diseases. Because WM is usually diagnosed in older adults, it is difficult to screen the general population and quantify the number of individuals with a given genotype that will develop the cancer. As a surrogate, the frequency of a given genotype in the WM population can be compared with that in the overall population to ask whether or not it has been enriched among WM patients. If so, one can infer that those harboring the genotype in question are at higher risk of having WM than those who do not. Cognizant of this constraint, we analyzed HAS1 SNPs in sporadic WM and other systemic B cell disorders (CLL, MM, sporadic MGUS) to determine whether or not these SNPs are enriched in patient populations.

For any given SNP, there are at least two alleles, one on each chromosome, termed major and minor alleles, with genotypes of homozygous major, homozygous minor, or heterozygous. Although alleles harbor the SNPs, the genotype is the genetic unit subject to selection. We analyzed a region of *HAS1* that we knew to be rich in mutations and to be important for aberrant splicing of HAS1 [22, 23, 35]. For over 1400 patients and healthy control groups, from genomic DNA we sequenced *HAS1* from exon 3 through intron 3, to genotype the SNP alleles present in these regions [23]. Of the HAS1 SNPs listed for this region in the NCBI dbSNP database, only five were in Hardy–Weinberg equilibrium, an essential criterion for analysis [39]. This includes two unlinked SNPs in exon 3 and three linked SNPS in HAS1 intron 3 (linkage disequilibrium).

For all of the systemic B cell malignancies analyzed, including MM, CLL, and WM as compared to their age-matched healthy controls, there was a significant association between the minor allele genotypes of the linked *HAS1* intronic SNPs and presence of B cell malignancy [23, 40]. However, for breast cancer patients compared to their matched control group, the presence of *HAS1* SNPs had no predictive value and did not associate with the presence of breast cancer [23]. As well, for all groups there was no association with the *HAS1* exon 3 SNPs, providing an internal negative control. A highly significant risk of having a minor allele genotype was associated with sporadic WM and CLL, with lesser risk for MM and no significant risk for sporadic MGUS. To evaluate familial inheritance patterns in a group with a more homogeneous genetic background, we also sequenced *HAS1* gene segments from a four generation Icelandic family that is prone to monoclonal gammopathies [25, 26]. Hyper-Ig synthesis from hyperactive B cells defined affected members of the family [26]. The minor allele genotypes of the linked intronic 3 SNPs, but not the exon 3 SNPs, were highly significantly associated with affected members of this family as compared to unaffected members who served as the control group. It is interesting that for the Icelandic kindred, the presence of familial MGUS or its surrogate, hyper-Ig synthesis, is strongly correlated with the minor allele genotypes of the intronic HAS1 SNPs [23]. Extrapolating from the Icelandic familial analysis, it seems likely that the infrequent sporadic MGUS having a high probability of transforming to frank malignancy would also have a high frequency of the minor allele genotypes as an indicator of that increased risk. Taken together, our work suggests that intronic *HAS1* SNPs are strongly associated with the presence of systemic B cell malignancies but are not associated with a solid tumor.

3.5 Originating Events in WM

3.5.1 How Might *HAS1* SNPs Promote Cancer?

The effects of *HAS1* SNPs are likely to be multifactorial [29]. Certainly, one consequence of harboring the minor alleles of the linked HAS1 intronic SNPs is a predisposition to aberrant splicing of HAS1 [22, 35] and the resulting changes in enzymatic function by the aberrantly spliced HAS1 variants [30, 31]. These include altered localization of HAS1 family proteins to intracellular compartments [31], de novo synthesis of intracellular HA, and increased synthesis of extracellular HA [30, 31], all of which potentially dysregulate mitosis [32] or alter motility and malignant spread.

The potential dysregulation of mitosis by intracellular HA provides a compelling rationale for the apparent impact of *HAS1* intronic SNPs as predisposing elements in the genome of WM patients [23]. In the context of other genetic events [22, 35], intronic *HAS1* SNPs promote aberrant splicing of HAS1 pre-mRNA to generate aberrant proteins that localize inside the cell. Aberrant HAS1 splice variants synthesize de novo HA, a polysaccharide known to have complex and

multifactorial effects in cancer [29]. Provocatively, aberrantly spliced HAS1 variants appear to synthesize intracellular HA [30, 31]. Although the functional outcomes of intracellular HA are not well described, in addition to their impact on mitosis, they may include interference with the activities of HA binding proteins inside the cell, including altered signaling, remodeled cytoskeletal architecture, and modulated miRNA activities.

It is reasonable to speculate that at least one mechanism through which HAS1 aberrant splicing and intracellular HA could promote cancer may involve mitotic events. The presence of HA within the cell will almost certainly have a functional impact on HA-binding proteins that participate in mitotic events. One such protein is the HA-binding receptor RHAMM (HMMR, CD168) which binds to the mitotic spindle through its HA-binding domain [41]. Intracellular HA may act as a competitor for RHAMM binding to mitotic spindle sites, thereby downregulating the mitotic toxicity of hyperexpressed RHAMM [29, 32, 42].

One potential pathway involves synthesis of intracellular HA by HAS1 splice variants as an enabler of oncogenic activities by RHAMM, an oncogene that is hyperexpressed in WM and MM [28, 42, 43]. Unbalanced RHAMM isoform expression predicts for poor outcome in MM [44]. But perplexingly, RHAMM which is hyperexpressed at levels comparable to those found in ex vivo WM and MM cells is toxic in transfectants, causing mitotic arrest and cell death [41]. Oncogenesis involving RHAMM may be critically dependent on rescue from RHAMM-mediated toxicity. This in turn may facilitate the emergence of viable cancer variants with chromosomal abnormalities that confer enhanced growth potential and escape from normal control mechanisms, thereby facilitating development, clonal expansion, and progression of WM and other cancers [42]. Without a rescue mechanism, potentially malignant cells that hyper-express RHAMM will die, an outcome which benefits the patient who hosts the transformation process. However, if incipient malignant cells gain an ability to implement “rescue” mechanisms that modulate the toxicity of too much RHAMM, they will survive and may lead to frank malignancy, clearly benefiting the cancer at the expense of the patient. We speculate that intronic *HAS1* SNPs promote development of HAS1-mediated rescue mechanisms that abrogate the toxicity of hyperactive RHAMM.

3.5.2 Potential Role of HAS1 in Systemic B Cell Malignancies

Intronic *HAS1* SNPs are associated with the presence of WM [23, 40], with a high degree of significance. A primary functional impact of these intronic SNPs may be to alter HAS1 splicing and thus promote the generation of aberrant HAS1 splice variants. If so, HAS1 splice variants may then act to dysregulate intracellular events and, in particular, promote the survival of genetically abnormal WM cancer variant clones. A variety of evidence discussed above supports this idea. These events may have their greatest impact in early stage progenitor populations that are actively proliferating and undergoing differentiation, cellular processes that may be ripe for disruption by aberrant HAS1 splice variant proteins and their HA products.

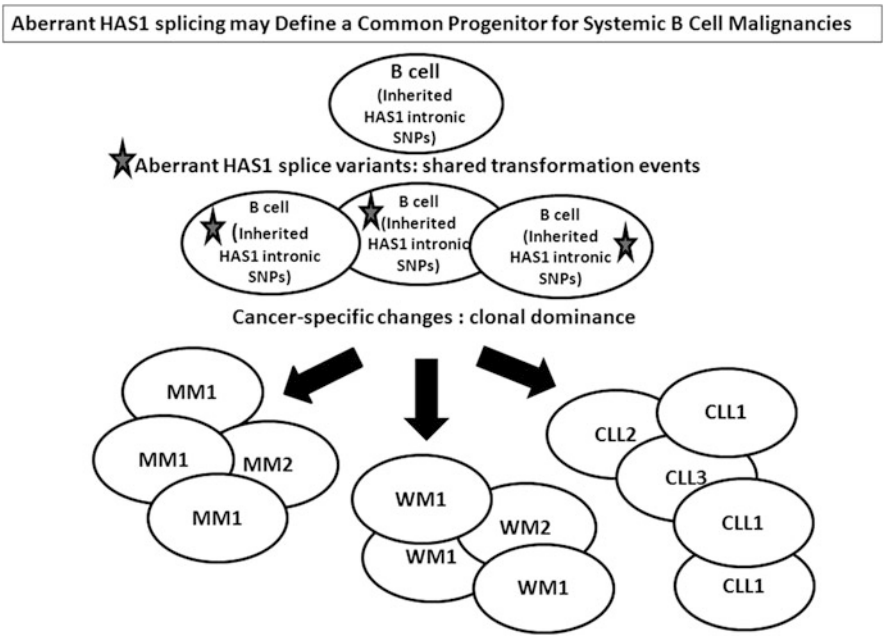

Fig. 3.1 Potential common progenitor for systemic B cell malignancies. Star indicates the presence of the three linked SNPs in HAS1 intron 3

Genetic analysis of WM, MM, and CLL indicates that intronic *HAS1* SNPs strongly associate with the presence of malignancy for all three cancers with the highest risk accruing to WM and CLL [23]. This suggests that the earliest transforming events and the first progenitor population in the hierarchy leading to frank malignancy may be shared by all three of these systemic lymphoid malignancies (Fig. 3.1). For any given patient, the malignancy that ultimately develops from this common progenitor will likely depend on cancer-specific events and the differentiation stage at which they occur.

Divergent commitment to a path that leads to systemic WM, CLL, or MM must occur later in development when disease-specific genetic changes begin to accumulate. However, WM continues to share many novel and recurrent HAS1 mutations with MM [21–23], suggesting that a close genetic relationship persists. In addition, all three cancers, WM, MM, and CLL, are characterized by the frequent occurrence of additional unrelated partner clones that have undergone sufficient expansion to be readily detectable against the significant background of the primary malignant clone. For all three cancers, partner clones lacked intraclonal diversity and in WM failed to undergo Ig class switching, both attributes being characteristic of malignant WM clones, suggesting relatively frequent transformation events. The partner clones in WM arise from independent transformation events. This leads to the supposition that multiple transformation events may continuously occur as WM progresses, with the strongest clone imposing dominance on the majority of partner

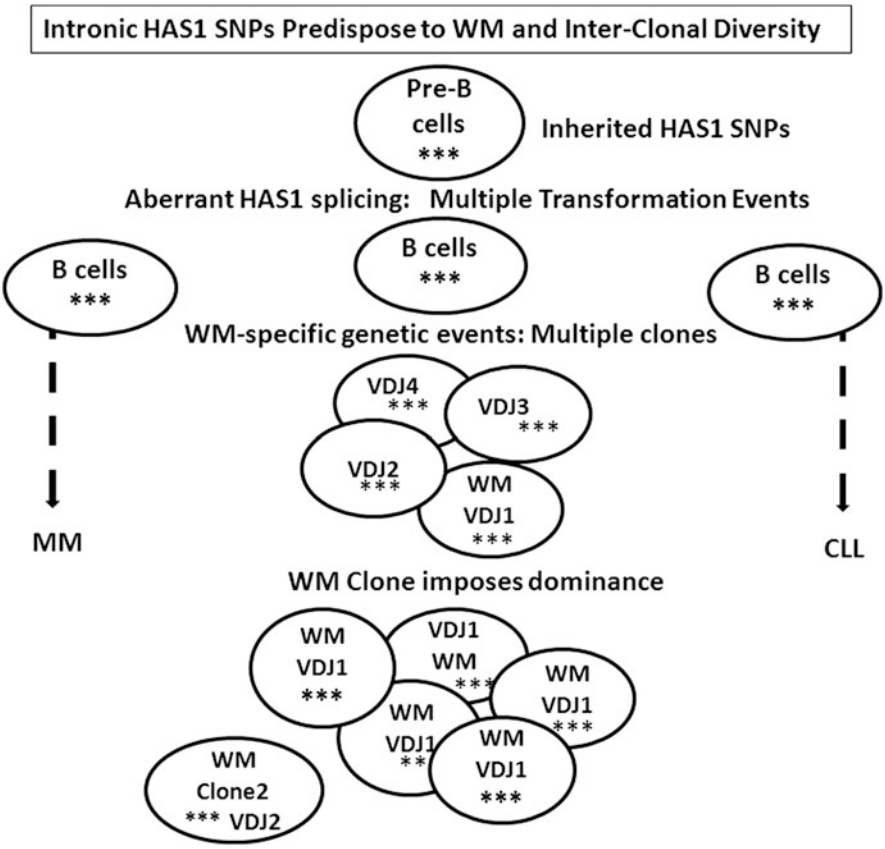

Fig. 3.2 Multiple transformation events and clonal dominance may characterize WM. *** indicates linked HAS1 intron 3 SNPs

clones (Fig. 3.2). Over time and with treatment, this dominance may begin to weaken, thereby allowing subservient clones to expand, as discussed above. This kind of clonal dynamic may occur both within and without the primary clone to expand intra-clonal and inter-clonal diversity.

3.5.3 Hypothesis: WM Undergoes Multiple and Continuing Transformation Events

Overall then, we speculate that transformation events occur on a continuous basis throughout the course of WM. The earliest stages are clinically invisible as the emerging clones "jockey" for dominance, a process that likely involves accumulation of additional genetic changes that promote malignancy and clonal survival. A primary WM clone eventually takes over and expands to the point where it becomes

clinically detectable and has pathological consequences. Initiation of treatment is predicted to alter the clonal balance and may or may not disrupt patterns of established clonal dominance, depending on the way in which it affects the primary WM clone. Partner clones, unrelated or distantly related to the primary WM clone, also may expand and become detectable, but their clinical significance remains unknown.

3.6 Conclusion

Our work suggests that HAS1 plays a central predisposing role in the originating events that lead to WM, as well as participating in transformation and emergence of frank malignancy. It also suggests that HAS1 plays a role in events that underlie disease progression. Aberrant intronic splicing, as occurs in WM, is unique to cancer cells or abnormal clinical conditions. The aberrant HAS1 splice variants are transforming, indicating their ability to transfer malignant characteristics to otherwise "normal" cells. We speculate that the expression of intra-cellular HAS1 splice variants leads to multiple transformation events from which the primary WM clone ultimately emerges. Sequencing has revealed a series of novel *HAS1* mutations that are independently acquired by more than one WM patient, termed "recurrent," indicating that acquisition of *HAS1* mutations must be highly significant for the disease. The fact that some acquired mutations are recurrent means that they are selected among those in whom the disease eventually develops, leading to a high frequency in individuals who have WM. This is characteristic of a genetic event that, once acquired, leads to a high probability of developing WM. Understanding these events is critical for WM patients and has high clinical relevance. The properties of aberrant HAS1 splice variants provide a rationale for the highly significant correlation between the presence of WM and the inherited minor alleles for a set of linked *HAS1* intronic SNPs. There is thus a high probability that *HAS1* is clinically important as a predisposing gene imposing risk for WM as well as being a contributor to oncogenesis and to progression of WM. More precise understanding of the genetic events leading to aberrant splicing in WM may identify novel therapies that disrupt them, for example, use of inhibitory RNAs, and thereby halt the progression of malignant disease in WM patients.

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Cytogenetics in Waldenström Macroglobulinemia (WM)

4

Florence Nguyen-Khac

Conventional Cytogenetic (CC), the first technics allowing whole genome analysis, has been hampered by the low mitotic activity of the malignant B-cells of Waldenström Macroglobulinemia [1]. The use of B-cell mitogens, especially the immunostimulatory CpG-oligonucleotide in combination with IL-2, has increased the rate of successful analysis [2]. Fluorescence in situ hybridization (FISH), a targeted approach, has also improved the rate of detection of cytogenetic abnormalities in WM because of both higher sensitivity and resolution. Overall, up to 50 % of cases exhibit abnormalities by CC and/or FISH. High-resolution genomic technics, such as aCGH (array-based comparative genomic hybridization) and SNPa (single nucleotide polymorphism array), detect genomic abnormalities in 61–83 % of WM patients, with a median of two or three abnormalities per patient according to the series [3, 4]. Of note, more than three abnormalities are detected in symptomatic WM [4].

If specific abnormalities were not recognized until now, the frequency and the association of chromosomal abnormalities distinguish WM from other B-cell malignancies.

Deletion of the long arm of chromosome 6 (6q) is the commonest recurrent chromosomal abnormality identified by several studies. Its frequency ranges from 22 to 54 % depending on the cohort (indolent patients, symptomatic patients either at diagnosis or relapse) and the technics used (cell sorting, cytoplasmic immunoglobulin m-FISH, FISH probes, whole genome arrays) [2–5]. Four separate minimal deleted regions were identified on 6q, two of these including *PRDM1* (*BLIMP1*) on 6q21 and *TNFAIP3* on 6q23, but their direct role in WM pathogenesis

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_4

has yet to be determined [3]. Although 6q deletion is associated with features of adverse prognosis (anemia, hypoalbuminemia, high B2-microglobulin), this abnormality has no adverse impact on patients' outcome [2, 6, 7].

The trisomy 4 was initially described in 8–20 % of WM, involving the whole or partial gain of chromosome 4 [2, 8]. Poulain et al. reported partial gain of chromosome 4q that did not include the centromere [4]. Trisomy 4 is not specific to WM, since it is also observed in myeloid leukemia, but has not yet been reported in other low-grade B-cell malignancies [1]. Trisomy 18 is also frequent in WM (15 %), and we have shown that trisomy 4 was significantly associated with trisomy 18 in WM [2]. The clinical implication of trisomy 4 is not well understood and no candidate gene has been identified to date [8].

Other recurrent abnormalities are described in WM, such as deletion 13q14, deletion 17p13 (involving the *TP53* gene), and deletion 11q22 (involving the *ATM* gene) and trisomy 12, which are observed in less than 13 % of WM cases. Translocations involving *IGH* genes are very rare (2 %) [2]. One study described a gain on 6p (short arm), always secondary to a deletion of 6q, in 16.6 % of WM patients [3]. In our large series of previously untreated patients, we have shown that none of the chromosomal abnormalities influences overall survival. Patients with *TP53* deletion had short progression-free survival and short disease-free survival, and trisomy 12 was associated with short progression-free survival [2]. In WM patients with *TP53* deletion, the status of the remaining copy, wild type and functional or not, still has to be fully established. Deep sequencing, epigenetic status, and functional studies are required to address this point and establish clinical value of P53 (haplo-) deficiency.

Cytogenetic studies have first described recurrent abnormalities, which may play a role in progression and drug resistance of WM. While recent technological advances over the past decade have dramatically improved our understanding of the molecular pathogenesis of the disease, it remains important to investigate additional abnormalities, such as gains and deletions, at both cytogenetic and molecular levels, in order to better characterize individual malignancies, toward personalized therapy.

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Genetic and Signaling Abnormalities in Waldenstrom's Macroglobulinemia

5

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5.1 Introduction

With the advent of next generation sequencing technology permitting large-scale genomic interrogation, many aberrations with implications for the diagnosis, clinical presentation, treatment, and overall survival in Waldenstrom's Macroglobulinemia (WM) patients have been revealed [1–3].

5.2 Somatic Mutations in MYD88

Whole genome sequencing of $CD19^+$ bone marrow (BM) cells from patients with WM led to the discovery of a somatic heterozygous NM_002468.4:c.978T>C mutation in MYD88 (rs387907272) resulting in a leucine to proline substitution p.Leu265Pro (L265P) in over 90 % of WM patients [1]. While not a kinase, MYD88 acts as an adaptor for the Toll-Like Receptor (TLR) and IL1R families by binding directly to the receptor or, as in the case of TLR4, through the adaptor molecule TIRAP. Upon receptor activation, MYD88 undergoes a conformational change promoting homodimerization and subsequent recruitment of signaling proteins including IRAK4 and IRAK1 [4–6]. Somatic activating mutations in MYD88 induce constitutive homodimerization and downstream MYD88 signaling independent of receptor activation [1, 7, 8]. This leads to the recruitment and activation of TRAF6 with subsequent nuclear factor-kappa B (NF-kB) activation (Fig. 5.1a) [8–10]. $MYD88^{L265P}$ can also trigger IRAK-independent NF-kB signaling through direct interaction with BTK [8]. The importance of NF-kB signaling in the growth

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_5

and survival of WM has been well documented in vitro, and inhibition of MYD88 dimerization results in loss of NF-kB nuclear localization and promotes apoptosis (Fig. 5.1b) [1, 11]. The high levels of phosphorylated IRAK4, IRAK1, and BTK are reduced by MYD88 inhibition and are all directly associated with the MYD88 complex [1, 8]. The knockdown or kinase inhibition of BTK is sufficient to reduce NF-kB signaling and promote apoptosis [8]. Moreover, a clinical trial of the BTK inhibitor ibrutinib resulted in a major response (>50 % reduction in serum IgM) in 46/57 (80.7 %) of patients with activating MYD88 mutations while none of the 5 patients who were wild type for MYD88 achieved that level of response ($P < 0.001$) [12, 13]. Activated MYD88 can also promote the transcription of IL6 and HCK in WM lymphoplasmacytic cells leading to the activation of HCK via autocrine signaling through IL6R and IL6ST (GP130 at the protein level) as illustrated in Fig. 5.1a [10, 12]. HCK signaling through the mitogen activated protein kinase pathway (MAPK), and the phosphoinositide 3-kinase (PI3K) pathway has been shown to promote the growth and survival of WM cells making HCK a relevant therapeutic target downstream of MYD88 [12].

The presence of MYD88 L265P in over 90 % of WM patients has been confirmed in large independent series, using multiple modalities including Sanger, next generation sequencing, and polymerase chain reaction (PCR)-based testing [10, 14–18]. MYD88 mutations are typically heterozygous though copy number amplifications and acquired uniparental disomies (aUPD) affecting the MYD88 locus resulting in increased/homozygous mutant expression have been reported [2, 17].

MYD88 activating mutations are not unique to WM and were first described in activated B-cell (ABC) subtyped diffuse large B-cell lymphoma (DLBCL) where they were observed in 29 % of patients and subsequently in 3 % of chronic lymphocytic leukemia (CLL) and up to 75 % of immune privileged DLBCL (i.e., testicular and primary central nervous system lymphoma) [7, 19, 20]. Somatic MYD88 mutations in hematological malignancies are not limited to L265P. In WM, other documented MYD88 mutations include p.Ser219Cys, p.Met232Thr, and p.Ser243Asn, all of which have also been have observed in DLBCL (Fig. 5.1c) [7, 13]. However, WM is distinct in the prevalence of the L265P mutations. In ABC DLBCL, L265P makes up approximately 76 % of MYD88 mutations [7]. In WM, a study of WM patients lacking the c.978T>C L265P mutation revealed alternative mutations in 3/19 (15 %) of patients [13]. With over 90 % of patients expressing the L265P mutation, $MYD88^{L265P}$ likely accounts for over 95 % of the MYD88 mutations in WM [14].

The prevalence of $MYD88^{L265P}$ relative to other MYD88 mutations in WM makes possible its use in diagnostic testing and as a biomarker of disease burden. Highly sensitive $MYD88^{L265P}$ detection techniques such as allele-specific PCR (AS-PCR) can be used to help distinguish WM from a number of related hematological malignancies with lower incidences of $MYD88^{L265P}$ mutations including marginal zone lymphoma (6–8 %), CLL (3 %), and IgM expressing multiple myeloma where $MYD88^{L265P}$ has not been observed (Fig. 5.1d) [18, 19, 21]. This technique can also aid in the diagnosis of central nervous system (CNS)-involved

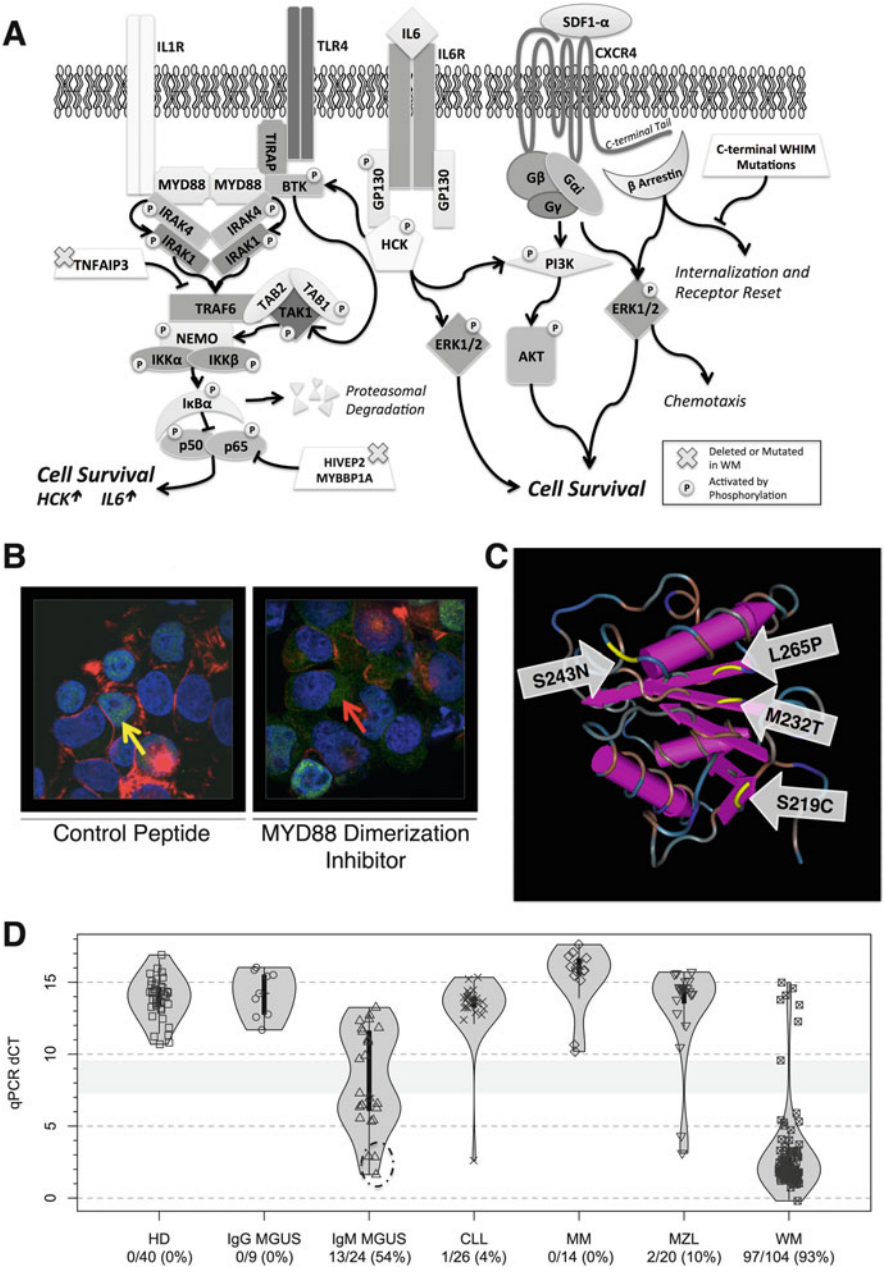

Fig. 5.1 MYD88 and CXCR4 mutations in Waldenström's macroglobulinemia. (**a**) Diagram of MYD88 and CXCR4 signaling in WM. (**b**) Merged data of DAPI nuclear staining (*blue*), plasma membrane F-actin (*red*), and NFkB-P65 (*green*) shows the expulsion of NFkB from the nucleus of BCWM.1 cells following incubation with a MYD88 dimerization inhibitor IMG-2005-5 (Imgenex, San Diego, CA) compared to a scrambled peptide control. (**c**) Structure of human MYD88 TIR domain (MMDB: 108985) showing the location of the somatic mutations observed in

WM, known as Bing Neel syndrome, where $MYD88^{L265P}$ can be detected in the spinal fluid [22]. Notably, $MYD88^{L265P}$ can be detected in approximately 50–80 % of IgM monoclonal gammopathy of undetermined significance (MGUS) cases using AS-PCR assays but not in IgG or IgA MGUS [15, 16, 18, 23]. Likewise, it is IgM, not IgA or IgG MGUS, that typically precedes WM diagnosis, suggesting that $MYD88^{L265P}$ occurs very early in WM oncogenesis and may have some prognostic significance for patients with IgM MGUS [24].

The genetic alteration associated with $MYD88^{WT}$ WM disease is an active area of research. One study of $MYD88^{WT}$ patients reported that two of three (66.7 %) had non-synonymous somatic mutations in MLL2 [2]. These two patients were further noted to have high levels of circulating WM $CD23^{+}$ lymphoplasmacytic cells which is an uncommon finding in WM [25, 26]. MLL2 mutations are also associated with other lymphomas including 89 % of follicular lymphoma and 32 % of DLBCL [27].

5.3 CXCR4 Somatic Mutations

CXCR4 is the next most commonly mutated gene in WM and can be observed in 29 % of patients [2, 3, 28]. These mutations occur nearly exclusively in patients harboring MYD88 mutations. CXCR4 is a G protein coupled receptor (GPCR) that in WM induces cell survival and migration to the BM mediated by its ligand, CXCL12 (SDF-1α at the protein level) [29]. CXCR4 consists of seven transmembrane helices, the loops of which form the extracellular ligand-binding pocket for SDF-1α and the intracellular signaling region responsible for downstream signaling through heterotrimeric G proteins (Fig. 5.1a). The intracellular carboxyl terminal tail (C-terminal) is important for receptor regulation, but does not participate in G protein signaling [30]. Following SDF-1α binding, CXCR4 activates $G_{\alpha i}$ by catalyzing the exchange of its guanosine diphosphate (GDP) for guanosine triphosphate (GTP), releasing the heterotrimer from the receptor and dissociating the $G_{\beta}G_{\gamma}$ heterodimer from $G_{\alpha i}$ [31, 32]. The $G_{\beta}G_{\gamma}$ subunits signal through the phospholipase C (PLC) and phosphoinositide 3-kinase (PI3K) pathways until inhibited by another GDP bound $G_{\alpha i}$. The $G_{\alpha i}$ subunit remains active, inhibiting adenylate cyclase and signaling through PI3K until the GTP is hydrolyzed to GDP by a regulator of G protein signaling family member (RGS). This G protein-based signaling is terminated when the C-terminal tail of CXCR4 is phosphorylated and the receptor internalized. Once the receptor is activated, several kinases including G protein receptor kinase (GRK), and protein kinase C (PKC) family members phosphorylate

Fig. 5.1 (continued) WM. (**d**) Violin plots displaying the density of delta cycle threshold (dCT) values from allele-specific PCR experiments detecting MYD88 L265P in healthy donors (HD), monoclonal gammopathy of undetermined significance (MGUS), chronic lymphocytic leukemia (CLL), multiple myeloma (MM), marginal zone lymphoma (MZL), and WM. Lower dCT values indicate a stronger MYD88 mutant signal

serines in the C-terminal tail. This recruits β-arrestin, inhibiting further signaling and transports the receptor to clathrin coated pits for internalization. During this process, β-arrestins signal through the MAPK pathway [33].

The CXCR4 somatic mutations in WM manifest as nonsense or frameshift mutations exclusively within the C-terminal domain (Fig. 5.2a) [2, 3]. The mutations are typically heterozygous though homozygous and compound heterozygous cases have been observed. Not only are these mutations typically found in MYD88-mutated patients, they are typically subclonal to MYD88 as well [34]. Highly sensitive techniques such as next generation sequencing and AS-PCR for highly recurrent mutations such as p.Ser338Ter have documented subclonal mutation rates of up to 43 %, far higher than the 29 % reported by Sanger sequencing studies [2, 28, 34]. While the clinical significance of these subclonal findings remains to be determined, their presence has implications for tumor evolution and the emergence of a more dominant CXCR4 mutant clone in the future.

The somatic mutational pattern in WM mirrors the germline variants found in familial warts, hypogammaglobulinemia, infection, and myelokathexis (WHIM) syndrome. In both diseases, these mutations occur after the last of the seven transmembrane helices, destroying the C-terminal tail but leaving the regions responsible for ligand binding and downstream signaling via G proteins intact (Fig. 5.2) [2, 35]. The loss of regulatory serines in the C-terminal tail of CXCR4 impairs receptor internalization via β-arrestins, thereby prolonging G protein and β-arrestin signaling [30, 33, 36]. In WHIM syndrome, this results in impaired B-cell class switching and constitutive homing to the bone marrow leading to peripheral neutropenia [37]. While this observation of somatic activation of a GPCR was first described in CXCR4 in WM patients, it affects a regulatory mechanism common to many GPCR chemokine receptors and a similar somatic mutation pattern can be found in the CCR4 c-terminal domain in up to 26 % of patients with adult T-cell leukemia/lymphoma [2, 38, 39].

WM cell lines transduced to express these WHIM-like CXCR4 mutations ($CXCR4^{WHIM}$) exhibited impaired receptor internalization and increased signaling through AKT and MAPK1 compared to CXCR4 wild-type ($CXCR4^{WT}$) or empty vector lines when stimulated with SDF-1α [28, 39]. Pre-incubating with a CXCR4 inhibitor prior to stimulation can block these effects. More robust signaling in the cell lines transduced with the most common nonsense mutation, p.Ser338Ter, compared with two separate CXCR4 frame shift lines was also observed [40].

5.4 Clinical Features Associated with MYD88 and CXCR4 Somatic Mutation Status

The clinical presentation of WM patients at diagnosis is partly determined by MYD88 and CXCR4 mutation status [3, 16, 18]. Patients harboring the $MYD88^{L265P}$ mutation have significantly increased bone marrow involvement, higher serum IgM levels, and lower serum IgA levels. However, those patients who are wild type for

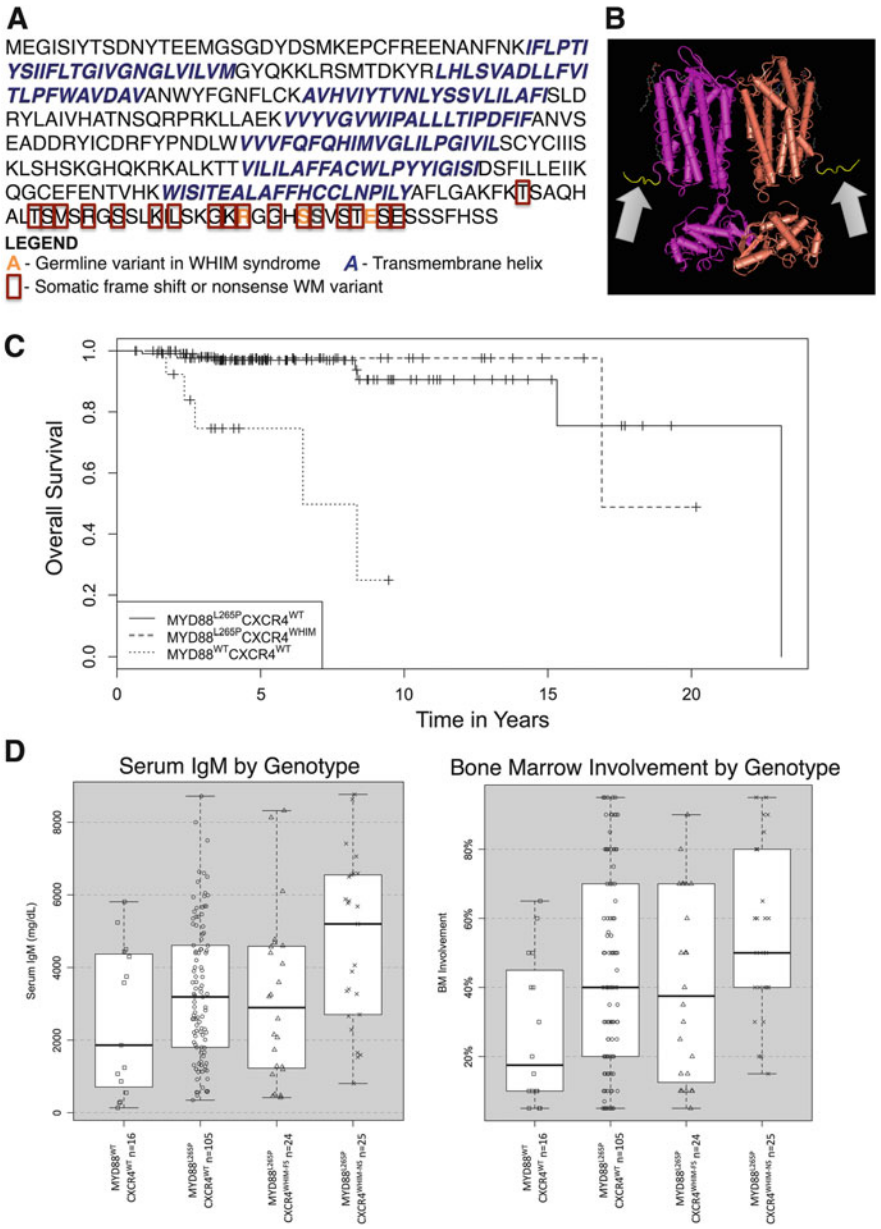

Fig. 5.2 Genomic and clinical findings for MYD88 and CXCR4 mutations in Waldenström's macroglobulinemia. (**a**) Location of the somatic CXCR4 nonsense and frameshift mutations found in 177 WM patient samples compared with germline variants associated with warts, hypogammaglobulinemia, infection, and myelokathexis (WHIM) syndrome based on the full length protein transcript NP_003458.1. (**b**) Crystal structure of homodimerized CXCR4 (MMDB ID: 85801) demonstrating the relative location of the regulatory carboxyl terminal tail domain. (**c**) Log-rank analysis of overall survival based on MYD88 and CXCR4 mutation status of 174 WM patients. The analysis was equally significant when stratified solely on $MYD88^{L265P}$ ($P < 0.0001$ for both). Box plots indicating median, interquartile, and range values by CXCR4 and MYD88

MYD88 have shorter overall survival with a tenfold higher risk of death compared with patients with MYD88 mutations (Fig. 5.2c) [3]. No differences in overall survival based on CXCR4 mutation status were observed. Mirroring the in vitro observations, differences between nonsense ($CXCR4^{WHIM\text{-}NS}$) and frame shift ($CXCR4^{WHIM\text{-}FS}$) can be seen in patient characteristics at diagnosis. Patients with any $CXCR4^{WHIM}$ mutation have decreased rates of adenopathy and patients with $CXCR4^{WHIM\text{-}NS}$ mutations have significantly increased BM involvement and serum IgM levels compared with $CXCR4^{WHIM\text{-}FS}$ or $CXCR4^{WT}$ patients (Fig. 5.2d) [3]. $CXCR4^{WHIM\text{-}NS}$ patients also presented with significantly higher rates of symptomatic disease and serum hyperviscosity syndrome.

WM cell lines BCWM.1 and MWCL-1 transduced with the S338X $CXCR4^{WHIM\text{-}NS}$ mutation demonstrate resistance to a variety of anti-neoplastic agents including the alkylator bendamustine, the nucleoside analog fludarabine, the proteasome inhibitor bortezomib, and the PI3K inhibitor idelalisib in the presence of SDF-1α [39]. In a clinical trial of BTK inhibitor ibrutinib in relapsed or refractory WM, patients with any type of $CXCR4^{WHIM}$ mutation had lower rates of overall and major response [41]. The protective effect of $CXCR4^{WHIM}$ is not universal and one clinical trial using a combination of rituximab, dexamethasone, and carfilzomib, demonstrated no differences in response based on CXCR4 mutation status [42]. Based on this data, it is strongly encouraged that CXCR4 and MYD88 genotyping be conducted as a part of all prospective clinical trials.

5.5 Other Recurrent Mutations

The chromatin remodeling proteins, ARID1A and ARID1B, are also somatically altered in WM [2, 43]. In one WM whole genome sequencing study, ARID1A mutations were found in 5/30 (17 %), one of which was opposite a somatic deletion and another made homozygous by an aUPD. The alternate family member ARID1B is encoded within the region of chromosome 6q that is commonly deleted in WM patients [43–46]. Both ARID1A and ARID1B are members of the SWI/SNF family of proteins which and are mutated in other neoplastic malignancies wherein they exert their effects through regulation of TP53 and CDKN1A [47–49]. TP53 itself is either somatically deleted or mutated in 5–10 % of WM patients [2, 45, 50].

Two studies identified somatic mutations in CD79B in 7–9 % of WM patients [2, 51]. CD79B is mutated in 12–18 % of ABC DLBCL were it activates B-cell receptor signaling [20, 52]. CD79B mutations were also reported in 23 % of immune privileged DLBCL cases with one study reporting CD79B mutations in 11/18 (61 %) of cases of primary DLBCL of the CNS [20, 53]. These same DLBCL populations are the ones with the highest rate of $MYD88^{L265P}$ mutations indicating possible synergistic interaction between the two genes. Many mutations associated with WM affect NF-kB signaling and the MYD88 pathway. In one study,

Fig. 5.2 (continued) genotype. $P = 0.05$, 0.01, and 0.01 for serum IgM in $CXCR4^{WHIM\text{-}NS}$ versus $CXCR4^{WHIM\text{-}FS}$ $MYD888^{L265P}CXCR4^{WT}$, and $MYD88^{WT}$, respectively. $P = 0.05$ and 0.005 for WM BM involvement in $CXCR^{WHIM\text{-}NS}$ versus $CXCR4^{WHIM\text{-}FS}$ and $MYD88^{WT}$, respectively

MYBBP1A was mutated in 2/30 (7 %) of patients and may inhibit NF-kB activity by repressing RELA [2, 54]. Somatic copy loss of HIVEP2, which competes with NF-kB for DNA binding sites, was present in 23/30 77 % of patients [2, 55]. Biallelic loss of TRAF3, a negative regulator of NF-kB, has been reported in two studies at a frequency of 2–3 % of WM patients [2, 56]. Finally, partial methylation of DLEU7 was reported in a study of 12/12 patients [51]. DLEU7 inhibits TNFRSF13B (also known as TACI) and TNFRSF17 (also known as BCMA) leading to suppression of NF-kB signaling [57].

5.6 Structural Variants

No recurrent translocations have been described in WM, further distinguishing it from IgM myeloma and other B cell malignancies [2, 58, 59]. However, karyotyping, FISH, and array-based studies have identified recurrent chromosomal abnormalities. These include deletions in chromosome 6q21-23 in 40–60 % of WM patients, with concordant gains in 6p in 41 % of 6q deleted patients [44–46]. Gains in chromosomes 3q, 4, 18, 8q, and Xq as well as losses of 11q23, 13q14, and 17p have also been described in up to 20 % of WM cases [45, 50]. As 6q deletions represent the most recurrent cytogenetic finding, there has been a great deal of interest in identifying the minimally deleted regions and target genes within this locus. Two such candidates are TNFAIP3, a negative regulator of NF-kB, and PRDM1, a master regulator of B-cell development [44, 56]. The removal of an NF-kB negative regulator is of particular interest as the phosphorylation and translocation of NF-kB into the nucleus is a crucial event for WM cell survival [11]. This has led to speculation that the success of proteasome inhibitor therapy in WM is due in part to inhibition of the degradation of negative regulators of NF-kB such as inhibitor of kappa B (IkB) [11, 42, 60].

5.7 Summary

Waldenström's Macroglobulinemia has a unique mutational profile that distinguishes it from other B-cell malignancies. In addition to serving as an important diagnostic measure, MYD88 mutation status can impact disease presentation, response to therapy, and survival status, while CXCR4 status has important implications for disease presentation and treatment resistance. MYD88 and CXCR4 signaling pathways also represent novel targets for the development of targeted therapeutics for WM.

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Molecular Pathways in Growth and Survival: Epigenomics

6

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6.1 Introduction

The term "epigenetics" has been first described in 1939 by C.H. Waddington to define "the causal interactions between genes and their products, which bring the phenotype into being." This definition has been subsequently modified in order to describe as "epigenetics" those heritable changes in gene expression that are not due to any alteration in the DNA sequence [1]. Among the epigenetic markers, histone acetylation represents one of those that have been well described and studied in the context of tumorigenesis and tumor progression, both in solid tumor and hematologic malignancies, including several B-cell lymphoproliferative disorders, including Waldenstrom's macroglobulinemia (WM) [2]. Specifically, primary WM tumor cells present with a microRNA (miRNA) profiling that differentiates bone marrow (BM)-derived WM cells from BM-derived B cells obtained from healthy individuals [3]. miRNAs are small, noncoding RNAs, described for the first time in the nematode Caenorhabditis elegans [4]. They act as negative regulators of gene expression and have been implicated in several biological processes, both in physiological and pathological conditions, due to their role in modulating development, cell differentiation, apoptosis, and cell proliferation [5–7]. The literature has widely accepted the concept that miRNAs play a crucial role in modulating the pathogenesis of tumors, including both solid

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_6

tumors and hematologic cancers; and in the specific context of WM [2, 3, 8], deregulation of miRNAs in WM cells has been reported, demonstrating, among others, a reduced expression of miRNA-9*, responsible for modulation of histone acetylation in lymphoplasmacytic clonal cells [2, 3]. Taken together, these evidences suggest that epigenetic modifications occur in WM cells, in terms of enhanced histone acetylation, and that miRNAs may be responsible for regulating the histone acetylation status within the tumor clone.

6.2 miRNA Signature in WM Cells: Role of the Onco-miRNA-155

We have previously shown that WM cells are characterized by a specific miRNA signature that differentiates BM-derived WM cells from B cells obtained from healthy individuals [3]. Specifically, increased expression of miRNA-363*, miRNA-206, miRNA-494, miRNA-155, miRNA-184, and miRNA-542-3p was documented in WM cells, together with reduced levels of miRNA-9*. Predicted targets of the increased miRNAs in WM patients included cell tumor suppressors and inhibitors of cytokine-induced signaling and of cell cycle progression. In contrast, oncogenes and transcription factors were listed among the predicted targets of WM-decreased miRNAs. Overall, these findings support the concepts that deregulated miRNAs may be crucial elements that support WM pathogenesis. Among those miRNAs that presented with enhanced expression in WM cells, miRNA-155 has been shown to act as onco-miRNA in WM, as demonstrated both in vitro and in vivo [3]. Importantly, the oncogenic properties of miRNA-155 were also demonstrated in the context of other B-cell lymphoproliferative disorders, including primary mediastinal B-cell lymphomas, diffuse large B-cell lymphomas, as well as chronic lymphocytic leukemia [9, 10]. The oncogenic role of miRNA-155 in WM was supported by the demonstration of reduced WM cell proliferation both in vitro and in vivo in WM cells that were silenced for miRNA-155. The phenotype of WM-silenced miRNA-155 cells was also characterized by cell cycle arrest in G1 phase, leading to reduced number of tumor cells in the S phase. These findings were also supported by demonstrating the increased expression of p18, p19, p21, and p27, known to act as cyclin-dependent kinase inhibitors, together with decreased expression of cyclin-dependent kinase-2, kinase-4, and kinase-6, as well as cyclins D1, D2, D3, and E, known to be positive cell cycle regulators. Importantly, WM cells lacking of miRNA-155 presented with reduced activation of pro-survival signaling pathways, including both MAPK/ERK and AKT and AKT-downstream targeted proteins [3]. It has been clearly demonstrated that the BM milieu acts as a positive modulator of tumor growth, due to its ability to confer growth advantages and to induce drug resistance in malignant B cells [11]. Therefore, we further defined the role of miRNA-155 in possibly targeting WM cells in the context of the BM microenvironment and found that miRNA-155-silencing in WM cells led to inhibition of WM cell proliferation even in the context of the supportive BM milieu, together with

reduced WM cell adhesion, supported by downregulation of Rho GTPase activating proteins, p21 (CDKN1A) activating protein, and p21-activated kinase (PAK) 1-interacting protein, known to be modulators of cell adhesion. In addition, miRNA-155-lacking WM cells presented with reduced migratory abilities [3]. These in vitro findings were also corroborated by the in vivo demonstration of inhibited WM tumor growth in those mice that were harboring miRNA-155-silenced WM cells. Subsequent studies have further demonstrated inhibition of WM tumor growth in vivo obtained by treating WM-bearing mice with the LNA-anti-miRNA-155 [12], thus providing the preclinical rationale for using anti-miRNA-155-based therapeutic strategies in WM.

6.3 miRNA-9* and Regulation of Histone Acetylation in WM Cells

Transcriptional regulation of many genes is regulated by nucleosomal histone acetylation homeostasis: particularly, hypoacetylation leads to a condensed chromatin structure responsible for gene transcription repression, while hyperacetylation is associated with a more open chromatin structure leading to transcription activation [13, 14]. This balance is modulated by a tight regulation of histone deacetylase (HDAC) and histone acetyl transferases (HATs) levels. An unbalanced expression of HDACs and HATs occurs in several malignancies, where the clonal cells are characterized by an increased expression of HDACs that will be responsible for aberrant gene expression, leading to enhanced tumor cell proliferation [15, 16]. Most of the aberrant HAT and HDAC activity has been due to translocation, amplification, overexpression, or mutation in many tumors, including hematological malignancies; and recent studies report miRNAs to be able to interfere with the epigenetic machinery, thus modulating the expression of enzymes regulating DNA methylation or histone modification [15–18]. For instance, miRNA-101 is able to target the zeste homolog 2 (EZH2) enhancer, leading to increased expression of EZH2 in aggressive tumors with an invasive phenotype [18, 19]. Recent evidences have demonstrated that increased histone acetylation characterizes WM patient-derived tumor cells where enhanced expression of HDAC-2, HDAC-4, HDAC-5, HDAC-6, HDAC-8, and HDAC-9 and reduced expression of HAT-1, HAT-2, and HAT-3 were observed as compared to control cells. This was further sustained by a higher HDAC activity in WM tumor cells as compared to their normal cellular counterpart [2]. Of note, miRNA-206 and miRNA-9* were shown to be increased and decreased, respectively, in WM cells, as compared to normal cells [2]. Predicted targets for miRNA-206 and miRNA-9* include HATs and HDACs, respectively, suggesting a possible role of miRNA-206 and miRNA-9* in modulating histone acetylation in WM cells. Functional studies further confirmed that acetyl histone-H3 and histone-H4 were upregulated in pre-miRNA-9*-transfected cells and anti-miRNA-206-transfected cells, with a higher acetyl histone-H3 and histone-H4 upregulation upon miRNA-9* modulation. Moreover, miRNA-9*-dependent modulation of HDAC activity lead to

reduced WM cell proliferation and increased WM cell toxicity, suggesting a key role of miRNA-9* in regulation WM progression [2].

6.4 Conclusions

miRNA modifications have recently gained considerable attention in the field of cancer research. In the specific field of WM, it has been shown that clonal lymphoplasmacytic cells present with a miRNA signature that specifically characterizes WM cells from their normal cellular counterpart. Among those deregulated miRNAs, miRNA-155 and miRNA-9* have been demonstrated to act as crucial regulator of WM pathogenesis, as supported by their influence on WM cell growth both in vitro and in vivo. Notably, miRNA-9* has been proven to enhance HDAC-4 and HDAC-5 expression in WM cells, further demonstrating the role of epigenetics in the pathogenesis of this disease. These findings may provide the preclinical rational for testing novel miRNA-based therapeutical strategies in WM as well as in other IgM-secreting low-grade lymphomas.

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The Bone Marrow Microenvironment and Tumor Cells Interactions in Waldenström's Macroglobulinemia

7

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Waldenström's macroglobulinemia (WM) is a lymphoplasmacytic lymphoma characterized by the secretion a monoclonal immunoglobulin IgM. The bone marrow (BM) is always involved and infiltrated by the monoclonal lymphoplasmacytic cells. BM infiltration and the production of IgM are causing most of the clinical features and complications of the disease. Other features which characterize this disease also include lymphadenopathy and organomegaly (especially liver and spleen) and, rather uncommonly, extramedullary and extralymphatic tissues (skin, pleura, etc.) [1]. Tumor microenvironment of WM contributes to malignant cell viability as well as immunoglobulin production. Furthermore, it has been postulated that BM microenvironment may play a critical role in the progression of the disease from the stage of MGUS to asymptomatic and symptomatic WM, and a permissive BM microenvironment has been postulated.

Waldenström's macroglobulinemia (WM) is characterized by widespread infiltration of the bone marrow with LPL cells, at the time of diagnosis, implying continuous trafficking and homing of LPL cells into the marrow. BM microenvironment has an important role in controlling this migration of LPL cells and also the survival of these cells in WM. Thus, there is continuous trafficking of WM cells in and out of the BM, leading to cell dissemination. Many of the biological features associated with this disease, such as cell survival, tumor cell proliferation, and drug resistance, may come as a result of the direct interactions of malignant LPL cells with the bone marrow microenvironment [2–4]. BM is a complex environment populated by several different cells types with different origins and is composed of a cellular and a noncellular compartment. The noncellular compartment is composed of the

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_7

extracellular matrix and soluble elements such as cytokines, growth factors, adhesion proteins, and so forth [5].

Oncogenesis in WM is a complex procedure which involves genetic and epigenetic events within the tumor clone [6–9]. WM arises from a cell, probably a B cell that has undergone somatic hypermutation prior to transformation and before the initiation of class switch recombination [10, 11], in contrast to IgM-secreting multiple myeloma where there is evidence of isotype switch transcripts. The incidence of IgH rearrangements is low in WM (<3 %). Although several genetic and cytogenetic events have been shown to be involved in WM on cogenesis, these events may not be sufficient, and a permissive microenvironment may be required for frank malignancy to emerge [12]. In this chapter, we analyze the interactions that is observed between bone marrow microenvironment and WM cells that are crucial for WM pathogenesis and its impact on the treatment of patients with WM.

7.1 The Cellular Compartment of the BM and Its Role in WM

The cellular compartment of the BM is composed of hematopoietic cells, including hematopoietic stem cells; mesenchymal progenitor cells, fibroblasts, and BM stromal cells; endothelial precursor cells; mature endothelial cells; immune cells (B lymphocytes, T lymphocytes, natural killer cells, macrophages, mast cells, monocytes, natural killer T cells); erythrocytes, megakaryocytes, and platelets; and chondroclasts, osteoclasts, and osteoblasts [5, 13]. Under normal conditions, the cellular compartment of the BM is in a dynamic equilibrium but in disease, either primary BM disease or when extramedullary conditions affect the BM, the cellular compartment may undergo changes either due to adaptation in the new conditions or as a response to specific stimuli.

7.1.1 Stromal Cells

The role of BM stromal cells has been extensively studied in WM and other hematologic malignancies. Several different groups have shown that stromal cells are critical for the growth of WM cells [14–16]. Many of these data come from coculture of WM cells with stromal cells which show that may lead to resistance of WM cells to therapeutic agents, including rituximab [14–16]. Importantly, certain drugs may overcome the resistance to WM cell apoptosis caused by these interactions, through disruption of the pathways involved in the WM cell to stromal cell interaction.

7.1.2 Mast Cells

The presence of excess BM mast cells is a common finding in WM. These cells are in continuous interaction with LPL cells in patients with WM, and their abundance

is a clue supportive of the presence of the disease. Coculture of tumor cells from patients with WM with mast cells has induced proliferation of WM patient BM LPL cells, demonstrating that mast cells may support tumor cell expansion in WM [17].

7.1.3 Endothelial Cells

Interaction between endothelial cells and WM cells also plays an important role in WM cell growth. This interaction is mediated through different pathways involving a variety of cytokines and other ligands and different receptors. Eph receptors and ephrin ligands are overexpressed in several cancers, and the Ghobrial group has shown that ephrin-B2 (the ligand of the Eph-B2 receptor, a tyrosine kinase) is highly expressed on endothelial cells and BMSC isolated from the BM of patients with WM compared with controls [18]. In addition, Eph receptors are activated in WM patients compared with controls. Both the receptor and the ligand are membrane bound, and they play a role in cell–cell interaction. Activation of the Eph-B2 receptor with its ligand ephrin-B2 did not affect WM cell proliferation and cell cycle; however, it induced signaling in the endothelial cells that promotes adhesion of WM cells to endothelial cells and induces angiogenesis program on endothelial cells (Fig. 7.1). Signaling through Eph/ephrins can induce cell adhesion. This adhesion promotes WM cell proliferation through cell-cycle transition. Ephrin-B2–Eph-B2 receptor interaction in WM was found to affect regulation of cell–cell interaction through an integrin-dependent system that involves downstream activation of focal adhesion kinase (FAK) and Src. Inhibition of either Eph-B2 on WM cells or inhibition of ephrin-B2 on endothelial cells or both reduced the adhesion of WM cells to endothelial cells and reduced activation of the cytoskeletal signaling. Knockdown of Eph-B2 in WM cells prevented the proliferative effect and cell-cycle regulation in WM cells previously induced by coculture with endothelial cells. These findings were also confirmed in *in vivo* studies which showed that inhibition of Eph-B2 in WM cells reduced BM infiltration by these cells. The link between adhesion-regulating molecules and proliferative signaling pathways in WM is interesting and may lead to discovery of new therapeutic agents.

Endothelial cells may also interact with WM cells through additional pathways, involving cytokines that are directly or indirectly implicated in angiogenesis.

7.2 Noncellular Compartment of the BM and Its Role in WM

The noncellular compartment of the BM niche consists mainly of the extracellular matrix and a variety of cytokines, growth factors, and adhesion molecules that are secreted by the cells that compose the cellular compartment of the microenvironment. Cytokines are protein mediators that are known to be involved in many biologic processes including cell growth, survival, inflammation, and differentiation. Several studies have focused on the role of cytokines in the pathogenesis of WM. In many hematologic malignancies, cytokines are essential in the paracrine or

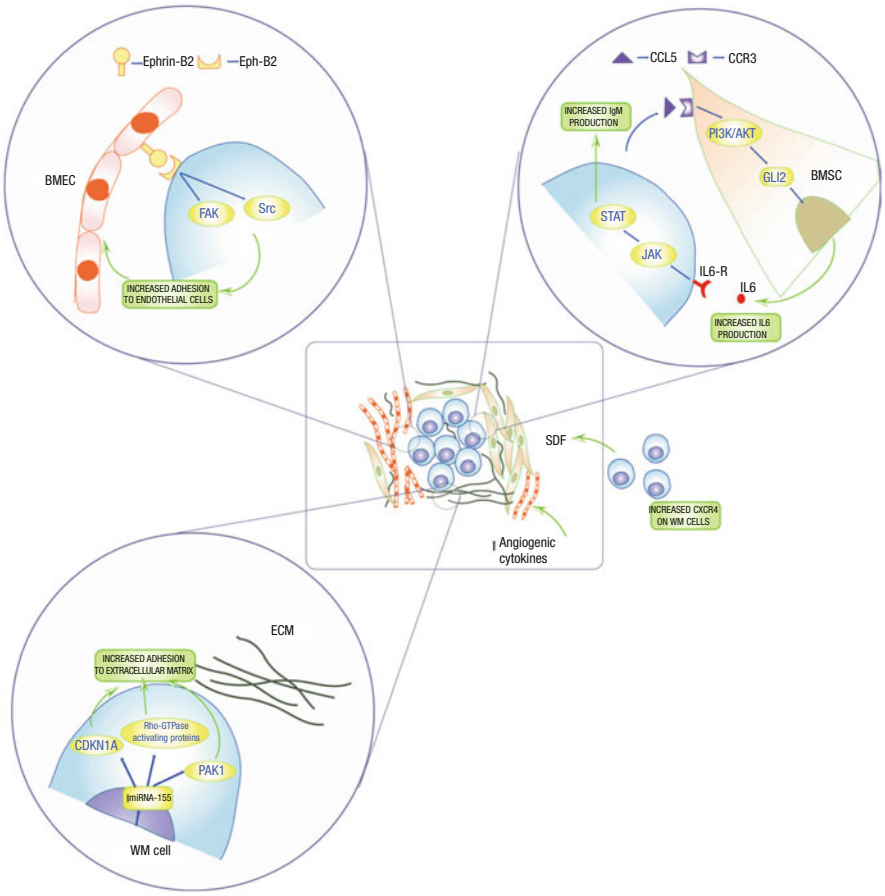

Fig. 7.1 Short description of the BM microenvironment–WM cell interactions and their role in the pathogenesis of Waldenström's Macroglobulinemia (WM). The bone marrow endothelial cells (BMEC) interact with the WM cells via the ephrin-B2–Eph-B2 interaction that increases WM cell adhesion to the endothelial cells. The bone marrow stromal cells (BMSC) and WM cells seem to stimulate each other via paracrine signaling by secretion of cytokines (CCL5 and IL6). WM cells also interact with the extracellular matrix (ECM) through several signaling pathways that may be initiated by miRNA-155 within the WM cell. The signaling of WM cells in and out of the bone marrow is partly regulated by the SDF-1 secreted by the bone marrow and the CXCR4 receptor on the WM cells (Adapted from Agarwal A and Ghobrial IM, Clinical Lymphoma, Myeloma & Leukemia, Vol. 13, No. 2, 218–21)

autocrine signaling pathways that are important for cell proliferation and survival. Microvessel density is increased in 30 % of patients with WM, but there is limited information about the role of angiogenic cytokines in this disease. Anagnostopoulos et al. [19] found increased levels of angiogenin, vascular endothelial growth factor, vascular endothelial growth factor A (VEGF-A), and basic fibroblast growth factor (FGF) in the sera of 56 patients with WM and 11 patients with IgM MGUS

compared with controls. A lower level of angiopoietin-1 (Ang-1) was also noted in WM compared with controls. Angiogenin levels correlated with disease status. Ang-1 is an antagonist of neovascularization, and microvessel density in the BM showed strong inverse correlation with Ang-1 [20]. These high levels of almost all angiogenic cytokines in patients with WM suggest that there is a role of angiogenesis in the biology of WM. This study raises the possibility of the cellular compartment of the BM microenvironment that supports neovascularization and contributes to WM pathogenesis through these angiogenic cytokines.

An interesting observation has been the association of the levels of von Willebrand factor (vWF) measured in the plasma of patients with WM to the prognosis. Some patients may have very low levels due to acquired vWF disease but in a study by Hivert et al., 59 % of patients with WM had increased levels of vWF. vWF is increased under conditions that lead to endothelial cell activation (such as inflammation or other stimuli). In this study, BM microvascular density was higher in patients with more elevated vWF levels, indicating a potential association between vWF levels and angiogenesis in the BM. The increased levels of vWF in patients with WM were also confirmed in another study, as well as the prognostic implications of this increase. In the study by Hivert et al. [21], both lymphoplasmacytic cells and mast cell stained positive for VEGF, indicating that these cells may also be a source of angiogenic cytokines. vWF is produced constitutively in the megakaryocytes and ECs where it is stored in the Weibel–Palade bodies together with several other mediators, including several with angiogenic activity (such as angiopoietin-2) and others associated with inflammation, hemostasis, and vascular tone [22]. vWF has a role in the trafficking and storage of these factors within ECs, including the maturation of adhesion molecules such as integrin αvβ3, while it colocalizes with molecules such as angiopoietin-2 [23]. The ECs release the content of Weibel–Palade bodies in response to stimuli, such as VEGF. vWF and other molecules are released simultaneously and a temporal association of vWF and angiopoietin-2 release has been shown [23, 24]. These data indicate that elevated levels of vWF are rather a marker of the increased endothelial cell activity due to increased "pro-angiogenic" signaling.

Elsawa et al. [25] performed a comprehensive cytokine analysis on serum and BM biopsy samples from patients with WM and from healthy donors. They found C-C motif ligand 5 (CCL5), granulocyte colony-stimulating factor, and soluble interleukin (IL)-2 receptor elevated in patients with WM, whereas IL-8 and epidermal growth factor levels were found to be lower compared with healthy controls [25]. CCL5 expression was higher in the BM microenvironment of patients with WM compared with controls. CCL5 levels were shown to correspond with disease aggressiveness, and there was a functional correlation between CCL5 and IL-6 levels. IL-6 is a proinflammatory cytokine mainly produced by BM stromal cell and has a role in normal and malignant B-cell biology. CCL5 stimulated IL-6 secretion from BM stromal cells by binding to a receptor C-C motif receptor 3 (CCR3) and induction of GLI2, a transcription factor via the PI3K-AKT-IkBα-p65 pathway [26]. Increased IL-6 production in turn resulted in increased IgM production by WM malignant cells via the JAK/STAT pathway (Fig. 7.1). The CCL5–IL-6

interaction may be a mechanism by which the WM cells and BM stromal cells stimulate each other wherein the WM cells produce CCL5, which stimulates the stromal cells to produce IL-6, and IL-6 in turn stimulates IgM production by the WM cells. IgM is a mediator of WM morbidity, and this study highlights the importance of the BM microenvironment in the pathogenesis of WM. B-lymphocyte stimulator (BLys) is a TNF family member that is critical for maintenance of B-cell homeostasis and is overexpressed in a variety of B-cell malignancies. BLys expression was found in bone marrow samples, and also serum levels were elevated in patients with WM.

Chemokine (C-C motif) ligand 3 (CCL3) (formerly known as Macrophage inflammatory protein-1 alpha, MIP-1a) has also been assessed in the serum of patients with WM. CCL-3/MIP-1a was elevated and serum levels correlated positively with disease activity [27, 28]. An immunohistochemical study, performed in bone marrow biopsies of WM patients, showed that all neoplastic cells expressed CCL3 in all cases. This finding was constant in newly diagnosed patients with both symptomatic and asymptomatic WM and also in patients with active disease post-therapy [29]. These data suggest that the WM cells are probably a major source of CCL3. Also, CCL3 may have a significant role in WM biology while CCL3 could be a potential target for developing novel drugs against WM. CCL3 is also a potent MIP-1a that is also one of the most potent osteoclast activators and has been implicated in the pathogenesis of myeloma bone disease. However, the role of osteoclast and osteoblast in WM has not been studied extensively, and only some data regarding bone metabolism have been published. Lytic bone lesions are not a common finding in WM. Indeed, although Receptor activator of nuclear factor-kappaB ligand (RANKL) serum levels was elevated in WM patients, there was also a balanced elevation of its decoy receptor osteoprotegerin production in a study of patients with WM. Subsequently, indices of bone formation and bone destruction were elevated, indicating increased bone turnover but no major shift towards bone destruction [28]. These findings may explain the absence of lytic lesions in WM patients.

The homing of LPL cells through the blood to the BM microenvironment is carried out through adhesion molecules that are expressed on the cellular compartment of bone marrow. Homing is a coordinated multistep process that involves signaling by stromal-derived factor (SDF-1), activation of lymphocyte function-associated antigen 1, very late antigen (VLA)-4/5, and activation of matrix metalloproteinase (MMP) 2/9 [30]. Ghobrial's group has shown that WM cells express high levels of CXCR4 (a receptor for SDF-1) and VLA-4 [2]. CXCR4 was found to be essential for the migration and transendothelial migration of WM cells under static and dynamic shear flow conditions, with significant inhibition of migration by using the CXCR4 knockdown or the CXCR4 inhibitor plerixafor. Inhibition of CXCR4 or VLA-4 led to decreased adhesion to fibronectin, stromal cells, and endothelial cells. Most importantly, decreased adhesion of WM cells to stromal cells by plerixafor led to increased sensitivity of these cells to cytotoxicity by bortezomib. PI3K and ERK signaling pathways were found to be activated by CXCR4 activation in this study. Polymorphisms in the SDF-1 gene may also have an impact in the pathogenesis and disease progression of WM [31]. These studies

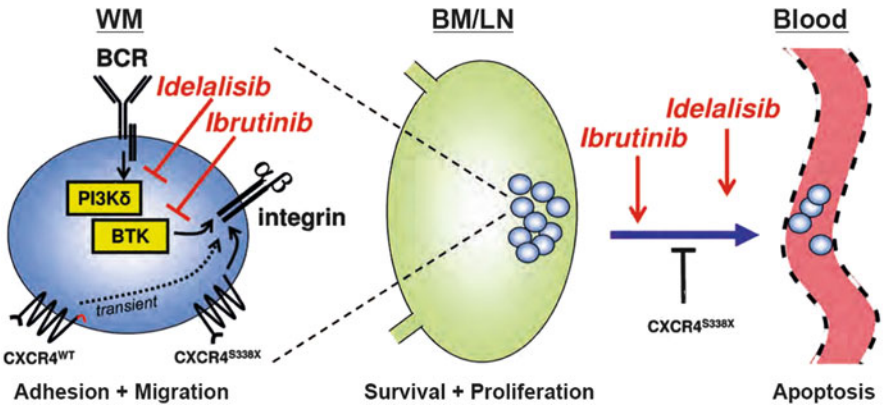

Fig. 7.2 Model of the potential mechanism of action of ibrutinib and idelalisib in WM. Inhibition of BTK by ibrutinib or PI3Kδ by idelalisib impairs BCR-controlled integrin-mediated adhesion of WM cells in bone marrow (BM) and lymph nodes (LN), which results in their egress from these protective niches into the circulation, resulting in WM regression. The homing receptor CXCR4 is normally desensitized upon binding of CXCL12, which is highly expressed within the lymphoid organ microenvironment. However, in the presence of mutation affecting the regulatory domain (such as the WHIM-like mutation S338X), CXCR4 may aberrantly support retention of WM cells in the lymphoid organs. Since CXCR4-controlled integrin-mediated adhesion is insensitive to ibrutinib and idelalisib, this would counteract their ability to inhibit BCR-controlled integrin activation, thus explaining the clinically observed ibrutinib resistance of WM patients with the $CXCR4^{S338X}$ mutation. (Figure adapted from de Rooij MF, Kuil A, Kraan W, Kersten MJ, Treon SP, Pals ST, and Spaargaren M. Ibrutinib and idelalisib target B cell receptor- but not CXCL12/CXCR4-controlled integrin-mediated adhesion in Waldenström macroglobulinemia. Haematologica. 2016 Mar;101(3):e111-5.)

showed the importance of the SDF-1–CXCR4 axis in the pathogenesis of WM and confirmed the protective effect of the BM microenvironment toward WM cells, providing a strong rationale for disrupting the WM–BM interaction to improve therapeutic efficacy. Subsequently, the clinical use drugs, such as BTK inhibitors, that disrupt this communication was associated with unprecedented activity. BTK inhibitors target signaling through B-cell Antigen Receptor (BCR), and their use was associated with very high response rates in patients with WM [32]. Recent data indicate that the BTK inhibitor ibrutinib modulate the adhesion and communication of WM cells with their microenvironment and that at least part of their activity is due to this effect. WM cells express a signaling-competent B-cell antigen receptor (BCR) which controls integrin-mediated adhesion. Both ibrutinib and idelalisib (a selective PI3k delta inhibitor) inhibit BCR-controlled signaling and integrin-mediated adhesion, whereas chemokine (CXCL12/CXCR4)-controlled signaling, adhesion, and migration are not affected. Thus, ibrutinib and idelalisib target BCR-controlled retention of WM cells in the lymphoid organs, explaining the clinically observed mobilization of malignant cells from these protective niches into the circulation. This "mobilization" of the WM cells may deprive the tumor cells from essential microenvironmental growth and survival factors [33]. The relative resistance of WM harboring activating mutations in CXCR4 may also be explained [34] (Fig. 7.2).

Micro-RNA expression profiling of bone marrow-derived CD19+ WM cells was performed in a study by Roccaro et al. miRNA-155 was increased in WM compared to normal controls [3]; miRNA 155 has been shown to play an important role in the oncogenesis of other B-cell malignancies, such as diffuse large B cell lymphoma, primary mediastinal B-cell lymphomas, and Hodgkin lymphoma [3]. In this study, a significant inhibition of adhesion to fibronectin was observed in the miRNA-155 knockdown cells compared with controls. This was further supported by the down-regulation of genes such as several Rho GTPase activating proteins, p21 (CDKN1A) activating protein, and p21-activated kinase 1 interacting protein, which are known to be involved in the adhesion process (see Fig. 7.1). Similarly, miRNA-155 knockdown significantly inhibited WM cell migration in response to SDF-1. By using an in vivo homing model, the study also showed that miRNA-155 knockdown WM cells homed and proliferated in the BM at a lower rate than control WM cells and led to decreased IgM production and a significant survival benefit in mice.

7.3 Summary

The role of the microenvironment in the BM and the lymphoid organs is key to the development and progression of WM. The accumulated data have shown that key players include certain cytokines and adhesions molecules. The modulation of the communication/interactions of the WM cells with their microenvironment is a therapeutic target which has recently been exploited with the use of BTK inhibitors.

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Waldenstrom's Macroglobulinaemia: Immunosurveillance and the Immune Micro-environment

8

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Waldenstrom's macroglobulinaemia (WM) is a lymphoplasmacytic lymphoma that involves the bone marrow and is associated with a monoclonal immunoglobulin of the IgM class [1]. The cytological spectrum of Waldenstrom's macroglobulinaemia reflects cellular B lymphocyte differentiation that ranges from small B-cells to atypical lymphocytes and fully differentiated plasma cells. Atypical lymphocytes are usually referred to as plasmacytoid or plasmacytic lymphocytes [2]. It is a rare disease with an incidence one tenth that of myeloma with the median age of onset of approximately 70 years. The strongest risk factor for WM is the precursor condition of monoclonal gammopathy of undetermined significance(MGUS) of the IgM class which is associated with an average annual risk of developing WM of approximately 1.5 % per year. It is associated with somatic mutations in the MYD88 gene [3].

The immune environment in WM is clinically characterised by susceptibility to infection [4]. Chronic immune stimulation associated with recurrent infections or autoimmune disease appears to be a predisposing factor to WM, reinforced by the fact that the immunoglobulin genes in WM cells have somatic mutations suggesting that the cell of origin of WM is a post-germinal-centre lymphocyte which has undergone antigenic stimulation and selection. However, unlike myeloma, WM arises from somatically mutated cells prior to immunoglobulin switch recombination [5–7]. The nature of the putative antigenic stimuli remains unclear, but the association with clinical syndromes, such as cryoglobulinaemia, cold agglutinin disease and neuropathy, suggests that autoimmune factors may be involved [8–10]. Recently it has been suggested that a T-cell-dependent response to phosphorylated autoantigens may be critical in the development of WM [11].

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_8

Immunological control of the disease is supported by the clinical features of stable or smouldering disease.

8.1 Antigenic Stimulation and Pathogenesis of WM

Large nationwide studies have explored the role of antigenic stimulation in the pathogenesis of WM and confirmed its significance. In US veterans, there was a two- to threefold risk of WM in patients with a personal history of autoimmune disease. Elevated risks of WM are also seen in association with hepatitis, HIV and certain infections. Studies in Sweden have shown that an increased risk of WM occurs with chronic immune stimulation with diseases such as Sjogren's syndrome, autoimmune haemolytic anaemia and polymyalgia rheumatica and certain infectious diseases such as a history of pneumonia, septicaemia hepatitis and herpes zoster being associated with an increased risk of disease [12, 13]. For example, hepatitis C confers a 20–30 % increased risk of lymphoma overall and an approximate threefold risk of WM [14].

Patients with WM are more likely to have first-degree relatives with a history of pneumonia and diabetes [15] also reinforcing concepts of the association with an autoimmune diathesis and immune stimulation, perhaps associated with genetic factors. Research data showing hyper-responsiveness of B-cells as an endophenotype, a functional phenotype, has been described suggesting a combination of exogenous and intrinsic features which may relate to the onset of this disease [16]. These observations point to the role of secondary or chronic immune stimulation interacting with familial susceptibility to account for the familial clustering of WM and suggest that there is a number of common susceptibility genes associated with WM and other lymphoproliferative diseases.

8.2 The Bone Marrow Micro-environment

Interactions in the marrow micro-environment in WM are an intrinsic part of the immune host-tumour relationship. The bone marrow environment consists of stromal, cellular and soluble components. To date most studies have reported on the cellular compartment of the bone marrow concerning lymphocytes, especially T-lymphocytes, natural killer cells, macrophages and mast cells. Less data is available on the interaction of the other stromal compartments comprising extracellular matrix, cytokines and growth factors with the malignant tumour.

Stromal cells have been shown to be critical for the growth of malignant cells as well as playing a role in drug resistance [17]. Mast cells support growth of WM cells through CD154/CD40 signalling [18]. CD27-CD70 interactions form a positive feedback loop via the secretion of the TNF family of proteins [19]. Endothelial cells also play an important role via the ephrin-B2 ligand, a pathway for adhesion of tumour cells to endothelial cells which has significant implications for drug

resistance [20]. Recently it has been shown that eosinophils play a supportive role to the malignant plasma cell in myeloma, although their role in WM remains unclear [21].

Studies on the role of cytokines in the tumour environment in WM have identified elevated levels of CCL5, G-CSF and soluble IL-2 receptor. CCL5 levels correlate with IL-6 levels, an important growth factor for plasma cells and immunoglobulin secretion, and high CCL5 levels correlate with features of disease aggressiveness such as IgM levels and bone marrow infiltration. This effect could be mediated via the JAK/STAT pathway [22] and is supported by the demonstration of constitutive activation of STAT5 in WM and evidence that inhibition of STAT5 significantly decreased IgM secretion [23].

Another important cytokine in the bone marrow micro-environment is IL-21 which contributes both to IgM secretion and proliferation of malignant cells in WM. IL-21 is secreted by activated T-cells. It activates the STAT pathway and results in phosphorylation of STAT 2 and increases expression of downstream targets of STAT 3, including IL-6, the main growth factor for plasma cells [23].

The overall role of IL-21 in the immune system is not fully characterised but IL-21 is produced by natural killer cells as well as Th17 cells and plays diverse roles including immunoglobulin production (as demonstrated in WM) and drives terminal differentiation of B-cells to plasma cells. It also regulates Th17 development and affects CD8 T-cells which may be playing a critical role in the immune micro-environment response to WM [24].

MicroRNAs are abnormally expressed in WM [25–29]. For example, overexpression of a family of seven miRNAs is related to prognostic factors including age, haemoglobin and IgM level [26]. Specifically miRNA-155 is increased and regulates proliferation of WM cells in vitro by inhibiting signalling cascades. The importance of the miRNA-155 in the immune system is suggested by a role in dendritic cell differentiation and function and its ability to stimulate T-cells [30]. Moreover, B-cells lacking miRNA-155 generate reduced extra-follicular and germinal centre responses upon stimulation with both thymus-dependent and thymus-independent antigens suggesting that miR-155 directly regulates class switching recombination in secondary lymphoid organs [31].

8.3 Clonal T-Cell Expansion in Waldenstrom's Macroglobulinaemia

Approximately 50 % of patients with myeloma have expansions of cytotoxic CD8 T-cells which are not associated with viral infections. These expanded T-cell clones are predominantly CD8+, CD57+ and CD28− and have an immunophenotype of terminally differentiated effector memory cells [32–34]. The presence of clonal cytotoxic T-cells in myeloma is associated with survival advantage and suggesting they may have an antitumour role or a role in tumour surveillance [35]. Clonal cytotoxic T-cells are stimulated by thalidomide, and the combination of thalidomide and the presence of clonal T-cells were a major positive prognostic factor in a

maintenance study of thalidomide in myeloma and correlated with an improvement in progression free survival [36]. Such cells also exist in patients with WM but are largely eliminated by nucleoside analogue therapy [37]. Their elimination by drugs such as fludarabine and cladribine suggest that clonal T-cells may play a significant role in tumour surveillance as illustrated by case reports of rapid transformation to large cell lymphoma following the introduction of cladribine or fludarabine [[38], [39]] spontaneous remission of transformed lymphoma following their cessation [40] and increased incidence of transformation in patients treated with fludarabine [41, 42]. An immunosurveillance role for cytotoxic T-cells in myeloma is supported by data showing that high numbers of T-cell receptor excision circles (TREC), demonstrating a previous proliferative history, are related to an improved prognosis in myeloma, both before and after autologous stem cell transplantation [43].

In both WM and in myeloma, such T-cell clones are in a state of proliferative hypo-responsiveness to T-cell receptor stimulation [32, 37]. Gene set expression analysis (GSEA), which detects changes in the expression of genes in entire pathways and is more robust than analysis based on single genes, has identified several up-regulated gene sets not specifically related to cytotoxic T-cells. These include RAS, CSK and TOB pathways and an unregulated TGF beta receptor. PTPN7, which plays a role in inactivating ERK, was also increased [37]. Up-regulation of TGF beta has been shown to be responsible for impaired dendritic cell function in myeloma and may play a similar role in Waldenstrom's [44–46]. A more precise characterisation of this dysfunctional signalling pathway may provide a target for restoring T-cell responsiveness.

Whether such T-cell hypo-responsiveness is a result of anergy, exhaustion (as seen in chronic viral infections) and immunological senescence or whether all processes are active is currently under active investigation [47, 48]. Current evidence suggests that these cells are not directly comparable to the exhausted T-cells seen in chronic viral infections as the hypo-responsiveness is not reversible by agents such as 1BBL, OX40, IL-2 and IL-15 [49]. In addition they do not exhibit high expression of PD-1, primarily associated with immune exhaustion. The expression of high levels of CD57 suggests that in myeloma and WM these cytotoxic T-cells may have been switched from a highly proliferative/low cytotoxic profile to a low proliferative/highly cytotoxic profile, which is reflective of senescence-associated secretory phenotype (SASP) [50]. Further categorisation of these T-cells is needed to investigate techniques to restore their function.

Relevant to understanding cytotoxic T-cell activity in myeloma and WM are the results of a study which demonstrated that 100 % of MM patients who had survived for more than 10 years since their diagnosis of MM had T-cell clones [49]. Interestingly, the T-cell clones found in the 10-year survivors were not hypo-responsive as they were found to be proliferative after T-cell receptor stimulation. The discovery of T-cell clones which could still respond to stimulation provides a unique opportunity to identify the lesion in the non-responsive T-cell clones found in the majority of patients and conduct functional assays to restore the proliferative capacity of T-cell clones in WM and myeloma.

8.4 Natural Killer Cells in Waldenstrom's Macroglobulinaemia

Unlike T- or B-cells which require gene rearrangements and costimulatory signals to initiate a cytotoxic response, cytotoxicity by NK cells is determined by a balance between inhibitory and stimulatory signals received via receptors on the surface of the cell. Despite the large amount of literature showing that NK cells play a critical role in the regulation of immune responses, in particular immune responses against tumours [51], there is little clinical evidence for a role in WM. In a study of an Icelandic family with WM, hyper-reactive B-cells were identified but no evidence for depressed NK function or NK numbers was seen [52].

In myeloma NK cells may play a protective role, and several studies have demonstrated in vitro cytotoxic anti-myeloma responses [53, 54].

NKT cells are true T-cells that recognise lipid antigens in the context of CD1d restriction and may play a role in tumour immunity. Type I NKT cells, defined by their invariant T-cell receptor (TCR), are protective, but contrastingly type II cells characterised by diverse TCRs are inhibitory. Subsets of NKT cells may thus play opposing roles in cancer [55].

Type I NKT cells recognise alpha galactosylceramide. In a mouse model, myeloma cells loaded with alpha galactosylceramide promoted therapeutic NKT-dependent antitumour immunity including the development of myeloma-specific antibodies and cellular immune responses including cytotoxic T-lymphocytes and memory T-cells. In addition these studies demonstrated the presence of decreased regulatory T-cells pointing to the complex interactions that are involved in antitumour immunity. Longitudinal studies have demonstrated a prolonged deficiency of NKT cells after high-dose chemotherapy; however, this appears to be unrelated to the clinical response. In myeloma there was no measurable effect of lenalidomide on NKT cell numbers, despite clinical response, and the clinical value of NKT cells as a marker of clinical activity as opposed to laboratory evidence of antitumour activity remains unclear.

The NK2 D receptor, one of the natural killer cell-activating receptors, is also expressed on the surface of cytotoxic T-cells as well as gamma delta T-cells and NKT cells. Blocking of the NK2 D receptor using antibodies will reverse in vitro cytotoxicity of cytotoxic T-cells to autologous myeloma cells and demonstrates the complex interactions of T-cells, NK cells and NKT cells [56]. Such findings do indicate a possible way in which a defective NKT cell pool may be associated with progressive disease and may be a target for therapy. For example, drugs like lenalidomide promote NKT cell activity in relapsed refractory myeloma and may play a similar role in WM.

The PD-1/PD-L1 axis has also been implicated in multiple myeloma and WM. PD-1 has been demonstrated to be expressed on NKT cells in myeloma, and NKT activation results in vitro toxicity against myeloma cells, including autologous myeloma cells. It has been demonstrated that PD-L1 is highly expressed on malignant cells of patients with WM and that they promote malignant B-cell growth [57]. Such data provide evidence for the possible efficacy of blockage of the PD-1 axis by monoclonal antibodies such as CT-101 [57, 58].

Thus the clinical importance of NKT cells in myeloma and WM remains uncertain at this time. Therefore, unlike cytotoxic T-cell populations which have been shown to have a clinical protective effect in vivo, the activity of NKT cells has yet to be shown to have clinical significance.

8.5 Treg and Th17 Cells: Myeloid-Derived Suppressor Cells

The importance of Treg cells is well documented in myeloma in which the Treg/Th17 ratio is altered to an immunosuppressive state. Long-term survival is associated with reversal of this finding. Recent publications have also noted increased MDSC cells in both the blood and bone marrow of myeloma patients, although their role in WM is not clear [59–63].

8.6 Differences Between Tumour-Induced Immunosuppression in Myeloma and Waldenstrom's Macroglobulinaemia

The homeostatic balance between the activation and suppression of immune antitumour effector cells in vivo is controlled by a network of cytokines and regulatory cells in a defined micro-environment. In myeloma, malignant plasma cells appear to have multiple mechanisms of inhibiting the antitumour immune response. These include inactivation of clonal cytotoxic T-cell, depression of NK and dendritic cell function and altering the regulatory T-cell and pro-inflammatory Th17 cell balance in favour of a suppressive state [60]. In addition, in myeloma, trogocytosis (demonstrated by malignant membrane transfer to T-cells) of plasma cells by T-lymphocytes, results in the development of inhibitory Treg cells [64]. In addition myeloid-derived suppressor cells (MDSC) are numerically increased, localised to the bone marrow micro-environment and functionally more active in patients with myeloma as compared to normal [61]. The state of the immune homeostatic balance in WM has not yet been so clearly defined.

With the exception of clonal cytotoxic T-cells, which are present in the blood of 70 % of patients with WM ($n = 20$), 48 % of all myeloma ($n = 120$) and 100 % of 10-year myeloma survivors ($n = 19$) [37, 49], inhibition of the immune antitumour response was shown to be less active in WM compared to myeloma. In patients with WM, there is a normal Treg/Th17 ratio which in patients with myeloma is significantly increased. WM patients have a normal percentage of Treg cells in the blood, and an absence of trogocytosis which can affect an average of 12 % of MM T-cells to become acquired regulatory T-cells. Treg function is significantly enhanced in both myeloma ($p = 0.05$) and WM. In addition absolute numbers of G-MDSC are highly elevated in myeloma, while in WM they are within the normal range. Furthermore, there are major differences in therapy in Waldenstrom's such as the use of nucleoside analogues which has many immunosuppressive actions including the depletion of cytotoxic clonal T-cells (Fig. 8.1).

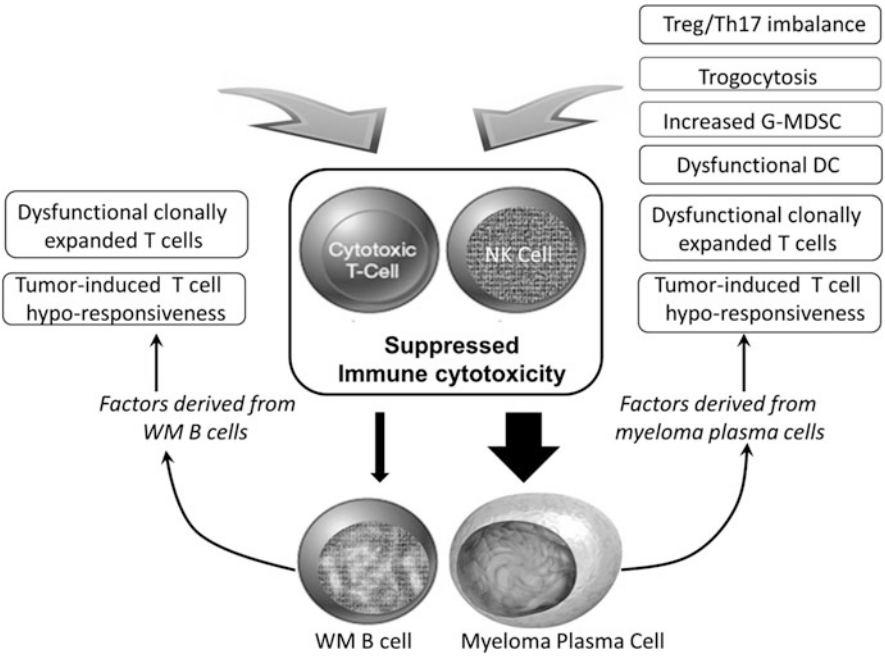

Fig. 8.1 Differences in host-tumour interactions in WM and myeloma. The malignant plasma cell has more effector mechanisms to protect itself from the host immune system compared to the WM malignant B-cell

Differences in the constitutive activation of signalling pathways of the Janus kinase family may also be associated with differences in immune modulation. pSTAT5 is constitutively activated in both WM [23] and myeloma [65]. While phosphorylated STAT5 does not have any prognostic significance in myeloma, its inhibition decreases the capacity of the malignant cell to secrete IgM in WM and suggests more active involvement of this pathway in the disease pathogenesis of WM and a possible target to control IgM levels with pSTAT5 inhibitors.

8.7 Summary

The ability of the host to control the malignant cells in Waldenstrom's macroglobulinaemia appears to be less compromised than seen in myeloma, suggesting that active immune therapy, such as immunisation and T-cell therapy, may be more successful in WM than in myeloma (Table 8.1). However, considerations around the optimum therapy in WM must take into account the immune environment implying that while immunosuppressive drugs such as nucleoside analogues are highly effective in their cytotoxic effect, but the effect on immune surveillance may be counterproductive. Understanding the clonal

Table 8.1 Relative potency of T-cell inhibitors

	Myeloma	WM
MDSC	+++	+
Trogocytosis (acq reg)	+++	+
Dysfunctional DC	++	+
Treg imbalance	+++	+

cytotoxic T-cell response and the role of NK cells may provide more insights into the immune interactions present in B-cell tumours and facilitate the development of effective immune therapies in the future.

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Part II

Epidemiology and Genetic Predisposition

Epidemiology of Waldenström Macroglobulinemia

9

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9.1 Introduction

Waldenström's macroglobulinemia (WM) is a chronic lymphoproliferative disorder characterized by an IgM monoclonal gammopathy in the presence of lymphoplasmacytic lymphoma (LPL) in the bone marrow [1]. Recently, Treon et al. identified a common somatic mutation, MYD88 L265P, to be found in over 90 % of WM patients [2]. It is also found in over 50 % of patients with IgM monoclonal gammopathy of undetermined significance (MGUS) [3, 4] which is the strongest risk factor for developing WM [5]. Another somatic mutation that frequently presents in WM is a mutation in CXCR4 [6].

LPL/WM is a rare malignancy, accounting for only 1–2 % of all hematologic tumors, with an incidence of 3–4 per million people per year [7, 8]. Due to the rarity of WM, acquiring epidemiological knowledge of the disease has been a challenge. In the beginning, much of the information was obtained from small clinical series but have recently been largely replaced by large population-based studies.

WM is a disease of the elderly, with less than 1 % of patients diagnosed before 40 years of age [9]. The median age of diagnosis is around 70 years [9–11]. The incidence increases sharply with advancing age until the age 60–69 years, after

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_9

which, there is a slower increase in incidence [11]. Blacks have the youngest median age at diagnosis (63 years) whereas median age of diagnosis in whites is the highest (73 years) [12].

Difference in the incidence of diseases in various subgroups can provide information on the occupational or biological risk factors. WM is up to twice as common in males [7, 11], and the male predominance is consistent in all age categories [11] and also found in Asian countries [13]. In the USA, white males represent 52 % of WM cases and white females represent 36 % of cases, while blacks represent 5 %, other races account for approximately 4 % and in 3 % of cases the race was unspecified [7]. The incidence rate in whites is more than twice as high as in other racial groups [11]. The incidence is even more variable between continents, and WM is up to 10 times less common in some Asian countries [13], and interestingly this incidence is lower than expected compared to Asian immigrants in the USA [14]. This supports that environmental and lifestyle factors are important in the pathogenesis of WM.

Trends in incidence over time can be difficult to estimate due to many factors, such as the possible increase in diagnosis due to more routine blood tests and electrophoresis and specialized health care due to the growing number of elderly people in Western countries. The age-adjusted incidence has been relatively stable since 1988, although there has been an increase in incidence in whites in the USA [11] and there has been some trend to increased incidence in males after 2007 in Sweden [11, 15]. There was also a significant increase in incidence found in Japan but not in Thailand [13].

9.2 Familiality

A role for genetic factors in the etiology has been implicated in researches for a long time. The first report on familiality in WM was published in 1962 [16], and since then papers on families with multiple cases, as well as small case-control and cohort studies, have been published showing family clustering in LPL and WM [16–27]. Treon et al. characterized clinicopathologic patterns of familial disease in a clinic-based series of 257 WM patients and found that 19 % of the patients had at least one first-degree relative affected by WM or another B-cell disorder, including non-Hodgkin lymphoma, multiple myeloma, chronic lymphocytic leukemia, MGUS, acute lymphoblastic leukemia, and Hodgkin lymphoma [26]. They also found patients with a familial history of WM or a plasma cell disorder to have greater bone marrow involvement.

Clinical-based studies are, however, likely to be subjected to selection bias and could overestimate the familial component in WM. In a large population-based case-control study on 2144 LPL/WM patients (1539 WM [72 %] and 605 LPL [28 %]) and over 8000 first-degree relatives and more than 30,000 controls, the familiality of WM was thoroughly estimated [9]. First-degree relatives of LPL/WM patients had a 20-fold increased risk of developing LPL/WM and, furthermore, an increased risk of developing non-Hodgkin lymphoma, chronic lymphocytic

leukemia, and MGUS. There was, however, no excess risk of multiple myeloma or Hodgkin lymphoma. There was a similar excess risk among parents, siblings, and offspring, which favors the operation of dominant or codominant gene effects, rather than recessive genes. These results imply that gene-environment interaction is likely as WM has been associated with immune-gene polymorphisms [17, 28–33] as well as autoimmunity and chronic infections [17, 34, 35]. Interestingly, family history of lymphoproliferative disorders in LPL/WM patients is associated with a modest, but significant, increased risk of death [36], indicating that there might be biological differences between familial and sporadic disease and that genetic susceptibility predisposes patients to a more severe form of LPL/WM.

IgM-MGUS is a precursor to WM and has been observed in about 10 % in first-degree relatives of WM patients in multiple-case WM families [37]. IgM-MGUS is more common in first-degree relatives compared to more distant family members and is most commonly observed in siblings of cases.

Epidemiological studies on WM have also shed some light on other genetic mechanism of the disease. For example, some evidence supporting genetic anticipation has been shown in earlier studies. In their study, Treon et al. [26] showed that WM patients with a family history of WM or a plasma cell disorder were diagnosed at a younger age than sporadic WM cases. In a large population-based study, offspring of LPL/WM patients were diagnosed with LPL/WM at an earlier age than the parent group [9]. However, the age at diagnosis of LPL/WM was similar in offspring of controls and of cases, indicating that this difference is likely due to differences in follow-up time between generations and not explained by earlier diagnosis in children of parents with LPL/WM [9]. The possibility of anticipation in WM needs to be studied in more detail.

Taken together, there is convincing evidence that first-degree relatives of LPL/WM patients are in increased risk of developing LPL/WM and certain other types of lymphomas/leukemia. However, because of the low baseline risk of LPL/WM and other lymphoproliferative malignancies in the general population, the absolute risk for a relative of a LPL/WM patient to develop LPL/WM or another lymphoproliferative malignancy is still very low.

9.3 Risk Factors

The etiology of WM is to a large extent unknown. Many studies have estimated the role of environmental and biological factors on WM. The best-known risk factor for WM is IgM-MGUS, accounting for about 14 % of all MGUS [38]. A personal history of IgM-MGUS has been associated with increased risk of developing WM [39]. Based upon long-term follow-up data from the Mayo Clinic, a history of IgM-MGUS is associated with a 1–1.5 % yearly risk of developing WM [5] and a significant additional risk of developing chronic lymphocytic leukemia and non-Hodgkin lymphoma (NHL) [5].

IgM-MGUS can in many ways be thought of as an early state of WM, rather than a cause of the disease, similar to IgG and IgA MGUS in multiple myeloma [40].

Chronic antigen stimulation is likely to have a role in the development of WM. Studies have reported evidence of somatic immunoglobulin gene mutations, suggesting that the WM cells originate from B cells that have undergone repeated antigenic stimulation and selection in the germinal centers of lymphoid follicles [41, 42]. A number of epidemiological studies [17, 22, 34, 35] have assessed the role of various types of chronic antigenic stimulatory condition in relation to the risk of developing LPL/WM; however, the results have been somewhat conflicting.

In a hospital-based study on 65 patients and 213 hospital-based controls, no association was found between autoimmune diseases and subsequently developing WM [22]. Interestingly, WM patients were more likely than controls to have first-degree relatives with a history of pneumonia, diphtheria, rheumatic fever, and diabetes mellitus, with relatives of two WM cases having IgM MGUS and about 40 % having diverse immunologic abnormalities.

Two nationwide US veterans studies [17, 34] were designed to explore the role of antigenic stimulation in the pathogenesis of WM. In the first study, including 146,394 patients infected with hepatitis C virus (HCV) and 572,293 controls [34], HCV infection confers a 20–30 % increased risk of NHL overall and a threefold higher risk of WM. In the second study, WM risk was assessed in relation to a variety of chronic immune stimulatory conditions among 4 million US veterans and among 361 patients with WM with up to 27 years of follow-up, and revealed a two- to threefold elevated risk of WM in individuals with a personal history of an autoimmune disease and notably increased risks associated with hepatitis, human immunodeficiency virus, and rickettsiosis [17].

In a questionnaire-based study of 103 patients with WM and of 272 unaffected relatives [43] (35 patients with multiple cases of WM, 46 with mixed WM/related B-cell disorders kindred, and 28 patients with sporadic WM), familial WM patients were more likely than unaffected relatives to report a history of autoimmune disease and infections. Familial WM patients were also more likely to report exposure to farming, pesticides, wood dust, and organic solvents compared with unaffected family members.

In a large case–control study from the Swedish population that included approximately 2500 LPL-WM patients, more than 9000 matched controls, and approximately 30,000 first-degree relatives, personal history of certain immune-related and inflammatory conditions was associated with increased risk of LPL/WM, supporting the theory that chronic immune stimulation plays a role in the pathogenesis of LPL/WM. These patterns were also observed with previous studies [35, 44, 45] focusing on other subtypes of non-Hodgkin lymphoma. An increased risk of LPL/WM was associated with a personal history of the following autoimmune diseases: systemic sclerosis, Sjögren's syndrome, autoimmune hemolytic anemia, polymyalgia rheumatica, and giant cell arteritis. An increased risk of LPL/WM was associated with a personal history of the following infectious diseases: pneumonia, septicemia, pyelonephritis, sinusitis, herpes zoster, and influenza. Interestingly, an increased risk of LPL/WM was associated with a family history of Sjögren's syndrome, autoimmune hemolytic anemia, Guillain-Barré syndrome, cytomegalovirus, gingivitis and periodontitis, and chronic prostatitis.

The observed relationship between LPL/WM and autoimmune diseases/infections could reflect a premalignant stage of LPL/WM with a disruption of the immune system [46]. Association of family history of autoimmune diseases and infections to LPL/WM could be caused by polymorphisms common to relatives. A third explanation, like earlier mentioned, is that chronic stimulation of B cells make malignant mutation more likely, leading to the development of WM.

These findings were further supported by a recent, large, population-based, case-control study that evaluated 5403 patients with MGUS and 21,209 matched controls with their respective first-degree relatives (14,535 for MGUS and 58,164 for controls) to assess for the association of MGUS with a personal or family history of infections and autoimmune and/or inflammatory conditions. In this study, both a personal history and a family history of autoimmune disease were independently associated with an increased risk of MGUS. Furthermore, a personal history of infections and inflammatory conditions also was associated with an increased risk of MGUS, which supports a role for shared susceptibility for these conditions [47].

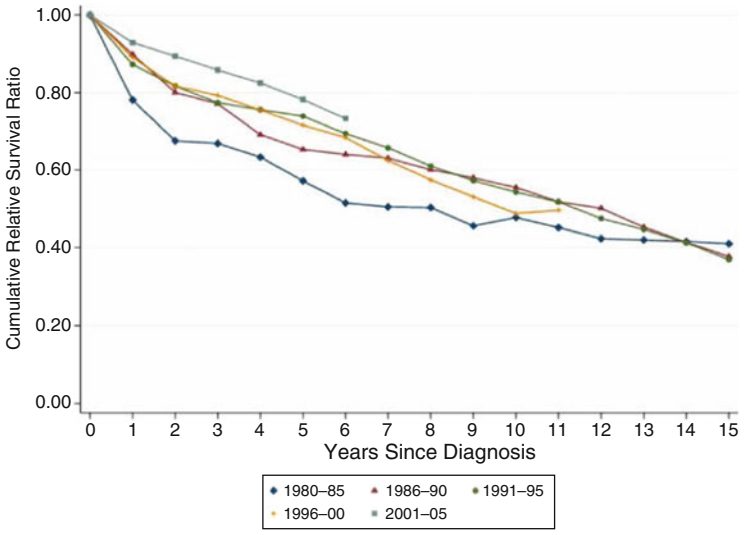
A pooled analysis on 374 cases and 23,096 controls from 11 countries investigated associations with medical and family history, lifestyle, and occupational history [48]. Increased risk of LPL/WM was found in those with history of Sjögren's syndrome, systemic lupus erythromatosus, HCV infection, and a family history of hematologic malignancy. Furthermore, decreased risk was found for history of hay fever and high adult weight. The risk was increased for smoking history exceeding 40 years and occupation as a medical doctor.

9.4 Survival

Due to the rarity of WM, very few phase III randomized trials have been performed to compare different treatment strategies and estimate overall survival. Traditionally, treatment in WM is based on results in phase II trials and expert recommendation [49, 50]. Therefore, thorough observations of survival over time becomes of greater importance.

WM is a slowly progressive disease with good prognosis in asymptomatic patients. Series have shown that the median overall survival is 60–120 months [10, 51–58]. Based on 48 WM patients, a Mayo Clinic study showed an overall survival of 83 % at 5 years and 50 % at 9.6 years in asymptomatic patients [59]. A study of 345 patients showed no improvement over a 25-year period, between 1985 and 2010 [60]. The study included only symptomatic patients and therefore not likely to be affected by lead-time bias. It was, however, based on a referral center and thus subjected to selection bias and, furthermore, the follow-up after 2000 was only 39 months. As pointed out by the authors, longer follow-up is probably needed.

In a population-based study with 1555 WM/LPL patients diagnosed between 1980 and 2005, there was evidence of increased survival over time [61], and it is speculated to be due to introduction to new therapies, such as rituximab, and improvement in supportive care (Fig. 9.1). The improvement was found between



Calendar period	1-year RSR	5-year RSR	10-year RSR	15-year RSR
1980–1985	0.78 (0.69–0.85)	0.57 (0.46–0.68)	0.48 (0.36–0.60)	0.41 (0.28–0.56)
1986–1990	0.90 (0.84–0.84)	0.65 (0.57–0.73)	0.56 (0.45–0.66)	0.38 (0.28–0.49)
1991–1995	0.87 (0.83–0.91)	0.74 (0.68–0.80)	0.54 (0.47–0.62)	0.37 (0.28–0.47)
1996–2000	0.89 (0.85–0.92)	0.72 (0.66–0.77)	0.49 (0.41–0.56)	NA
2001–2005	0.93 (0.89–0.96)	0.78 (0.71–0.85)	NA	NA

Abbr.: RSR=relative survival ratio; NA=not applicable

Fig. 9.1 Cumulative relative survival of LPL/WM patients diagnosed in Sweden 1980–2005, stratified by calendar period at diagnosis [61]

all calendar periods, the greatest improvement in survival was from 1980–1985 to 1986–1990. The improvement in survival was also found in the oldest patients (>80 years old), a trend that has not been found in many other hematologic malignancies, for example, multiple myeloma [62]. There was a consistently better survival in women, which might be contributed to different stage at diagnosis and distribution of prognostic factors in women compared to men. This is similar to observations made in other hematologic malignancies [62, 63] and also found in a large study from the Surveillance, Epidemiology, and End Results (SEER) data on overall survival [64]. Increased age at diagnosis was also, as seen in other studies [64, 65], associated with worse overall survival, in concordance with other hematologic malignancies [62, 63].

A difficult issue to address in many epidemiological studies on survival in WM is the potential effect of lead-time bias. This issue is raised by a possible increase in the number of serum electrophoresis performed and the change in diagnostic criteria over time.

A SEER-database study on 6231 WM diagnosed between 1980 and 2010 found improved relative survival in 2000–2010 compared to 1980–2000 (Fig. 9.2) [66]. Consistent with the Swedish study [61], there was improvement overtime in all age categories. Also, both studies found worse relative survival in oldest

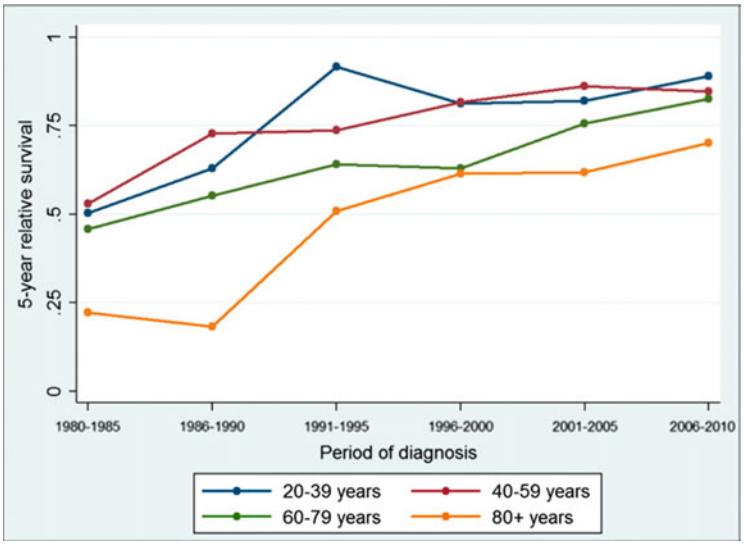


Fig. 9.2 Five-year relative survival in patients with Waldenström's macroglobulinemia from the SEER database 1980–2010, stratified by age at diagnosis [66]

patients; however, there did not appear to be a consistently worse overall survival in males when compared to females.

Another SEER-database study showed improved overall survival independent of age, sex, race, and site of involvement in a competing risk analysis [64]. Furthermore, they found that the overall survival had improved over the last decades in the oldest patients, agreeing with earlier studies.

Changes in survival are multifactorial and can be based on the stage of diagnosis, and furthermore, therapeutical, biological, economical, and environmental. When survival difference between ethnic groups was studied, Hispanics had the worst overall survival while the highest overall survival was in whites [12]. Another study on WM/LPL showed that black patients had worse overall survival [64], and these findings were ascribed to difference in socioeconomical status, health insurance coverage, attitude towards therapy, and biological factors. Improvement in relative survival over the recent years was found in whites and other races, but not in blacks [66].

9.5 Related Cancers and WM Complications

Given the implications for future studies aimed at uncovering underlying susceptibility genes, it is important to define the spectrum of tumors associated with LPL/WM. Hanzis et al. found in a single center study of 924 WM patients and their relatives that 24.3 % of patients had an additional cancer diagnosis, with 63 % preceding the diagnosis of WM [67]. Familial WM was more likely to be associated

with lung cancer, and furthermore, myeloid leukemias were more likely to be found in relatives of WM patients. However, no comparison was made with the US population in general [68].

Other small studies have also estimated the association with various cancers. Garzía-Sanz et al. found in a study of 217 patients that the most common associated malignancies in WM were lung cancer, colon cancer, and myelodysplastic syndrome [55]. An Italian study of 230 WM patients found that they had a 69 % increased risk of developing a second cancer [69]. The highest risk was for diffuse large B-cell lymphoma, myelodysplastic syndrome/acute myeloid leukemia (all three cases in patients treated with alkylators), and brain cancer. This study estimated the incidence in treated vs. untreated patients and found that it was not statistically significant, likely due to few numbers. A large study, using the SEER data, was conducted with 1618 WM patients [70], and results were consistent with the Italian study regarding the increased risk of acute myeloid leukemia; however, it did not support the increased risk of brain cancer. They were unable to estimate the risk of diffuse large B-cell cancer; however, risk of NHL was also increased. Furthermore, there was an increased risk of multiple myeloma, melanoma, and cancers of the colon, lung, and kidney following the diagnosis of WM.

A more recent study based on the SEER data [68] found that WM patients had a 49 % higher risk of secondary malignancy than the general population, and the median time from diagnosis to a second malignancy was 3.7 years. The risk was significant for lungs, urinary tract, thyroid, melanoma, aggressive, lymphoma, and acute leukemia. The risk was higher in patients younger than 65 years compared to older patients, and it was also higher in women. The authors speculated that the increased risk was due to genetic predisposition, immunologic dysfunction, therapy, and other environmental factors. Finally, another study from the SEER database showed that survival in WM patients with colorectal cancer, melanoma, and diffuse large B-cell lymphoma was worse than a matched population with the same cancers [71].

Several issues need to be taken into account when interpreting these results. A transformation of WM into more aggressive malignancy is well known, most commonly to diffuse large B-cell lymphoma [72, 73]. However, the increased risk of acute myeloid leukemia has mainly been attributed to therapy, i.e., nucleoside analogs and alkylating agents.

The Swedish study group analyzed the risks of myeloid hematological malignancies and solid tumors among first-degree relatives of LPL/WM patients [74]. First-degree relatives of LPL/WM patients did not have an increased risk of myeloid malignancies, including acute myeloid leukemia, myelodysplastic syndromes, chronic myeloproliferative neoplasms, and chronic myeloid leukemia. In addition, first-degree relatives of LPL/WM patients had no increased risk of solid tumors taken together (28 solid tumors tested), and relatives of LPL/WM patients who had only a borderline significantly increased risk of pancreas cancer.

It is known that patients with WM may have an increased risk of bleeding due to the IgM paraproteinemia-associated hyperviscosity. Furthermore, many malignancies, including multiple myeloma and its precursor, monoclonal

gammopathy of unknown significant, are associated with an elevated risk of thromboembolism [75]. In a population-based study from Sweden, patients from WM/LPL had a fourfold increased risk of venous thrombosis during the first year, and although the risk was highest during the first year after diagnosis in the study, the risk of venous thrombosis was elevated 5 and 10 years after diagnosis [76]. There was, however, no increased risk of arterial thrombosis. The authors concluded that there was a potential role for prophylaxis, especially in the first year after diagnosis.

9.6 Implications and Future Directions

Recognizing familial association in WM is important for better understanding of the pathogenesis of the disease. However, its use in early detection of LPL/WM is not likely to affect outcome because, currently, asymptomatic LPL/WM is typically not treated. Instead, while relatives of patients with LPL/WM can be informed that they have a higher risk of developing LPL/WM and other lymphomas (compared with family members of unaffected individuals), it should be emphasized that the absolute risk for developing LPL/WM and other hematologic malignancies is very low, there is no treatment for early lesions, and thus no increased medical surveillance is necessary at this time.

The observation that immune-related and inflammatory conditions are associated with an excess risk of developing WM and other lymphomas might have clinical implications regarding the treatment of those conditions. Indeed, it has been suggested in some previous studies that autoimmune disease therapy (such as methotrexate and tumor necrosis factor-alpha blocking agents) might play a role in the development of subsequent lymphoma [77–79].

Studies showing improved survival are important in estimating development in WM therapy over recent decades. Building on the insights regarding activated pathways in WM (e.g., MYD88 and CXCR4), there are ongoing studies with drugs targeting active pathways. At this time, it is too early to tell whether such an approach will continue as a single drug approach or if targeted drugs will require combinations with other more broad acting drugs to prevent resistance and/or escape mechanisms by the tumor cells.

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Genetic Predisposition to Waldenström Macroglobulinemia

10

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10.1 Introduction

The presence of the product of a single clone of antibody-producing cells in the blood is termed paraproteinemia or monoclonal gammopathy. The identification of paraproteinemia is often an incidental finding and can involve immunoglobulins of all classes. Its prevalence increases with age and is estimated to be about 1 % by the age of 50 and about 3 % at 70 years [1]. Usually, no lymphoid infiltrate or clinical symptoms are associated with the electrophoretic findings, and this presentation is then referred to as monoclonal gammopathy of undetermined significance (MGUS). Paraproteinemia may also reflect the presence of malignant clonal growth, typically either IgG/IgA-producing plasma cells in multiple myeloma or IgM-producing lymphoplasmacytic cells in Waldenström macroglobulinemia (WM). WM is a rare disease, but the precursor condition, IgM-MGUS, occurs with increasing prevalence above the age of 50 (See Chap. 11 for an in-depth discussion of IgM-MGUS). It is now recognized that these disorders form a continuum and that IgM-MGUS will progress to WM at an annual rate of about 1.5 % [2]; however, progression to malignancy cannot be predicted on an individual basis.

The recent identification of mutations in the *MYD88* gene in WM tumor cells is providing insights into WM tumor biology. Meanwhile, the host-related and

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_10

environmental factors predisposing to WM development remain active areas of research. A number of putative risk factors have been evaluated and are discussed elsewhere in this volume. Mounting interest in a potential genetic component of WM susceptibility was sparked as familial clustering of IgM paraproteinemias—both IgM-MGUS and WM—has been reported periodically over the last six decades. Observations from studies of these families provided the impetus for population-based epidemiological studies, which have provided further support for a hereditary component in the etiology of IgM-related monoclonal gammopathies. Over the past 15 years, an expanding array of technological tools has permitted more intensive genetic evaluation of familial clusters, and various approaches are being used to better characterize the phenotypic spectrum of WM. The ultimate goal is to establish genotype–phenotype correlations that will pave the way towards understanding the underlying pathogenetic mechanisms. Moreover, because monoclonal gammopathies are associated with advancing age, the study of the genetic mechanisms contributing to these disorders may lead to important insights into the aging immune system, which is highly relevant as older age groups continue to account for an increasing proportion of many populations.

10.2 Evidence Supporting Genetic Predisposition to WM

10.2.1 Familial Clustering of WM

Reports of familial clusters often provide the initial indication of a potential role for genetic factors in disease susceptibility. The familial occurrence of Waldenström macroglobulinemia was first reported by Massari [3], who published an example of WM occurring in two brothers more than 50 years ago. Familial clusters have subsequently been the subject of dedicated case reports [4–10] or embedded within larger family studies [8, 11–15]. Table 10.1 summarizes the most important studies of families published to date, some including extensive literature surveys. The inheritance pattern of WM within families is variable (Fig. 10.1). Many WM families include cases in multiple generations, thereby strengthening the argument for inherited factors rather than shared environment.

These studies generally predated the genomic era and therefore included only limited genetic analysis (reviewed in [31]). Some of these early studies were notable for extensive investigation of the characteristics of the IgM molecule in the WM and IgM-MGUS patients [6, 8, 10, 13]. In four of nine families where IgM was characterized, light chain typing was discordant among the cases [8, 13], including a family with two affected monozygotic twins [6]. Among the remaining five sets of familial WM cases, related patients differed in other characteristics of the IgM molecules, including light chain mobility, immunoglobulin allotyping, or idiotypic determinants [10, 13], suggesting that familial cases could not be explained by clonal B-cell expansion in response to some common exposure. In addition, HLA typing in a subset of WM families was also inconclusive, with only one family having a haplotype that co-segregated with disease [4].

Table 10.1 Reported familial clusters of Waldenström macroglobulinemia (WM) or IgM monoclonal gammopathy of undetermined significance (MGUS)

Author	Fam	Number of LPDs occurring in family				Relationships between all LPD cases[a]	Case Age and Gender		Symptomatic WM	Basis for WM diagnosis
		WM	Other LPD	MGUS			LPD[a]	IgM MGUS		
				IgM MGUS	Non-IgM MGUS					
Multiple-case WM families										
Masari [3]	1	2	0	1	0	**Brothers**—mother	**61 M, 61 M**	83 F	Yes, Yes	BMB, EP
Coste [11]	1	2	0	0	0	**Brother—sister**	**61 M, 54 F**	—	Yes, nr	BMB, EP, anemia
Getaz [7]	1	2	0	0	0	**Father—son**	**70 M, 47 M**	—	Yes, Yes	BMB, EP
Youinou [10]	MIN	4	0	1	0	**Brothers**	**61 M, 62 M, 64 M, 68 M**	—	nr	BMB, EP
Blattner [4]	1	4	0	1[b]	0	**Father—sons, daughter**, grandson	**77 M, 53 M, 42 M, 57 F**	52 M	No, Yes, Yes, No	BMB, EP
Fine [5, 16]	1	3	0	(2)[c]	0	**Brother—brother, sister**	**61 M, 65 M, 76 F**	58 M, 61 F	Yes, Yes, Yes	BMB, EP
Fine [6]	1	2	0	0	0	**Monozygotic twins**	**75 M, 75 M**	—	No, No	BMB, EP
Renier [8]	1	4	0	0	0	**Brothers**	**76 M, 75 M, 72 M, 69 M**	—	Yes, No, Yes, Yes	BMB, EP[d]

(continued)

Table 10.1 (continued)

Author	Fam	Number of LPDs occurring in family				Relationships between all LPD cases[a]	Case Age and Gender		Symptomatic WM	Basis for WM diagnosis
		WM	Other LPD	MGUS			LPD[a]	IgM MGUS		
				IgM MGUS	Non-IgM MGUS					
Jaccottet in [8]	1	2	0	0	0	**Father—son**	**74 M, 47 M**	—	Yes, Yes	BMB, EP
Du Pont de Romemont in [8]	1	2	0	0	0	**Mother—daughter**	**74 F, 52 F**	—	nr	nr
Taleb [9]	1	2	0	0	0	**Sisters**	**64 F, 43 F**	—	Yes, Yes	BMB, EP
Deshpande [17]	0047	2	1 MM; 1 HL[e]	0	1 IgG	**Aunt—niece**, granddaughter	**72 F, 53 F**, 65 M, nr F	—	nr	nr
McMaster [12]	B	4	0	1	0	**Mother—daughter, granddaughter, grandson**, grandson	**64 F, 51 F, 32 F, 57 M**	39 M	Yes, Yes, Yes, nr	BMB, EP; medical record review
McMaster [12]	C	3	1 NHL[f]	2	0	**Brother—brother, sisters**, son	**69 M, 69 F, nr F**, 34 M	59 M, 52 M	Yes, Yes, Yes	BMB, EP; medical record review
Single-case WM + MGUS families										
Spengler [18]	IV	1	0	1	0	**Father**—daughter	**69 M**	48 F	Yes	BMB, EP
Vannotti in [14]	ZA	1	0	1	0	**Son**—mother	**56 M**	82 F	nr	NR

Seligmann [14]	PE	1	0	1	0	**Son**—mother	**41 M**	70 F	nr	BMB, EP
Seligmann [14]	PO	1	0	1	0	**Son**—mother	**57 M**	87 M	nr	BMB, EP
Seligmann [14]	LA	1	0	1	0	**Brother**—brother	**72 M**	70 M	nr	BMB, EP
Seligmann [14]	MO	1	0	3	0	**Sister**—sister, son, daughter	**65 F**	68 F, 54 M, 49 F	nr	BMB, EP
Kalff [19]	Ru	1	0	1	0	**Brother**—sister	**61 M**	71 F	Yes	BMB, EP
Kalff [19]	Ho	1	0	1	0	**Son**—mother	**53 M**	75 F	Yes	BMB, EP
Nardoni in [8]	1	1	0	1	2 NT	**Mother**—son	**83 F**	42 M	nr	nr
Linet [20]	A	1	0	1	0	**Sister**—sister	**nr F**	>76 F	nr	BMB or LNB, EP
Linet [20]	B	1	0	1	0	**Father**—daughter	**nr M**	>43 F	nr	BMB or LNB, EP
Custodi [21]	1	1	0	1	0	**Brother**—sister	**73 M**	70 F	nr	nr
Manschot [22]	1	1	0	1	0	**Mother**—daughter	**70 F**	50 F	Yes	nr
Kalff [19]	Be	1	0	0	1 NT[g]	**Brother**—brother	**65 M**	70 M	Yes	BMB, EP
Kalff [19]	Ze	1	0	1	1 NT	**Brother**—brother—sister	**51 M**, 57 F	65 M	No	BMB, EP
Kalff [19]	Br	1	0	0	1 NT	**Uncle**—niece	**73 M**, 46 F	—	Yes	BMB, EP
Kalff [19]	Wij	1	0	0	1 IgA	**Brother**—sister	**74 M**, 62 F	—	Yes	BMB, EP

(continued)

Table 10.1 (continued)

Author	Fam	Number of LPDs occurring in family				Relationships between all LPD cases[a]	Case Age and Gender		Symptomatic WM	Basis for WM diagnosis
		WM	Other LPD	MGUS			LPD[a]	IgM MGUS		
				IgM MGUS	Non-IgM MGUS					
Kalff [19]	Vi	1	0	0	1 IgG	**2nd cousin**—2nd cousin	**75 M**, 85 F	—	Yes	BMB, EP
Single-case WM + LPD families										
Kalff [19]	Hn	1	1 CLL; 1 NHL	1	1 IgG; 3 NT[g]	Complex, no male:male[h]	**80 M**, 67 F, 62 M, 78 F, 55 F, 49 M	35 F, 55 F	nr	EP
Fraumeni [23]	1	1	4 NHL; 1 HL; 1 ALL	0	0	Complex, no male:male[i]	**70 M**, 75 M, 54 M, 50 M, 68 F, 23 M, 5 M	—	Yes	BMB, EP
Björnsson [24]	1	1	1 NHL; 1 MM[j]	2	0	**Sister**—brothers and niece	**71 F**, 72 M, 57 F	82 M, 77 M	Yes	BMB, EP
Steingrimsdóttir [25]	1	1	1 NHL; 3MM	0	1 IgA; 1 NT	Complex, no male:male[k]	**F**, F, M, M, F, F (ages nr)	—	nr	nr

Steingrimsdóttir [25]	3	1	2 NHL; 2 MM; 1 HL	0	2 IgG; 1 NT	Complex, no male:male[l]	**F**, M, M, F, F, M, M, F (ages nr)	—	nr	nr
Multiple-case IgM MGUS families										
Williams [26]	L'Her	0	0	2	0	Brother—sister	—	48 M, 56 F	—	nr
Kalff [19]	Re	0	0	2	0	Brother—sister	—	46 M, 46 F	—	BMB, EP; EP
Zawadzki [27]	VII	0	0	3	0	MZ twins—granddaughter	—	98 M, 98 M, 38 F	—	EP
Busis [28]	1	0	0	2	0	Mother—son	—	nr F, nr M	—	nr
Jensen [29]	1	0	0	2	0	Sister—brother	—	68 F, 55 M	—	BMB, EP
Petite in [8]	1	0	0	3	0	Brother—sisters	—	72 M, 75 F, 70 F	—	EP
Manschot [22]	2	0	0	2	0	Brother—brother	—	57 M, 65 M	—	EP

(continued)

Table 10.1 (continued)

Author	Fam	Number of LPDs occurring in family				Relationships between all LPD cases[a]	Case Age and Gender		Symptomatic WM	Basis for WM diagnosis
		WM	Other LPD	MGUS			LPD[a]	IgM MGUS		
				IgM MGUS	Non-IgM MGUS					

Abbreviations: WM, Waldenström macroglobulinemia; MGUS, monoclonal gammopathy of undetermined significance; Fam, family; LPD, lymphoproliferative disorder; M, male; F, female; nr, not reported; BMB, bone marrow biopsy; EP, electrophoresis; MM, multiple myeloma; HL, Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; ALL, acute lymphocytic leukemia; LNB, lymph node biopsy; NT, not typed; —, not applicable; >, greater than

[a]WM cases are shown in bold type; complex relationships are also notated regarding whether male-to-male (male:male) inheritance was observed

[b]Identified on follow-up of the family (A) by McMaster et al. [12]

[c]This family was originally reported as having one case of WM and two cases of IgM MGUS. The two patients with IgM MGUS progressed to WM 7 and 15 years later, respectively

[d]Bone marrow biopsy was reported only for the 72 year old brother

[e]The myeloma and Hodgkin lymphoma cases were both in the paternal bloodline of the IgG MGUS case, but not in the bloodline of either WM case

[f]A son of a WM case was diagnosed at age 34 with diffuse large B cell lymphoma and was treated without recurrence during 21 years of follow-up. Eighteen years following his NHL diagnosis, he was discovered to have an IgM MGUS at age 52. Both diagnoses appear independently in the table

[g]In Family Be, member II.2 (70 year old male) is provisionally categorized as IgM MGUS based on having an IgM level 600 % of the upper limit of the reference range, IgA level below the 95 % tolerance limit for the reference range, normal IgG level, and a monoclonal protein that was not typed. In Family Hn, member IV.4 (35 year old female) is definitively categorized as IgM MGUS on the basis of a serum monoclonal protein typed as IgM, while member III.3 (55 year old female) is provisionally categorized as IgM MGUS based on an untyped monoclonal protein in the presence of an IgM level 230 % of the upper limit of the reference range and normal IgA and IgG levels

[h]In this 3-generation family, the WM case had a sister with IgG MGUS (or possibly myeloma) a granddaughter with IgM MGUS, and a brother and a daughter with untyped monoclonal proteins. The sister with IgG MGUS had a son with an untyped monoclonal protein

[i]In this 4-generation family, the WM case had one sister and three brothers with NHL. The affected sister had a son with Hodgkin lymphoma and a great grandson with acute lymphocytic leukemia

[j]Multiple myeloma developed in a niece and was reported by Ögmundsdóttir et al. [30]

[k]In an extended family, the WM case had a sister with IgA MGUS and a father with untyped MGUS. On the maternal side were two cousins with NHL, three cousins with MM, and one cousin with acute myelogenous leukemia (AML)

[l]In an extended Icelandic pedigree, the WM case had a paternal aunt and cousin with MM, two paternal cousins with NHL, three paternal cousins with MGUS (IgG and untyped), one paternal cousin with HL, and one paternal cousin with AML

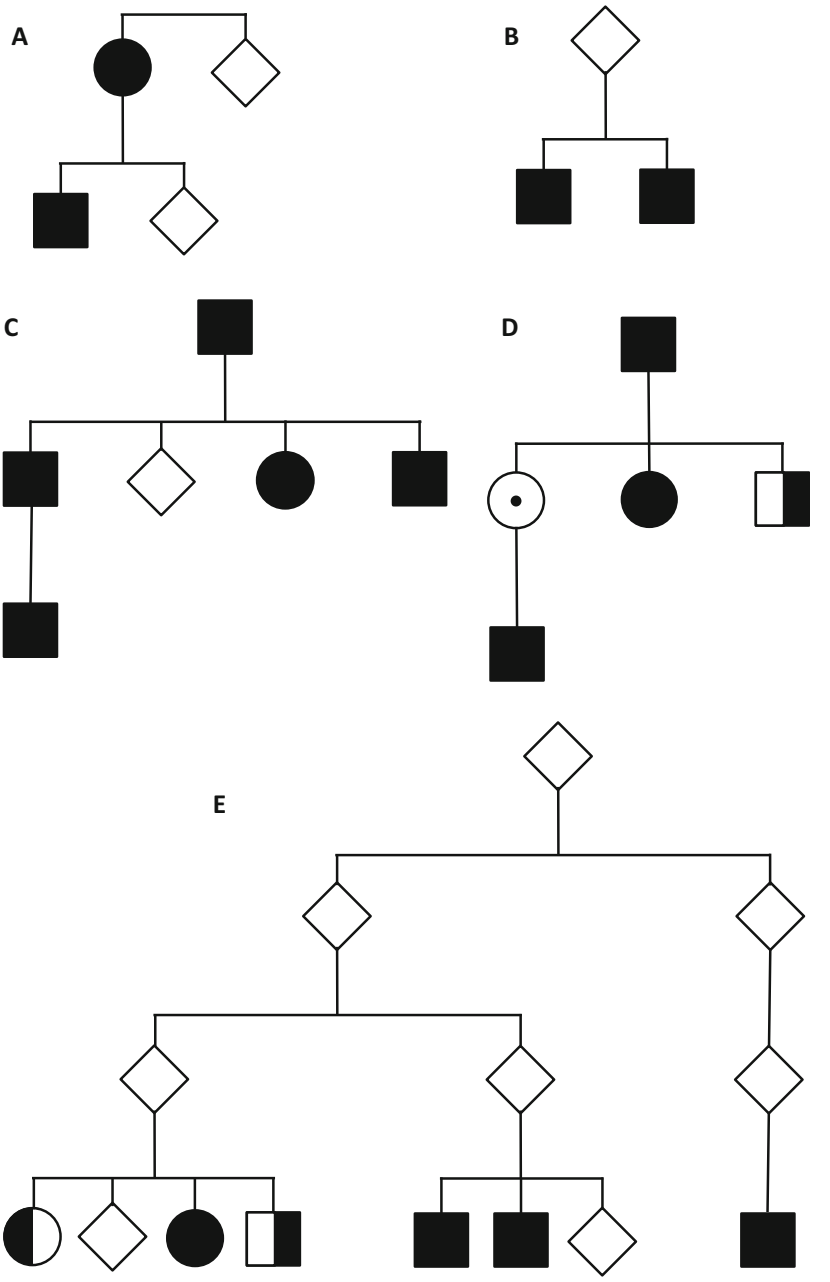

Fig. 10.1 The diversity of Waldenström macroglobulinemia (WM) pedigrees. Panel (**a**) depicts one of the most common configurations, a family containing two affected individuals, in this case parent and offspring. All possible gender configurations, i.e., father to son or daughter and mother to son or daughter, have been observed. Panel (**b**) shows another common configuration consisting of affected siblings. Panel (**c**) depicts an autosomal dominant pattern of inheritance, with three generations of male-to-male transmission. Panel (**d**) illustrates a family that demonstrates

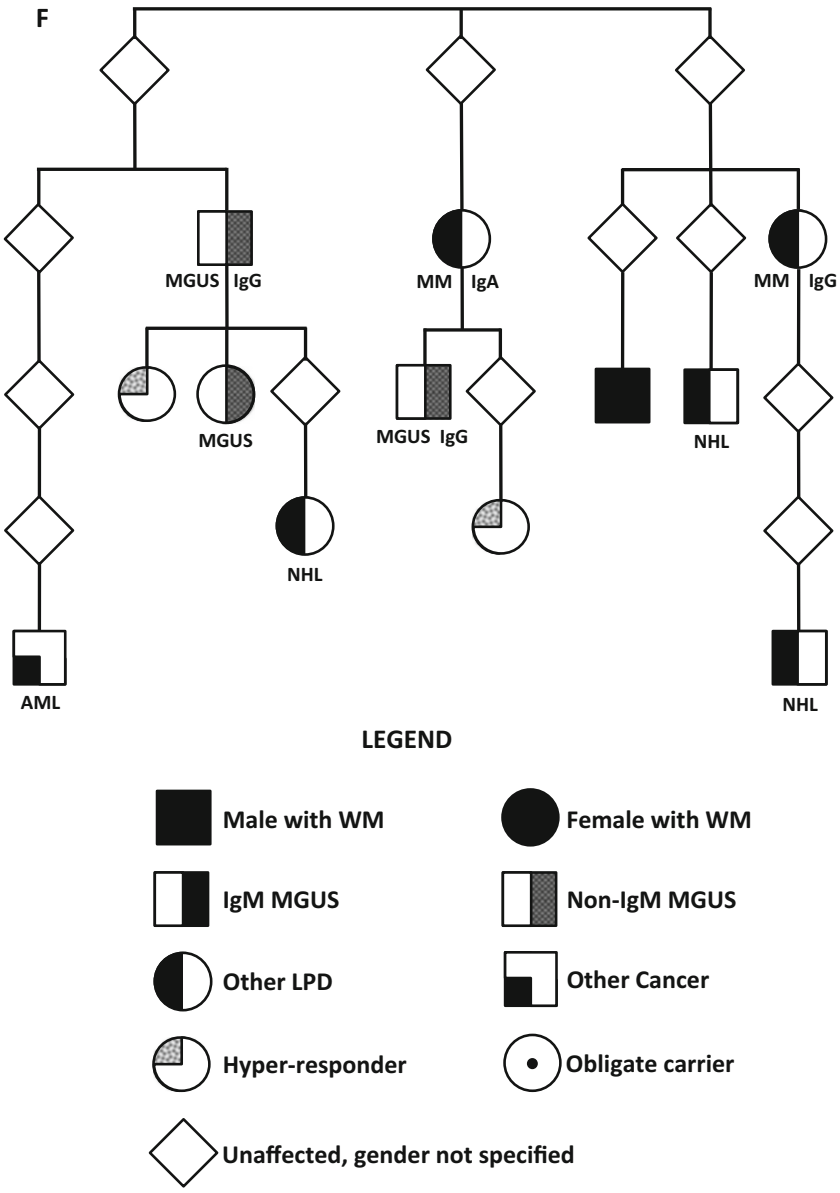

Fig. 10.1 (continued) incomplete penetrance. Panel (e) shows a pedigree configuration compatible with autosomal recessive transmission. Panel (f) demonstrates that a variety of lymphoproliferative disorders may be found in different combinations in some families. This family has been published previously [25]. Families C and E are remarkable for the large number of individuals affected with WM. However, often there is insufficient information about older generations to determine whether earlier family members may have been affected. *Abbreviations*: *NHL* non-Hodgkin lymphoma, *MM* multiple myeloma, *AML* acute myelogenous leukemia, *WM* Waldenström macroglobulinemia, *MGUS* monoclonal gammopathy of undetermined significance, *LPD* lymphoproliferative disorder

Although initial reports highlighted families defined by at least two cases of WM, investigators also recognized that WM might cluster with other B-cell lymphoproliferative disorders, including chronic lymphocytic leukemia (CLL), other subtypes of non-Hodgkin lymphoma (NHL), and possibly multiple myeloma (MM), as well as with monoclonal gammopathy of undetermined significance (MGUS) [17, 23, 24, 30]. Ascertainment strategy has been a critical component of study design and results. For example, several studies reported families with plasma cell dyscrasias, and there was often not a clear distinction made between IgA/IgG and IgM disorders. Many studies were prompted by clinical findings and were often based in one hospital or center. Moreover, WM was not registered as a malignant disease in cancer registries until 1988 or later, and this has limited case-finding efforts through population-based registries. Thus, the Icelandic cancer-registry-based study was performed by first identifying all cases of multiple myeloma between 1954 and 1989 and then cross-referencing a specially collected database of monoclonal gammopathies based on all serum electrophoreses between 1976 and 1997. Therefore, this study was biased towards identifying families with IgG/IgA and IgM disorders occurring together, a combination that is otherwise rarely reported [25].

10.2.2 Population-Based Evidence for Inherited Predisposition to WM

Further support for both an inherited predisposition to WM and familial co-aggregation of WM/LPL with other B-cell lymphoproliferative disorders (LPD) has come from population-based data. To date, large epidemiologic studies have included both WM and lymphoplasmacytic lymphoma (LPL) as cases, both to increase power and to mitigate misclassification. Interlinked population registries in Sweden have been an invaluable tool to investigate WM/LPL familial aggregation using standardized incidence ratio [32] and case-control [33] methodology. Studies conducted using such registries tend to minimize recall bias and to increase generalizability of results, although they cannot completely eliminate the possibility of histopathological misclassification that is inherent in current LPD diagnostic methods. These studies show that first-degree relatives of WM/LPL patients have increased risk of developing B-cell malignancy, a pattern that is common to other lymphoproliferative disorders. As illustrated in Table 10.2, the data supporting co-aggregation are remarkably similar irrespective of the proband's diagnosis. In the largest such study of relatives of WM/LPL patients (6177 first-degree relatives of 2144 WM/LPL patients; 27,609 first-degree relatives of 8279 controls), Kristinsson [33] found that the highest risk—20-fold—was for a first-degree relative of a WM/LPL patient to develop WM/LPL (odds ratio [OR] = 20; 95 % confidence interval [CI] 4.1–98.4). The risk was significantly increased, though to a lesser extent, for other B-cell malignancies, including CLL (OR = 3.4; 95%CI 1.7–6.6) and NHL (OR = 3.0; 95%CI 2.0–4.4). Family members of WM/LPL patients were also at increased risk for MGUS, but immunoglobulin isotype data were unavailable. The increased risk applies to all first-degree relatives (parents,

Table 10.2 Relative risk for lymphoproliferative and plasma cell tumors among first-degree relatives of patients with lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia or other lymphoproliferative or plasma cell tumors or MGUS, compared with relatives of matched controls[a]

Proband condition	Non-Hodgkin lymphoma OR (95 % CI)	Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia OR (95 % CI)	Chronic lymphocytic leukemia OR (95 % CI)	Hodgkin lymphoma OR (95 % CI)	Multiple myeloma OR (95 % CI)	MGUS OR (95 % CI)
Lymphoproliferative disorder						
Non-Hodgkin lymphoma	1.7	—	1.3	1.4	1.1	—
	(1.4–2.2)		(0.9–1.9)	(1.0–2.0)	(0.8–1.5)	
Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia	3.0	20.0	3.4	0.8	1.6	5.0
	(2.0–4.4)	(4.1–98.4)	(1.7–6.6)	(0.3–2.2)	(0.8–3.2)	(1.3–18.9)
Chronic lymphocytic leukemia	1.9	4.0	8.5	1.5	1.2	1.4
	(1.5–2.3)	(2.0–8.2)	(6.1–11.7)	(0.96–2.3)	(0.9–1.8)	(0.9–2.2)
Hodgkin lymphoma	1.3	—	2.1	3.1	1.0	—
	(0.9–1.8)		(1.2–3.8)	(1.8–5.3)	(0.6–1.8)	
Plasma cell disorders						
Multiple myeloma[b]	1.1	1.4	1.1	0.9	2.1	2.1
	(0.9–1.4)	(0.7–2.8)	(0.8–1.7)	(0.6–1.4)	(1.6–2.9)	(1.5–3.1)
MGUS	1.1	2.0	2.0	1.3	2.9	2.8
	(0.8–1.5)	(1.2–2.3)	(1.2–2.3)	(0.6–2.9)	(1.9–4.3)	(1.4–5.6)

OR odds ratio, *95 % CI* 95 % confidence interval, *MGUS* monoclonal gammopathy of undetermined significance

[a]Used with permission. Originally published in [34]. Obtained from the Haematologica Journal website http://www.haematologica.org

[b]A single study [35] subsequently reported a relationship between multiple myeloma in a proband and risk of LPL/WM in the offspring. See text

offspring, and siblings). There are suggestions that the risk may be modified by gender or age at diagnosis, but small numbers of cases may lead to imprecision in the estimates. Increased risks were not seen for Hodgkin lymphoma or multiple myeloma. A subsequent population-based study in two Swedish counties [36] confirmed a high prevalence of broadly defined familial WM. A more recent Swedish linkage study of 24,137 multiple myeloma patients found an increased risk of LPL/WM in offspring when a parent was diagnosed with multiple myeloma (standardized incidence ratio (SIR) = 3.47; n=8; 95% CI 1.82–6.61) [35]. Taken together, it appears that WM definitely co-aggregates with B-cell lymphoid disorders while co-aggregation of IgG/IgA and IgM disorders in the same family may occur. The relationship of multiple myeloma to familial WM remanis controversial.

Conventional case-control studies have provided some additional evidence supporting genetic susceptibility. An early hospital-based study of 65 cases of WM did not find increased risk among patients having a first-degree relative with hematolymphoproliferative malignancy, other cancer, or autoimmune disease but did document various immunologic abnormalities, including IgM-MGUS, in the families of those patients [20]. More recently, a large international study of 374 WM/LPL cases and 23,096 controls found a 64% increased risk for developing WM/LPL in individuals with a first-degree relative diagnosed with a hematologic malignancy [37]. Finally, WM patients in a large single-center series reported a high prevalence (18.7%) of either WM or another B-cell disorder in first-degree relatives [38]. These data are convincing for predominantly white, northern European populations, but similar studies have not yet been conducted in other demographic groups.

The data are less compelling for the co-segregation of WM and myeloid malignancies or solid tumors. Familial WM patients reported a proportional excess of myelopoietic cancers in their first- and second-degree relatives, compared to non-familial WM kindred [39]. The same study found no excess of solid tumors in WM families. A follow-up of the same clinically derived cohort used a population-based family history registry to conduct an observed-to-expected analysis and found that familial WM patients differed from controls in their reporting of family history of some solid tumors [40]. Selection and reporting bias could not be ruled out, since these observations were based on family report rather than actual incidence data and were not independently verified. In contrast, an early registry study of WM/LPL patients found no increased risk for non-CLL leukemias in their relatives but did find an increased risk of gastric cancer in their parents [32]. Subsequent analyses in the Swedish population registries were unable to confirm either of these findings and instead reported a possible association with pancreas cancer [41]; the investigators concluded that they could not rule out a chance association based on multiple testing. Thus, the preponderance of evidence supports an association of WM with other B-cell malignancies but not myeloid leukemias or solid tumors.

Overall, there is a strong indication for the involvement of genetic mechanisms in the pathogenesis of WM. The diversity of reported inheritance patterns involving cases in one or more generations suggest that several different genes might be involved, and the co-aggregation of different B-cell disorders implies that causative genes may be operating at the level of lymphopoietic stem cells.

10.3 Phenotypic Correlations with Genetic Predisposition

Both population and family studies suggest that predisposition to WM may be associated with a broader clinical phenotype than simply development of WM alone. Characterizing the clinical phenotype may aid susceptibility-gene-discovery efforts by more precisely identifying at-risk individuals for inclusion in genetic studies. From a clinical standpoint, familiality may have implications for screening, diagnosis, surveillance, and outcomes. Ultimately, the goal is to establish genotype–phenotype correlations to be able to address these issues. A classic paradigm is that of hereditary breast–ovarian cancer, in which the recognition that these two tumors clustered in families eventually led to the identification of *BRCA1/2*, which in turn led to: (1) a better understanding of the association of other cancer types (e.g., pancreas and prostate cancers) in some families but not others; (2) development of surveillance and prevention strategies for at-risk individuals; and (3) ongoing research to optimize treatment. At present, in the absence of a confirmed susceptibility gene(s) for WM, research efforts have focused primarily on family studies or cohorts of patients reporting a family history of WM. Until better treatments and/or prevention strategies are developed for WM, identification of genotype-phenotype correlations may have limited practical clinical utility. Notwithstanding, family studies provide an opportunity for the study of the natural history and pathophysiology of these disorders, which will also be of value for non-familial cases.

10.3.1 Descriptive Epidemiology of Familial WM

Descriptive studies of familial WM have included case studies of one or more specific family clusters [3] or studies of WM patients reporting a family history [38, 42]. In early studies of multiple-case WM family units, familial WM patients were noted to differ from non-familial WM in having a younger age at onset and stronger male predominance compared to expectations based on the medical literature for sporadic WM. Familial WM patients had similar presenting symptoms, signs, and laboratory findings as historical non-familial patients and were noted to have a frequent antecedent history of IgM monoclonal gammopathy. Laboratory studies revealed no distinguishing features of the IgM molecules isolated from familial patients. Limited cytogenetic studies found no chromosomal abnormalities that discriminated familial from sporadic cases [9, 10, 13, 43, 44].

More recent studies have focused on cohorts of patients who report a family history of WM or other B-cell LPD and have included non-familial WM cases as controls [38] and in some instances unaffected relatives [42]. These studies did not confirm younger age at diagnosis or skewed gender distribution for familial vs. -non-familial WM patients, although it should be noted that the median age at diagnosis in non-familial patients was substantially younger in both reports than expected in the general population [45] and may reflect ascertainment or access bias. In contrast, both studies confirmed no differences in presenting complaint, symptoms, signs, or routine laboratory results based on familial status, with the

exception that familial patients appeared to have higher IgM levels and a nonsignificant increase in tumor burden at referral. Cytogenetic analysis, including more sensitive FISH studies, of a subset of patients also confirmed no significant differences between groups.

Thus, while the question of age at onset remains unresolved, there appear to be no clinical features that reliably discriminate familial from non-familial WM at disease presentation. However, familial WM might be associated with a more indolent course, at least in its early stages, based on the observation that higher IgM levels at referral were not accompanied by more severe symptoms, signs, or other laboratory abnormalities. This conclusion has been challenged by more recent data from some of the same groups (see Section 10.3.6, below) and thus remains an active area of inquiry.

10.3.2 Autoimmunity and Familial WM

Multiple lines of evidence implicate a relationship between WM and autoimmunity (Table 10.3). Autoimmunity may be manifest either clinically, through diagnosis of a symptomatic autoimmune disorder, or sub-clinically, through demonstration of high titers of autoantibodies that have no—or minor—associated symptoms. While investigators reported instances of clinical autoimmune disease (e.g., rheumatoid arthritis) in family members, early family studies focused primarily on the latter phenomenon. A variety of autoantibodies were evaluated in a subset of multiple-case families and in families of sporadic WM. In nine reported multiple-case WM families that included assessment of first-degree relatives, 25 % of relatives were found to have at least one autoantibody [4, 8, 10, 13]. In two mixed WM/LPD families, 13 % of tested first-degree relatives had at least one autoantibody [21, 23]. Finally, in one study including first-degree relatives of sporadic WM, 18 % of those tested had at least one autoantibody [14]. In contrast, in an Icelandic WM/LPL/MGUS family, a slightly raised autoantibody level was detected in only 1 of 25 family members tested [30]. A clinical diagnosis of autoimmune disease was rarely reported in early studies, although in only one study relevant medical history data were explicitly sought and validated [4]. More recently, Brandefors and colleagues reported validated autoimmune disease in relatives among 5 of 12 (42%) WM families [36]. These results raised the possibility that family members of WM patients might be predisposed to clinical and subclinical autoimmune phenomena. However, the lack of controls precluded definite conclusions. Moreover, the prevalence of many of these autoantibodies in the general population is unknown, though some (e.g., antithyroid antibodies and low-level rheumatoid factor levels) are known to be relatively common among apparently healthy subjects.

These family-based observations provided the impetus for a large population-based study to evaluate family history of diagnosed autoimmune disease and risk of WM/LPL. In a case-control study of 2470 WM/LPL patients, 9698 controls and first-degree relatives of cases ($n = 5710$) and controls ($n = 22{,}799$), family history of Sjögren syndrome (OR = 5.0; 95%CI 2.1–12.0), autoimmune hemolytic anemia (OR = 3.8; 95%CI 1.1–13.2), and Guillain–Barré syndrome (OR 4.1; 95%CI

Table 10.3 Autoimmunity associated with familial Waldenström macroglobulinemia

Reference	Families studied	WM cases	Unaffected relatives studied			Findings related to autoimmunity in relatives				
			Total	# 1st degree	Age range	Clinical	Disease (n)	Biological	Laboratory assays reported	Abnormal assays (n)
	n	n	n	n		n		n		
Studies of aggregated WM families										
Seligmann [13, 14][a]	63	65	216	192	5–87	—	—	46	AγG, AGA, ANA, ATA, CA	AγG
Linet [20][b]	8	8	45	45	>9[c]	5	nr	—	—	—
Studies of individual WM families										
Fraumeni [23]	1	1	9	2	42–64	3	Raynaud (1), possible RA (2)	—	—	—
Youinou [10]	1	4	35	8	1–65	0	n.a.	5	ADA, RF	ADA (1); RF (4)
Blattner [4]	1	4	16	13	nr	2	Hashimoto thyroiditis (2)	8	AMC, AMt, ANA, APA, ASM, ATG, ATM, RF	RF (3); ATG (2); ATM (3); AMC (2)
Renier [8]	1	4	12	2	20–63	—	—	2	AMt, ANA, APA, ARA, ASM, ATG, ATM, RF	ANA (1); ATG (1)
Du Pont de Romemont in [8]	1	2	2	nr	nr	0	n.a.	0	nr	nr
Ögmundsdóttir [30]	1	1	34	1	nr	—	—	1	ANA, RF	ANA (1)
Custodi [21]	1	1	4	4	25–70	—	—	1	ATG, RF, cryo	ATG (1)

Brandefors et al.	12	19	59	45	nr	5	RA (2), Sjögren's syndrome (1), Hypothyroidism (1), PMR (1)	—	—	—
Studies of multiple-case IgM MGUS families										
Petite [46]	1	0	18	4	18–80	2	Pernicious anemia (1), RA (1)	2	ADA, A-IF, ANA, ANP, APA, ATG, RF	ANA (1); ADA/ANP/ APA (1)

Abbreviations: WM, Waldenstr-m macroglobulinemia; nr, not reported; n.a., not applicable; RA, rheumatoid arthritis; AγG, anti-γ-globulin factors; AGA, anti-gastric antibodies; ANA, anti-nuclear antibodies; ATA, anti-thyroid antibodies; CA, cold agglutinin titers; ADA, anti-DNA antibodies; RF, rheumatoid factor; AMC, anti-myocardial antibodies; AMt, anti-mitochondrial antibodies; APA, anti-parietal cell antibodies; ASM, anti-smooth muscle antibodies; ATG, anti-thyroglobulin; ATM, antithyroid microsomal; ARA, anti-reticulin antibodies; cryo, cryoglobulins; A-IF, anti-intrinsic factor; ANP, anti-nucleoprotein antibodies; cryo, cryoglobulins; —, evaluation or assay not performed; PMR, polymyalgia rheumatica

[a]Includes two multiple-case WM families reported by Massari [3] and Coste [11], respectively, and one WM + MGUS family reported by Vannotti [15]. Although 216 relatives were studied, immunoglobulin levels were reported only for the 192 first-degree relatives

[b]The study was based on the families of 31 WM patients. Results were reported as odds ratios for the aggregate study. Individual results were reported for 45 relatives (all first-degree) from 8 families and are included here

[c]Year of birth was reported. For this table, age was estimated using date of birth and last date of study entry (December 31, 1983)

1.8–9.4) were each significantly associated with risk of WM/LPL [47]. Family history of systemic lupus erythematosus was also associated with increased risk for WM/LPL, at a borderline level of significance (OR = 3.9; 95%CI 1.0–15.6). Since this was a registry study, it did not include a laboratory screening arm for subclinical manifestations of autoimmunity.

Taken together, these findings suggest that an underlying immunoregulatory defect is present in WM families. Since immune dysfunction is a known predisposing factor for lymphoma, carefully designed studies will be required to determine whether the immune dysfunction itself predisposes to WM or whether there is a more general immunogenetic defect that may manifest as malignancy, autoimmunity, or both.

10.3.3 IgM Monoclonal Gammopathy of Undetermined Significance in Familial WM

One of the most interesting discoveries to arise from detailed evaluation of WM families is the relatively frequent occurrence of other immunoglobulin abnormalities in apparently unaffected relatives of WM patients. The most striking of these is IgM monoclonal gammopathy of uncertain significance (MGUS) (Table 10.4). The very first description of a WM family noted the occurrence of asymptomatic IgM monoclonal gammopathy in the healthy parent of two WM patients [3]. Subsequent family studies revealed that IgM-MGUS is a frequent finding in WM families [8, 10, 14, 36], although its prevalence remains undefined. In the initial report, the monoclonal gammopathy was transient. However, long-term follow-up studies have shown that relatives with IgM-MGUS may be at high risk for progression to WM, perhaps at a rate exceeding that of progression of IgM-MGUS to WM in the general population. Fine et al. reported a family in which two relatives with IgM monoclonal gammopathy progressed to WM in 7 and 15 years, respectively [5]. McMaster and colleagues [12] described three multiple-case WM families that presented with a total of eight WM cases. Of seven relatives found on screening to have IgM monoclonal gammopathy, three were subsequently diagnosed with WM, three others had progressive increases in their monoclonal IgM, and one had persistent, stable IgM monoclonal gammopathy, with a median follow-up of 17 years. In an Icelandic family, progression was observed in one case from MGUS to WM in 3 years and in another from MGUS to WM in 6 years and after further 4 years to LPL [25, 30]. Four of the five families in these reports were unusual in having more than two members affected with WM, so it is not yet clear whether the observed risk of progression applies to all WM families. IgM-MGUS has been recognized independently as a risk factor for subsequent development of WM and other B-cell LPD at a rate of 1.7 % per year [2]. These observations sparked further population-based studies and together these studies have established IgM-MGUS as a precursor condition for WM. Active areas of research include establishing the prevalence of IgM monoclonal gammopathy in WM families, defining the spectrum of outcomes, and estimating the risk of

Table 10.4 Immunoglobulin abnormalities associated with familial Waldenström macroglobulinemia

			Unaffected relatives studied			Number of relatives found to have immunoglobulin abnormalities																	
						Monoclonal component				Polyclonal Ig increase						Decrease Ig						Ig abnl, other or NOS
														Multiple Ig						Multiple Ig		
	Families studied	WM cases	Total	# 1st degree	Age range	Any	IgM	Non-IgM		Any	IgM	IgA	IgG	Incl IgM	Excl IgM	Any	IgM	IgA	IgG	Incl IgM	Excl IgM	
Reference	n	n	n	n		n	n	n	Type	n	n	n	n	n	n	n	n	n	n	n	n	n
Studies of aggregated WM families																						
Seligmann [13, 14][a]	63	65	216	192	5–87	6	6	0		34	10	17	0	5	2	42	18	15	0	7	2	3
Kalff [19][b]	12	12	282	72	>10	11	3	8	1 IgA; 2 IgG; 5 NT	46	37	11	9	5	2	14	2	6	0	3	3	2[c]
Linet [20]	31	31	109	>45[d]	>9[e]	2	2	0		18	2	7	5	0	4	6	0	5	0	0	1	0
McMaster [12][f]	3	10	25	25	16–80	5	5	0		9	5	0	0	0	4	2	0	0	0	0	2	0
Brandefors et al.	12	19	59	45	nr	12[g]	8	4	3 IgG; 1 FLC	4	2	2	0	0	0	6	1	1	4	0	0	—
Studies of individual WM families																						
Spengler [18]	1	1	4	4	nr	1	1	0		0	0	—	—	—	—	0	0	—	—	—	—	—
Williams [26]	1	1	6	nr	nr	0	0	0		—	—	—	—	—	—	—	—	—	—	—	—	—
Fraumeni [23]	1	1	9	2	42–64	0	0	0		3	3	0	0	0	0	0	0	0	0	0	0	0
Zawadzki [27]	1	1	5	5	30–98	2	2	0		—	—	—	—	—	—	—	—	—	—	—	—	—
Youinou [10]	1	4	35	8	1–65	1	1	0		3	3	0	0	0	0	13	0	2	11	0	0	0
Fine [6][h]	1	2	6	6	nr	0	0	0		0	—	—	0	—	—	0	—	—	0	—	—	0
Renier [8]	1	4	12	2	20–63	0	0	0		6	1	1	2	1	1	0	0	0	0	0	0	0
Jaccottet in [8]	1	1	4	2	nr	1	1	0		0	0	0	0	0	0	0	0	0	0	0	0	0
Dupont de Romemont in [8]	1	2	2	nr	nr	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
Taleb [9]	1	2	7	7	8–67	0	0	0		4	4	0	0	0	0	0	0	0	0	0	0	0
Custodi [21]	1	1	4	4	25–70	1	1	0		0	0	0	0	0	0	1	0	1	0	0	0	2[i]
Nardoni in [8]	1	1	4	nr	nr	3	1	2	2 NT	0	0	0	0	0	0	0	0	0	0	0	0	0

(continued)

Table 10.4 (continued)

			Unaffected relatives studied			Number of relatives found to have immunoglobulin abnormalities																
						Monoclonal component				Polyclonal Ig increase						Decrease Ig						
														Multiple Ig						Multiple Ig		
	Families studied	WM cases	Total	# 1st degree	Age range	Any	IgM	Non-IgM		Any	IgM	IgA	IgG	Incl IgM	Excl IgM	Any	IgM	IgA	IgG	Incl IgM	Excl IgM	Ig abnl, other or NOS
Reference	n	n	n	n		n	n	n	Type	n	n	n	n	n	n	n	n	n	n	n	n	n
Fine [16]	1	1	27	7	1–67	2[j]	2	0		13	13	0	0	0	0	0	0	0	0	0	0	0
Björnsson [24]	1	1	49	5	nr	2	2	0		12	9	1	0	0	2	0	0	0	0	0	0	0
Studies of multiple-case IgM MGUS families																						
Williams [26]	1	0	10	5	13–56	2	2	0		0	0	0	0	0	0	0	0	0	0	0	0	0
Kalff [19][b]	2	0	16	11	≤72	2	2	0		4	3	0	0	1	0	1	0	0	1	0	0	0
Petite [46]	1	0	18	4	18–80	3	2	1	1 IgG	2	1	0	1	0	0	2	0	2	0	0	0	1[k]

Abbreviations: *Ig* immunoglobulin(s), *WM* Waldenström macroglobulinemia, *incl* including, *excl* excluding, *abnl* abnormality, *NOS* not otherwise specified, *NT* not typed, *nr* not reported, — assay not performed, *MGUS* monoclonal gammopathy of undetermined significance; FLC, free light chains

[a]Includes two multiple-case WM families reported by Massari [3] and Coste, respectively, and one WM + MGUS family reported by Vannotti. Although 216 relatives were studied, immunoglobulin levels were reported only for the 192 first-degree relatives

[b]The study included 353 relatives of 23 patients with IgM monoclonal gammopathy. The patients were categorized as having definite WM ($n = 11$), possible WM ($n = 6$), IgM monoclonal gammopathy associated with other cancers ($n = 4$) or autoimmune disease ($n = 1$) and "benign essential monoclonal gammopathy" or MGUS ($n = 1$). Results were reported for 282 relatives from 12 families having WM, including 6 families with definite WM and 6 families with possible WM. Results are also presented for the families with MGUS and autoimmune-related IgM monoclonal gammopathy

[c]One case of elevated IgM together with decreased IgA and one case of elevated IgG and decreased IgA

[d]Relationships were reported only for families with identified abnormalities

[e]Year of birth was reported only for families with identified abnormalities. For this table, age was estimated using date of birth and last date of study entry (December 31, 1983)

[f]Includes the family reported by Blattner et al. [4]

[g]IGM MGUS was included in the definition of familial WM in this study; therefore two families had only 1 case of WM and one or more cases of IgM MGUS, which may bias the results

[h]Subjects were tested for IgG levels only

[i]Two members of this family had high IgE levels; no other study reported IgE levels in participants

[j]The two relatives with IgM MGUS progressed to WM 7 and 15 years later [5]

[k]One case of elevated IgG and IgA together with decreased IgM

progression. In addition, the consequences of qualitative or small-volume IgM monoclonal gammopathy (defined as the presence of a monoclonal band on immunofixation that is not accompanied by a measurable M-spike on protein electrophoresis) have yet to be defined [48].

10.3.4 Other Immunoglobulin Abnormalities in WM Families

Relatives of WM patients may have other immunoglobulin abnormalities in addition to IgM monoclonal gammopathy (Table 10.4). In the largest published study, Seligmann et al. [14] screened 192 apparently healthy relatives of 65 WM patients. They found 27 instances (14 %) of polyclonal increases of IgM ($n = 10$), IgA ($n = 17$), or both. Similarly, there were 33 occurrences (17 %) of hypogammaglobulinemia affecting IgM or IgA. Abnormalities of IgG were not reported in this series. The polyclonal IgM gammopathy findings were corroborated by Kalff et al. [19], who studied 165 relatives of 11 WM patients and documented polyclonal IgM elevations in 22 (13 %). Several studies of multiple-case WM or WM/LPD families have also confirmed abnormalities affecting IgM [8–10, 12, 23, 24, 30, 36], IgA [8, 10, 12, 36], and IgG [10, 12, 14, 36].

These observations are intriguing as they raise the possibility that the observed predisposition to WM may actually reflect a more generalized predisposition to immunodysregulation. Because the overall prevalence of subclinical immunoglobulin abnormalities in the general population is unknown, it is difficult to conclude that they are more frequent in the familial context. In one study of three multiple-case WM families, first-degree relatives were compared with non-bloodline family controls (i.e., spouses) [12]. In that study, polyclonal immunoglobulin elevations were more frequent in first-degree relatives of WM cases compared to controls, although the difference was not significant. There was no difference in the prevalence of immunoglobulin deficiencies in these families. Higher prevalence of polyclonal immunoglobulin elevations might reflect an underlying condition of polyclonal B cell activation, which would accord with the findings reported by Steingrimsdóttir [25]. All of these studies have been limited, however. None has controlled for confounding variables or has followed the relatives longitudinally to confirm that the immunoglobulin abnormalities are persistent over time or to document outcomes. It is known, for example, that immunoglobulin concentrations are influenced by age, race, and gender [49, 50]. Moreover, none has included medical evaluation of the relatives to rule out a concomitant diagnosis of a known immunodeficiency syndrome. Well-designed case-control studies are needed to determine whether relatives of WM cases are at increased risk of immunoglobulin abnormalities, and a clinical evaluation component would provide insight regarding potential underlying immunodeficiency diagnoses.

10.3.5 The Hyper-responder Phenotype

Blood levels of immunoglobulins and autoantibodies reflect B-cell function and activity in vivo. The first report of in vitro studies on lymphocytes from members of WM families was published by Fraumeni et al. in 1975 [23] and showed impaired responses to stimulation with phytohemagglutinin, a T-cell mitogen. This was not followed up further. Extensive in vitro testing of peripheral blood lymphocytes from unaffected relatives in Icelandic families led to the identification of the hyper-responder phenotype. This is defined as increased production of immunoglobulins, IgG and IgM, in poke-weed-mitogen-stimulated cultures. Seventeen hyper-responders have been identified in four families, 12 of them in one large family where 40 persons were tested [25, 30]. Lymphocytes from hyper-responders show enhanced survival in stimulated cultures and this is associated with increased levels of the anti-apoptotic protein Bcl-2 [51].

10.3.6 Presentation and Outcomes of Familial WM

As noted earlier, the indolent nature of WM has resulted in limited data pertaining to disease-specific characteristics of familial WM as well as outcomes. Historical survey of 40 years of accumulated individual family reports suggested that most features of the WM disease process in familial WM did not differ substantially from that expected based on review of the literature [31]. The exceptions were a marked difference in both age and gender distribution between WM patients from multiple-case families and historical registry-based controls. Familial WM patients were diagnosed nearly a decade younger than sporadic cases and were much more likely to be male. However, other disease characteristics, including presenting symptoms and signs, were consistent with expectations for sporadic WM.

Following up on these case report observations, Treon and colleagues [38] reported a hospital-based series of 48 patients who had a family history of either WM ($n = 13$) or a related B cell LPD ($n = 35$) and a comparison group of 205 - non-familial patients. Familial patients were more likely than the non-familial group to present with a high IgM level (above 3000 mg/dL). Otherwise, there was no difference in age at diagnosis, gender, presenting complaint, extent of bone marrow involvement at diagnosis, lymphadenopathy, splenomegaly, concomitant IgA and IgG hypogammaglobulinemia, anemia, thrombocytopenia, or beta-2-microglobulin. Similarly, Royer [42] examined several disease-related variables in a large family-based case-control analysis of 131 WM patients (57 cases with a family history of WM, 46 cases with a family history of other B cell LPD, and 28 nonfamilial cases) and 272 unaffected relatives. That study confirmed no significant differences in disease features at presentation based on family history, including age at diagnosis, gender, prior history of MGUS, onset or spectrum of symptoms, anemia, thrombocytopenia, lymphadenopathy, or splenomegaly. In addition, time from diagnosis to first treatment did not differ between groups, and there were insufficient events to permit meaningful survival analysis.

Treon et al. [52] also studied 135 patients (36 cases with a family history of WM or B cell LPD and 99 nonfamilial cases) who had been treated with rituximab-containing regimen. Prior treatment history was not reported, and there was no difference between groups with respect to age at diagnosis, serum IgM level, hemoglobin, platelet count, prognostic score, or beta-2-microglobulin level. Familial patients had shorter time to progression and time to next therapy than nonfamilial patients, with too few events to assess survival. There was a suggestion that among familial patients, those treated with bortezomib may have had a better outcome than those receiving a regimen without a proteasome inhibitor. Although the subset analysis was limited by small numbers and relatively shorter follow-up, this observation may have important clinical implications and it deserves additional study.

Steingrímsson [53] recently performed a population-based study to address the impact of familial disease on survival. The investigators identified 2185 WM/LPL patients diagnosed between 1958 and 2007 and their 6460 first-degree relatives in Sweden. Overall survival in WM/LPL patients having a first-degree relative with any lymphoproliferative disease (WM/LPL, chronic lymphocytic leukemia, other non-Hodgkin lymphoma, Hodgkin lymphoma, multiple myeloma, or MGUS) was significantly inferior to patients without a family history (hazard ratio [HR] = 1.34; 95%CI 1.03–1.75). This finding could not be explained by differences in age or calendar period of diagnosis between groups; however, detailed clinical data (e.g., bone marrow results or treatment) were not available. Based on these findings, family history information should be routinely collected from all patients on clinical trials so that confirmatory analyses can be performed.

10.4 Genetic Studies in Familial Waldenström Macroglobulinemia

Recognition of WM familial clustering at the population level provided the foundation for studies directed at unraveling the genetic basis for WM. These efforts have proved challenging. Early reports suggested that the most common pedigree configuration consisted of two affected siblings, a pattern most consistent with autosomal recessive inheritance. As the National Cancer Institute WM family registry accumulated increasing numbers of families, it became apparent that multiple pedigree configurations exist (Fig. 10.1). A potential explanation for single-generation clusters is that affected individuals in earlier generations may have been undiagnosed or misclassified. In any case, the early evidence indicated that any susceptibility gene(s) will have low penetrance and possibly pleiotropic effects.

10.4.1 Cytogenetics in Familial WM

Unlike some hematologic malignancies, WM lacks a defining cytogenetic abnormality. The most common finding is the del6q21, which can be identified in the

tumor cells of about half of WM cases by sensitive techniques such as fluorescence in situ hybridization (FISH) [54]. Subsequent studies have not discovered any evidence suggesting that this abnormality occurs in the germline as a predisposing genetic event. Early cytogenetic studies in family clusters were incomplete and inconclusive [9, 10, 43]. In a prospective study using conventional Giemsa banding, spectral karyotyping, and comparative genomic hybridization assays of bone marrow and peripheral blood from 21 familial WM patients (7 treated; 11 untreated) and 3 IgM-MGUS patients, McMaster et al. [44] found a small number of recurrent deletion events affecting chromosome 7 in both treated and untreated patients. However, only one abnormality was found in both tumor and peripheral blood mononuclear cells, and no abnormalities were seen in bone marrow cells from IgM-MGUS patients. In addition, no abnormality was shared between WM patients from the same family (11 patients from five families). Another prospective study of bone marrow cytogenetics in 22 familial WM patients found del6q21 by FISH in tumor cells in a high proportion of both familial and non familial patients but did not assess the germline [38]. Thus, to date, cytogenetic studies have not identified any regions that may contain WM predisposition genes.

10.4.2 Linkage Analysis in High-Risk WM Families

Genome-wide linkage analysis is an established tool for identifying chromosomal regions that may harbor susceptibility genes that co-segregate with disease in informative families. A genome-wide linkage screen was conducted in 11 well-characterized, informative high-risk WM families using densely spaced microsatellite markers to localize susceptibility genes [55]. The investigators conducted parallel analyses that either included or excluded IgM-MGUS as a phenotype indicating affected status. This study had two key findings. First, evidence for linkage to four chromosomal regions argued strongly for potential genetic heterogeneity. Second, IgM-MGUS was clearly shown for the first time to be part of the familial WM spectrum, since including patients with IgM-MGUS strengthened the evidence for linkage.

10.4.3 Candidate Gene Association Studies

Another strategy to identify susceptibility genes is to use a candidate gene approach. Liang [56] genotyped single nucleotide polymorphisms (SNPs) in a panel of 152 candidate genes based on published and unpublished data from case-control genome-wide association studies (GWAS) of NHL. Candidates included genes implicated in apoptosis/cell cycle regulation, DNA repair, immune response, T-helper cell type 1/2 subsets, tumor necrosis factor/nuclear factor kappa B pathways, and oxidative stress. Twenty SNPs in five genes (*BCL6, IL10, IL6, IL8RA*, and *TNFSF10*) were significantly associated with WM following correction for multiple comparisons. Of these, two genes were also significantly associated

with CLL (*IL10* and *TNFSF10*), strengthening the hypothesis that CLL and WM are closely biologically related. Moreover, the results provide additional evidence supporting the notion that WM is characterized by genetic heterogeneity.

10.4.4 Other Genetic Markers of WM Risk

Early studies of individual families had limited genetic analysis tools. Human Leukocyte Antigen (HLA) typing was undertaken in a small number of families. HLA A9 was expressed in two families [4, 10], but no haplotype was shared among families [31]. The *A9-B8-DRw3* haplotype co-segregated with both WM and autoimmune thyroid disease in one family [4]. This observation is interesting in retrospect, as this haplotype has since been more extensively characterized and identified as the HLA 8.1 ancestral haplotype (AH), which is carried by 10 % of Northern Europeans and also includes the *TNF* locus [57]. The AH 8.1 is associated with many autoimmune diseases, including some that are also risk factors for WM, for example, Sjögren syndrome and systemic lupus erythematosus [37]. Moreover, the AH 8.1 locus has been shown to interact with a variety of non-Hodgkin lymphoma (NHL) risk factors, including family history, to modulate NHL risk [58]. In addition, the *TNF* G308A variant allele has been associated with diffuse large B cell lymphoma and marginal zone lymphoma [59]. Systematic evaluation of more WM families will determine whether the co-occurrence of the AH 8.1 with WM and autoimmune disease is a true and recurrent association.

An intriguing recent line of research has focused on the targets of paraproteins, termed "paratargs" by the investigators, as a risk factor for development of IgM-MGUS and WM. In an international study, the paraproteins from 11.2 % of patients with either IgM-MGUS or WM were shown to react specifically with hyperphosphorylated paratarg-y (pP-7) [60]. Chronic antigenic stimulation by lifelong exposure to a hyperphosphorylated target protein is the proposed mechanism. Constitutive hyperphosphorylation of these proteins appears to be inherited as an autosomal dominant trait in some families, raising the possibility that an inherited aberrant autoantigen might account for familial clustering of WM. WM patients whose paraprotein targets pP-7 have been shown to have apparently healthy relatives who carry pP-7 in an autosomal dominant fashion. Co-segregation of WM and pP-7 carrier state has not yet been demonstrated as a recurrent event in multiple-case WM families; such a finding would augment the evidence supporting a true association. Further research in this area is ongoing.

10.4.5 Genetic Polymorphisms: *HAS1*

Research on the role of stroma and extracellular matrix in MM led to the discovery of the gene coding for hyaluronsynthase 1, *HAS1*, as a potentially important gene in B-cell-derived malignancies, showing associations with both somatic and germ-line mutations [61, 62]. Interestingly, the effects are not related to the role of *HAS1* in

synthesis of extracellular matrix. The identified genetic variants in introns lead to alternative splicing yielding proteins with intracellular effects. Transfection with these *HAS1* variants induces a malignant phenotype, and the variants show different intracellular distribution and interaction with the cytoskeleton [63]. The potential role of *HAS1* splice variants in the pathogenesis of WM is discussed in detail in Chap. 3 by Pilarski et al. In a recent study, it was shown that single nucleotide polymorphisms in *HAS1* intron3 that are associated with B-cell-derived malignancies have significantly increased frequency in affected members of an Icelandic family [64]. Remarkably, affected members were identified not only as those with clonal B-cell expansion (MGUS, WM, or MM) but also included those with the hyper-responder phenotype.

10.4.6 Next-Generation Technologies: Ongoing Studies and Future Directions

Next-generation sequencing technology has rapidly expanded, and hopes are high that it will help to elucidate the genetic determinants of WM susceptibility. Whole genome sequencing of tumor tissue led to the discovery that *MYD88* mutation is a key somatic event in WM tumorigenesis [65]. Targeted sequencing has shown that the *MYD88* L265P mutation is not present in the germline of familial WM patients, effectively eliminating it as a WM susceptibility gene [66]. Whole exome and whole genome sequencing are also expected to be effective as part of a strategy to discover whether rare, high-penetrance germline mutations contribute to WM susceptibility. Such mutations have been convincingly demonstrated in Mendelian disorders. However, whether disease-related mutations can be identified and validated in complex diseases such as WM, even within large, multi-generational families, remains to be seen. Preliminary data suggest that there are significant challenges in the conduct and interpretation of large sequencing studies in cancer-prone families generally [67] and that genetic heterogeneity may be extensive in WM/LPL [68]. A recent study identifying potentially deleterious rare variants in two genes that co-segregated in the affected members of a single family and appeared to be enriched in familial versus non-familial WM cases demonstrates the power and the challenges of this approach [69]. It is likely that genomic sequencing will be best utilized by incorporating it into multi-disciplinary studies using multiple technological approaches such as epigenetic and expression studies and RNA sequencing in addition to the strategies already discussed here.

Unlike other B-cell lymphoproliferative disorders, there has not yet been a genome-wide association study (GWAS) performed in WM to assess the role of common genetic polymorphisms in WM susceptibility. This is understandable given that WM is rare and therefore it is very difficult to assemble an adequately powered sample of WM cases for study. Nonetheless, over time it is likely that such a sample will be accumulated, and it will be interesting to discover whether GWAS findings are similar to those in other B-cell malignancies. Results from GWAS may

also address known biologically-plausible candidate genes, potential differences between familial and sporadic WM cases, and other relevant subgroup issues.

10.5 Conclusion

The precise contribution of genetic predisposition to the development of Waldenström macroglobulinemia remains undefined. The bulk of the available evidence, including familial clustering, population data, associated findings in relatives of patients with WM, and some genetic data, supports the hypothesis that genetic factors are important determinants in the development of WM. Whether such factors are directly responsible for WM or are operating through intermediary conditions such as immunodeficiency is less clear. Both clinical and genetic studies suggest that there may be extensive genetic heterogeneity in this disease. If true, this may have profound implications for the power of gene-discovery efforts directed at detecting highly-penetrant rare gene variants. Alternatively, WM susceptibility may be mediated by common variants in multiple genes, variants in regulatory regions, or through gene-environment interactions.

Family studies provide the opportunity for long-term observation of the natural history of disease progression and may thus contribute to identifying additional risk factors, refining the clinical phenotype, and guiding gene-discovery and other efforts aimed at unraveling the molecular determinants of WM. The example of family studies of breast cancer leading to the discovery of the BRCA1/2 genes has demonstrated the power of this approach with clinical consequences for these families. Even more importantly, it has become apparent that these genes are also involved in the pathogenesis of sporadic cases of breast cancer, thus having wider implications for therapeutic development. In WM the acquired L265P activating mutation in the *MYD88* gene, found in a very high proportion of patients regardless of family history, is clearly a major determining event providing selective advantage. However, as yet no data support the L265P mutation as a likely germline mutation candidate in families with a predisposition for developing IgM-MGUS and WM as adults. The *MYD88* gene and its pathway remain of interest in this context, however, as it is widely expressed in a variety of tissues, mediating the initiation of inflammatory responses and innate immunity. Moreover, deficiency of *MYD88* is involved in inherited primary immunodeficiency disorders. Meanwhile, mutations in other rare, high-penetrance genes and more common, low-penetrance genes may be identified and will require careful study to delineate their role in WM susceptibility.

Finally, family studies were pivotal in the recognition of IgM-MGUS as a precursor condition for WM and may ultimately provide insights into determinants of disease progression. Further, in the research context, screening of high-risk family members for IgM-MGUS may identify a cohort that may benefit from future prevention strategies. Additional work in WM families may therefore hold the key to answering two remaining important questions: (1) identifying the link between age-related changes in the immune system and monoclonal gammopathies, and

(2) understanding what determines risk of malignant progression, there by paving the way towards prevention studies.

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Immunoglobulin Type M Monoclonal Gammopathy of Undetermined Significance (IgM-MGUS)

11

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11.1 Introduction

Monoclonal gammopathy of undetermined significance (MGUS) is one of the most common premalignant disorders in Western countries, affecting about 4 % of whites aged 50 years or older. It is characterized by the presence of a monoclonal immunoglobulin (also known as M-protein, M-spike, M-component or paraprotein) on serum electrophoresis without evidence of underlying lymphoproliferative malignancy. MGUS is known to affect all major classes of immunoglobulin (Ig), including light chains. Initially, studies analyzed all types of MGUS together, and investigators reported aggregate results or, at most, reported the distribution of Ig subtypes. Over the past 15 years, however, there has been emerging appreciation that MGUS is more heterogeneous than previously recognized. In particular, there has been growing interest in MGUS of the immunoglobulin type M (IgM) class that

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_11

has paralleled the enlarging body of research related to Waldenström macroglobulinemia, the typical malignancy associated with IgM-MGUS. In this section, we will focus on the prevalence, clinical aspects, and natural history of IgM-MGUS specifically and within the broader context of MGUS overall.

11.2 Definitions, Technical Considerations, and Caveats

Patients presenting with an IgM monoclonal gammopathy are classified according to the 2003 Consensus Panel Guidelines as having either IgM monoclonal gammopathy of undetermined significance (IgM-MGUS), an IgM-related disorder, or Waldenström macroglobulinemia (WM), which may be either asymptomatic or symptomatic [1]. Other indolent lymphoid malignancies, such as chronic lymphocytic leukemia and marginal zone lymphoma, may also be associated with an IgM M-protein. Patients who have symptoms attributable to the IgM monoclonal protein but no evidence of lymphoma are classified as having an IgM-related disorder. Examples of IgM-related manifestations include amyloid light chain (AL) amyloidosis, cold agglutinin disease, cryoglobulinemia, or peripheral neuropathy. Such patients constitute a clinically distinct group, and although they have no evidence of malignancy, they may be candidates for treatment to control their IgM-related symptoms. In contrast, IgM-MGUS is defined as a serum IgM monoclonal protein of any concentration in the absence of either unequivocal morphological evidence of bone marrow lymphoplasmacytic infiltration or constitutional symptoms, such as anemia, lymphadenopathy, hyperviscosity, or hepatosplenomegaly, related to the lymphoproliferative process or symptoms directly related to the IgM protein. The Consensus Panel noted the concentration of the IgM M-protein rarely exceeds 3 g/dL in MGUS. Further, the Consensus Panel recommended that patients with equivocal evidence of bone marrow infiltration, such as detection of clonal B-cells by flow cytometry or polymerase chain reaction in the absence of morphologically identifiable bone marrow infiltration, or equivocal bone marrow infiltrates without confirmatory phenotypic studies, should be classified as having IgM-MGUS. In contrast, the International Myeloma Working Group explicitly proposes an M-protein <3 g/dL and lymphoplasmacytic infiltration $<10\,\%$ as the threshold for a diagnosis of IgM-MGUS [2].

Methods to identify and type monoclonal proteins have evolved over time. Early diagnostic assays relied on low resolution techniques applied initially to paper and later to cellulose acetate and agarose substrates. These assays were supplanted by high resolution methods that improved detection of M-proteins of small size or that migrate outside the gamma region. Currently, serum protein electrophoresis (SPE), used to identify a monoclonal protein, followed by immunoelectrophoresis (IEP) or immunofixation electrophoresis (IFE) to determine isotype, is the mainstay of

diagnosis and remains the method of choice in many clinical centers. IFE avoids some of the limitations of IEP for identification of IgM [3]. Also, IFE is more sensitive than IEP for the detection of small monoclonal bands [4], but because the clinical and biological significance of these small bands has been unclear, many centers do not routinely employ IFE. However, the M-protein associated with MGUS is usually small and frequently below the densitometric threshold for measurement [5, 6]. Such small bands may be transient [7, 8]; however, progression to B-cell malignancy has been documented in patients whose M-proteins are <0.5 g/dL [9]. More recently, the serum free light-chain (FLC) assay has been developed as a very sensitive technique, though its utility in this setting is primarily related to the identification of light-chain MGUS [10–12].

When evaluating the MGUS literature, there are several points that must be kept in mind.

1. The specific definition of MGUS employed. Although there is substantial overlap in the diagnostic criteria proposed by the WM and myeloma research communities, there are subtle differences in phenotype definition, as outlined above. Thus, depending on the background and practical orientation of the investigators, studies have variously included all monoclonal gammopathies irrespective of disease status or all asymptomatic patients irrespective of disease status, considered all IgM-related disorders together or separately, or adhered strictly to one of the two current definitions.
2. Inclusion or exclusion of the subgroup of IgM-MGUS that is associated with such conditions as non-hematologic malignancy or systemic autoimmune disease, in which the occurrence of IgM-MGUS may share some mechanistic determinant(s) with the associated condition, but the IgM molecule does not appear to be integral to the phenotype. Clinically, such patients may be seen by different types of providers (e.g., rheumatologists rather than hematologists) and consequently, studies may or may not include them, depending on the setting.
3. Choice of M-protein detection assay. Studies using IFE or similar highly sensitive technologies may report higher prevalence rates, include a larger proportion of patients with small M-proteins, or introduce relative lead-time bias in outcome studies, compared to studies that use other methodologies.
4. As detailed below, MGUS has variable age- and gender dependence in different racial groups; consequently, study results may be influenced by the age-, gender-, and racial distribution of the study population and must be adjusted appropriately.
5. Until recently, most studies have not distinguished between MGUS of differing Ig subtype, in part due to statistical power issues. As will be seen, the ability of recent larger population-based investigations to stratify analyses based on Ig class is yielding some interesting observations regarding MGUS heterogeneity.

11.3 Epidemiology

11.3.1 Prevalence of IgM-MGUS

IgM-MGUS represents a fraction of all MGUS. True incidence rates are unknown, and differences in study design make generalizable estimates difficult. Prevalence data for IgM-MGUS are derived from studies of monoclonal gammopathy or MGUS overall, in which IgM M-protein frequency is usually expressed as a relative percent distribution of immunoglobulin isotypes across all MGUS in the study population. Numerous such studies have been reported and are reviewed by McMaster [13]. The prevalence of MGUS overall varies according to the age, gender, racial, and geographic distribution of the population under study. Among populations studied for MGUS overall, the proportion attributable to IgM varies widely, although IgM-MGUS is often, but not always, intermediate in frequency among the three major subtypes (IgG, IgA, and IgM).

Most studies of MGUS have been in white populations. The largest population-based study to date was performed in the USA in Olmsted County, MN, whose residents are overwhelmingly white [6]. In this well-characterized population, serum samples were obtained from 21,463 of the 28,038 (76.6 %) known residents who were at least 50 years of age. The overall prevalence of MGUS in this cohort was found to be 4.0 % in men and 2.7 % in women, and it increased in an age-dependent manner. Another recent large population-based study in the USA was conducted as a nested investigation within the National Health and Nutritional Examination Study (NHANES), a large cross-sectional cohort designed to select a nationally representative sample of the civilian US population [14], using the same screening methods as the Minnesota study. In a sample of 12,482 adults age $\geq$50 years for whom serum was available, MGUS prevalence in the white subgroup was 2.3 %, somewhat lower than seen in Olmsted County. Further stratification suggested geographic differences, with prevalence reaching 3.1 % in the North and Midwest compared to 2.1 % in the South and West. IgM accounted for 17.2 % of all MGUS in the homogeneous white upper Midwest population of Olmsted County and 15.4 % in whites in the NHANES study, corresponding to an estimated IgM-MGUS prevalence of 0.6 % and 0.4 %, respectively.

Studies in African American, native African, and Asian populations have suggested racial variations in MGUS prevalence. For example, both MGUS and multiple myeloma have been shown to be twofold more common in African Americans compared to whites in the USA [15, 16], and MGUS is more common in Ghanaian blacks compared with US whites [17]. This doubling of MGUS risk among African Americans relative to whites was unchanged when obesity, education status, and income status were considered in the same multivariate model applied in another large population-based study [18]. However, although MGUS overall is more common in black populations, the proportion due to IgM-MGUS is much lower than in whites. IgM accounted for only 5.6 % of MGUS in Ghanaians compared to 14 % of MGUS in a historical white comparison group in the Ghanaian study and 2.7 % in African Americans versus 15.4 % in whites in NHANES.

However, the absolute excess of IgM-MGUS among whites compared with blacks is somewhat attenuated because the frequency of MGUS overall is higher among blacks. Thus, in the NHANES study, which provides a direct comparison between racial groups, whites had an apparent 5.7-fold increase in IgM distribution, but a lower prevalence of MGUS overall, compared to blacks. This translates to an absolute excess of 3.6-fold increased prevalence of IgM-MGUS in whites relative to blacks. This finding is consistent with the racial disparity in incidence of WM that has been observed in US Surveillance, Epidemiology and End Results (SEER) data in the USA [19].

IgM-MGUS also appears to be uncommon in Asian populations, but the prevalence varies widely. In a large study, Iwanaga [20] screened 52,781 adults who had survived the atomic bomb detonation in Nagasaki, Japan. Within an overall MGUS prevalence of 2.1 %, IgM-MGUS comprised 7.5 %, yielding an estimated prevalence of 0.16 % for IgM-MGUS in this population. Men ($n = 50$, 8.7 %) were affected 1.4-fold more commonly than women ($n = 32$, 6.2 %). Studies in other Asian ethnic groups have been smaller and variable in design and outcome. A study within the ethnic Chinese population in Hong Kong surveyed 1000 male and female blood donors age 50–65 years and identified no IgM isotype among the eight patients found to have MGUS [21]. Similarly, IgM was not detected in a cross-sectional study of 3260 residents age $\geq$50 from urban, suburban, and rural communities in Thailand in which the overall prevalence of MGUS was 2.3 % [22]. In contrast, when Korean investigators screened 945 participants drawn mainly from a population-based, prospective cohort study of the elderly outside Seoul, Korea, they found an overall MGUS prevalence of 3.3 %, with IgM accounting for 19 % (estimated IgM-MGUS prevalence 0.6 %, similar to estimates in US whites) [23]. Notably, the Korean population was older ($\geq$65 years), which may contribute to the higher prevalence seen in that group overall. It is unknown whether observed variability in Asian populations relates to sample size or demographics. Larger studies are needed to confirm these patterns.

Finally, there is emerging evidence that both MGUS overall and IgM-MGUS may be less common in Hispanics than in whites. The first reports suggesting that the prevalence of MGUS and WM is very low in Mexicans were referral hospital-based surveys in Mexico. Based on a prospective screening study of 14,246 Mexican patients with hematological diseases, investigators identified 17 patients with MGUS and 20 patients with WM [24]. This observation was confirmed in the NHANES study, in which the subgroup of 2475 Mexican Americans was found to have an overall prevalence of 1.75 % for MGUS and 0.1 % for IgM-MGUS. Taken together, these data support a role for genetic factors and possibly as yet undetermined environmental exposures in the development of IgM-MGUS.

In addition to racial differences, there is also evidence supporting regional variation in IgM-MGUS prevalence. Among western European population-based studies, the highest proportion of the IgM isotype (25 %) has been reported in western France [25, 26] and the lowest (6–8 %) in the Mediterranean basin [27] and Sweden [28]. Some of these differences may relate to study design, and all of the caveats outlined earlier apply. Large studies across populations that employ

uniform methods are needed to confirm these observations. For example, an early study of 13,400 blood donors in France demonstrated remarkable variability by region [29]. While MGUS was unusually uncommon in this population, IgM frequency ranged from 0 % in a group of mostly urban dwellers in Paris to 33 % in residents of an agricultural district in western France. More recently, the previously mentioned NHANES study found evidence of regional variation in MGUS prevalence rates within the USA, although regional data stratified by isotype were not reported.

11.3.2 Risk Factors for IgM-MGUS

Risk factors for IgM-MGUS have been investigated primarily as subset analyses in investigations of MGUS overall. The prevalence studies discussed earlier established that risk for MGUS overall increases with age and male sex. A population-based screening study conducted in nearly 2000 white and African American women [18] addressed the racial disparity suggested by prior prevalence studies. The investigators confirmed a lower frequency of IgM-MGUS (8 % of total) in black women compared to white females (19 % of total), but small numbers limited the analysis. To date, there have been no other direct comparisons of IgM-MGUS in different racial populations.

A large population-based study in Sweden examined the relationship of personal and family history of autoimmune disease and MGUS [30]. The study included 5403 MGUS patients and 21,209 matched controls, together with 14,535 first-degree relatives of MGUS case patients and 58,164 first-degree relatives of MGUS controls. None of the analyses showed differences in risk based on immunoglobulin isotype, with IgM-MGUS constituting 10 % of the sample. Increased risk for all MGUS was associated with a personal history of autoimmune disease overall (odds ratio [OR] = 2.1; 95 % confidence interval [CI], 1.9–2.4), including autoimmune diseases characterized by either systemic involvement (OR = 2.4; 95%CI, 2.0–2.9) or organ-specific involvement (OR = 1.7; 95%CI, 1.3–2.2). Certain specific autoimmune disorders, including rheumatoid arthritis, systemic sclerosis, Sjögren syndrome, immune thrombocytopenia, Guillain–Barré syndrome, celiac disease, chronic rheumatic heart disease, ankylosing spondylitis, polymyalgia rheumatica, giant cell arteritis, and aplastic anemia, were found to confer disease-specific increased risk for subsequent development of MGUS. In addition, a family history of autoimmune disease was associated with a significantly increased risk of MGUS, though the magnitude was small (OR = 1.1; 95%CI, 1.0–1.2). The same study also investigated personal history of infections and inflammatory disorders. Significantly increased risk of MGUS was seen in patients with a history of infections overall (OR = 1.6; 95%CI, 1.5–1.7), inflammatory conditions overall (OR = 1.4; 95%CI, 1.2–1.5), and any of a variety of specific infections and certain inflammatory conditions. Other specific exposures have not been investigated with respect to IgM-MGUS. These findings suggest that chronic antigen stimulation may trigger the development of a MGUS or that treatment of

autoimmune disease might increase the risk. Furthermore, a common genetic or environmental susceptibility might play a role. There are numerous reports of familial clusters of MGUS, myeloma, and other hematologic malignancies, which are referenced in Chap. 10. These reports raised the question of whether familial clustering of these disorders occurs more frequently than expected by chance. The largest study addressing this question was conducted in a Swedish population and examined 14,621 relatives of 4458 patients with MGUS [31]. First-degree relatives of IgM-MGUS patients were found to have a significant fivefold (95%CI, 1.1–23) risk of developing chronic lymphocytic leukemia, as well as a nonsignificant excess risk of multiple myeloma (relative risk [RR] = 1.9; 95%CI, 0.3–10) and lymphoplasmacytic lymphoma/WM (RR = 3.5; 95%CI, 0.2–63), based on small numbers. While the Swedish study found increased risk for family history of MGUS overall (RR = 2.8; 95%CI, 1.4–5.6), it could not confirm increased risk in the smaller IgM subset. Investigators at the Mayo Clinic screened 911 relatives of multiple myeloma ($n = 232$) and MGUS ($n = 97$) patients by a two-step serum protein electrophoresis followed by IFE method [32]. Relatives of MGUS patients had a higher prevalence of MGUS relative to the general Olmsted County reference population (risk ratio = 2.6; 95%CI, 1.9–3.4). The MGUS risk in relatives did not differ by the proband's isotype, which was stratified as IgG vs non-IgG. Overall, these data confirm that family history of MGUS is a significant risk factor for an individual's development of MGUS. Overall, these data extend the evidence suggesting a role for genetic susceptibility or possibly shared environmental factors in the development of these conditions. However, the influence of family history on risk of IgM-MGUS remains to be clarified and would benefit from further study.

A series of studies has shown an interaction between monoclonal protein and hyperphosphorylation of a protein of unknown function, called paratarg-7 (P-7), which is ubiquitously expressed in all human tissues. The M-proteins from 9 of 51 patients with IgM-MGUS (17.6 %) were shown to react specifically with P-7, compared with 4 of 200 healthy controls (2 %) [33]. The investigators concluded that the P-7 marker is associated with a 6.2-fold increased risk of IgM-MGUS or WM ($P = 0.001$). A second molecule in this class, sumoylated HSP90 (HSP90-SUMO1), was recently reported to be a target of IgM paraprotein [34]. The investigators propose that these proteins represent a source of chronic antigenic stimulation that contributes to susceptibility to plasma cell malignancies. These interesting preliminary results await confirmation in larger, well-defined cohorts.

11.4 Genetics

Our understanding of the cellular origins of WM and IgM-MGUS continues to evolve. It is not clear that WM and IgM-MGUS share a common cell of origin, given that patients with IgM-MGUS may evolve into a spectrum of B-cell lymphoproliferative malignancies that includes, but is not limited to, WM (see Outcomes, below). Immunoglobulin heavy chain (*IGH*) gene sequence analysis has been used to provide evidence regarding the B-cell differentiation status at the

initiation of WM oncogenesis. Early studies in small numbers of patients suggested that WM may originate from a post-germinal center cell that has undergone somatic hypermutation but not class switch recombination (CSR) [35, 36]. Analyses of V_H variable region genes further revealed that WM cells were skewed strongly toward V_H3 family segments. Larger studies have confirmed the preferential usage of V_H3 family segments. In a study of 64 WM and 8 IgM-MGUS patients, Martín-Jiménez [37] found a bias toward V_H3-23 usage in >75 % cases, including 6 IgM-MGUS cases. In this study, WM and IgM-MGUS showed nonsignificant differences in frequency of antigenic selection (77 % vs. 40 %, respectively) and DJ_H rearrangements (42 % vs. 13 %, respectively), suggesting possible differences in their neoplastic origin. WM and IgM-MGUS also appear to diverge with respect to the mutational status of the switch mu (Sμ) region, suggesting that WM cells are arrested prior to CSR but that IgM-MGUS B-cells may retain CSR ability [35]. In another study, investigators were able to demonstrate clonal rearrangements in only 3 of 40 IgM-MGUS patients, presumably because of low numbers of monoclonal B-cells [38]. This study confirmed consistent somatic hypermutation and found evidence of intraclonal sequence variation in one case of IgM-MGUS (compared to zero of seven WM cases), suggesting that a portion of IgM-MGUS might be derived from cells under continued antigenic exposure. These data are interesting in that they provide a mechanism that might partially explain IgM-MGUS outcome heterogeneity. A more detailed explanation of clonal dynamics appears in Chap. 3.

To date, there have been no IgM-MGUS-specific genetic investigations, and until recently, investigators did not routinely include IgM-MGUS cases in their genetic studies of WM. McMaster [39] examined bone marrow and peripheral blood from 3 IgM-MGUS and 18 WM patients with a family history of WM using a combination of conventional G-banding, spectral karyotyping, and comparative genomic hybridization, supplemented with fluorescence in situ hybridization (FISH) to screen for translocations. The investigators found no evidence of rearrangements involving the *IGH* locus on chromosome 14q32, which have been shown to occur frequently in non-IgM-MGUS and multiple myeloma [40]. The only clonal abnormality identified in any of the IgM-MGUS patients was deletion of the Y chromosome in one patient. Following identification of 6q deletion as the most common recurring cytogenetic abnormality in WM, Schop and colleagues [41] were unable to detect loss of 6q by FISH in 12 IgM-MGUS patients, compared to detecting it in 21 of 38 WM cases (55.3 %). Both of these investigations were conducted in unsorted bone marrow cells.

In 2012, massively parallel sequencing identified *MYD88* L265P as a characteristic recurring somatic mutation in WM [42]. The initial study, using Sanger sequencing on CD19-selected bone marrow mononuclear cells, reported detection of the *MYD88* L265P mutation in 26 of 30 (87 %) WM cases and 2 of 21 (10 %) IgM-MGUS cases, raising the possibility that the mutation represented an oncogenic event in the transformation of IgM-MGUS to WM. Subsequent studies, using the highly sensitive allele-specific polymerase chain reaction (AS-PCR) assay, have demonstrated the *MYD88* L265P mutation in about half of IgM-MGUS patients [43–45]. Jiménez [46] reported a higher frequency of *MYD88* L265P in

IgM-MGUS (87 %); their selection criteria included ensuring that all cases had at least 1 tumor cell per 100 cells by flow, which may account for the higher rate of detection. In Xu's [45] follow-up study, 13 of 24 (54 %) of IgM-MGUS patients had *MYD88* mutations by AS-PCR but only 3 were positive by Sanger sequencing. These three patients also had higher quantitative levels of the mutation based on AS-PCR ΔC_T values (correlating with the number of tumor cells) and later progressed to WM. Of the 12 IgM-MGUS patients from these studies who have been reported to progress to WM, all but 1 carried the mutation at diagnosis of IgM-MGUS. Varettoni [44] screened 77 IgM-MGUS patients and detected the *MYD88* L265P mutation in 36 patients (47 %). During a median follow-up of 12 months, 9 patients evolved to WM ($n = 7$) or marginal zone lymphoma ($n = 2$). One patient who progressed to WM was *MYD88* wild type at the time of diagnosis of both MGUS and WM. Of the two patients who progressed to MZL, one had the *MYD88* mutation and one was *MYD88* wild type.

Very few studies have addressed germ-line genetics in IgM-MGUS. McMaster [39] found no germ-line cytogenetic abnormalities in IgM-MGUS patients. One genome-wide linkage association study was performed using parametric and non-parametric methods to analyze 1058 microsatellite markers in 11 families of probands with WM [47]. The study included 122 family members, including 10 with confirmed IgM-MGUS and 34 with WM. Among the findings was the observation that inclusion of the IgM-MGUS patients within the affected phenotype strengthened the evidence for linkage on chromosomes 1q and 4q. The 4q result is particularly interesting, given recent evidence that trisomy 4 may be unique to WM among low-grade B-cell lymphoproliferative disorders [48]. However, no relevant genes for either WM or IgM-MGUS have yet been identified on chromosome 4. Thus far, there have been no genome-wide association studies (GWAS) that include IgM-MGUS, likely due to power considerations. Genetic predisposition to WM is addressed more fully in Chap. 10.

11.5 Outcomes

11.5.1 Progression to Hematological or Lymphoid Malignancy

Outcome studies in IgM-MGUS fall into three broad categories: (1) studies analyzing MGUS overall that report findings for a limited number of IgM-MGUS patients; (2) studies of MGUS overall having sufficient numbers of IgM-MGUS patients to permit stratified analysis; and (3) studies of IgM-MGUS specifically. Numerous studies of MGUS overall containing limited numbers of IgM-MGUS patients provided the initial indication that these individuals are at risk for progression to B-cell malignancy [49–54]. Analyses performed for MGUS overall did not identify consistent predictors of prognosis, most likely because of differences in patient selection and limited power to detect differences between groups.

Among MGUS studies providing selected stratified analyses by Ig subtype, Ögmundsdóttir [55] performed an observed-to-expected (O/E) analysis and

determined that patients with IgM-MGUS were more likely to develop hematological malignancies compared to the general Icelandic population. The risk was higher for women (O/E = 11.2; 95%CI, 5.21–20.1) than for men (O/E = 6.88; 95%CI, 2.57–14.3). Gregersen [56] included 231 patients with IgM-MGUS in a study of 1247 MGUS patients defined by detection of an M-protein in the absence of a diagnosis of a plasma cell or lymphoproliferative malignancy within the next 100 days. Among the IgM-MGUS subset, the standardized incidence ratio (SIR) for malignant transformation was 17.2 (95%CI, 10.5–26.5) compared to the general Danish population. All subsequent malignancies were lymphoproliferative, with no cases of progression to myeloma observed. Poisson regression modeling identified female sex, increasing IgM concentration, younger age at diagnosis (<70 years), and the first year of follow-up as factors associated with risk of malignant transformation from MGUS overall. Cesana [57] reported outcomes for 130 IgM-MGUS patients. IgM-MGUS patients were characterized by a median age of 67 years (range, 23–91 years), median M-protein concentration of 0.75 g/dL (range, 0.16–2.95 g/dL), and median bone marrow involvement of 5 % (range, 0–10 %). During a median follow-up of 67 months (range, 12–180 months), 13 (10 %) patients progressed to a lymphoid malignancy, including WM ($n = 12$) and multiple myeloma ($n = 1$; immunoglobulin class not reported). Multivariate analysis identified increasing level of bone marrow infiltration, presence of Bence Jones proteinuria, serum polyclonal Ig reduction (immunoparesis), and erythrocyte sedimentation rate >40 mm/h as independent predictors of progression of any MGUS to lymphoid malignancy. Turesson [58] reported the characteristics of 118 - IgM-MGUS patients within a larger study of MGUS overall conducted in two regional Swedish hospitals. During a median follow-up of 10 years, 18 patients developed a lymphoid malignancy (WM, $n = 14$; chronic lymphocytic leukemia, $n = 3$) or a myeloid malignancy (acute myelocytic leukemia, $n = 1$). No IgM-MGUS patient developed multiple myeloma or Hodgkin lymphoma. The investigators found no difference in risk of progression for non-IgG-MGUS vs. IgG-MGUS. They pointed out that comparison of rates of progression among Ig subtypes of MGUS is difficult because of the different patterns of outcomes (predominantly lymphoid for IgM-MGUS vs. multiple myeloma in IgG/IgA-MGUS). In addition, they noted that IgM and IgA have been shown to have a higher catabolic rate compared with IgG; consequently, at a given serum M-protein concentration, there are more clonal cells in IgM- or IgA-MGUS than in IgG-MGUS [59]. Using Cox proportional hazards models for MGUS overall, they confirmed the previously identified association of serum free light-chain ratio and M-protein concentration with risk of progression [60] and confirmed immunoparesis ($P < 0.001$) as a prognostic indicator. Although these studies did not consistently analyze IgM-MGUS separately, they provided data on potential prognostic factors that informed subsequent studies targeting IgM-MGUS.

Beginning in 2003, some studies were designed to focus specifically on IgM-MGUS (Table 11.1). Kyle [61] reviewed 213 IgM-MGUS patients diagnosed during a 34-year period in southeast Minnesota. Patients were diagnosed based on having a serum IgM monoclonal protein <3 g/dL and no signs or symptoms

Table 11.1 Summary of IgM-MGUS-specific studies conducted to date, with malignant progression as the primary outcome

Author	Kyle [61]	Montoto [62]	Morra [63]	Baldini [64][a]
Study type	Retrospective	Retrospective	Retrospective	Retrospective
	Population-based	Single institution	Multi-institution	Multi-institution
Location	Southeast Minnesota	Spain	Italy	Italy, France
Acrrual period	1960–1994	1970–2001	1975–2001	1978–2003
IgM MGUS cases, *n*	213	52	138	217[b]
IgM MGUS diagnostic criteria				
IgM MC concentration	<3 g/dL	<3 g/dL	Any	<3 g/dL
BM infiltration	–	–	normal	<10 % BMLPC
Signs/symptoms of LPD	None	None	None	None
Patient characteristics				
Age, median (range), years	74 (24–94)	67 (46–84)	66 (30–85)	Mean, 63.7 (29.9–89.3)
Sex, M/F, *n*	123/90	27/25	62 %/38 %	131/86
Ratio	1.4	1.1	1.6	1.5
MC concentration, median (range), g/dL	1.2 (0–2.6)	1.45 (0.7–2.9)	0.9 (0.14–2.9)	Mean, 0.88 (0.2–2.86)
IgM concentration, median (range), mg/dL	675 (40–4800)[c]	–	–	885.8 (210–2990)
No. with bone marrow	27	39	138	217
BM involvement, median (range), %[d]	<10 (–)	2.6 (0.6–6.7)	5 (0–9)	Mean, 6.6 (2–10)
Decreased polyclonal Igs	35 % of 129 tested	↓IgG, 12; ↓IgA, 10[e]	9 % of 133 tested	–
Hemoglobin, median (range), g/dL	14 (5.9–18.9)	13.3 (9–16)	14 (–)	Mean, 14.0 (10.1–17.9)
Median follow-up (range)	6.3 years (0–20.6)	5 years (1–20.4)	80 months (12–19[*sic*])	56.1 months (12–242.1)
Outcome, *n* (%)				
MC resolved	9 (4.2)	0 (0.0)	12 (8.7)	–
Evolution to LPD	30 (14.1)	6 (11.5)	14 (10)	15 (6.9)
WM	6	5	13	13
NHL[f]	17	1	0	2
CLL	3	0	0	0
MM[g]	1	0	1	0
Amyloidosis	3	0	0	0
Time to LPD	1.5 % per year	Median, 3.6 years	Median, 75 months	Median not reached
Range	n.a.	2.3–12.4 years	12–117 months	n.a.

(continued)

Table 11.1 (continued)

Risk of evolution to LPD at 5, 10, 15, 20 years	10 %, 18 %, 24 %, –	–, 13.3 %, –, 27.7 %	8 %, 29 %, –, –[h]	–, 14.5 %, 24.9 %, –
Multivariate predictors of progression[i]	Serum MC concentration	%BMPC (<5 vs. ≥5)	Serum MC size	Serum MC concentration
	Serum albumin	%BML (<20 vs. ≥20)	ALC > 4 × 10 (9)/L	Hemoglobin
				Male sex
Median survival	7 years	–	–	Not reached
Survival at 5, 10, 15 years[j]	69 %, 48 %, 35 %	–	95 %, 83 %, –	–, 92 %, 75 %

MC monoclonal component; *LPD* lymphoproliferative disorder, *M* male, *F* female, *No.* number; *BM* bone marrow, *BMPC* bone marrow plasma cells, *BMLP* bone marrow lymphoplasmacytes, *BMLPC* bone marrow lymphoplasmacytic cells, *Igs* immunoglobulins, *WM* Waldenström macroglobulinemia, *NHL* non-Hodgkin lymphoma, *CLL* chronic lymphocytic lymphoma, *MM* multiple myeloma, *BML* bone marrow lymphocytes, *ALC* absolute lymphocyte count, *n.a.* not applicable, *vs.* versus, – not reported

[a]In this study, measures of central tendencies were reported as means rather than medians

[b]May include some patients ($n = 48$) reported in [63]

[c]15 % were normal at <300 mg/dL

[d]BM involvement reported as BMPC (Kyle, Montoto), BMLP (Morra), or BMLPC (Baldini)

[e]12 cases had decreased IgG and 10 cases had decreased IgA

[f]Reported types of NHL. Kyle reported lymphoplasmacytic ($n = 6$), diffuse large B cell ($n = 5$), mucosa-associated lymphoid tissue (MALT) ($n = 2$), small lymphocytic ($n = 1$), and B-cell unclassified ($n = 1$). Montoto did not report NHL subtype. Baldini reported lymphoplasmacytic ($n = 2$)

[g]Kyle reported one patient who initially had IgM MGUS, later developed biclonal gammopathy (IgM 386 mg/dL + IgAλ 2840 mg/dL), and ultimately progressed to smoldering multiple myeloma with 15 % BMPC 5 years later. Morra reported one case of IgM multiple myeloma

[h]Includes aWM

[i]Includes factors found to be statistically significant at the $P = 0.05$ level

[j]Kyle reported survival indirectly, as related to death from causes other than nonlymphoid malignancy, Morra reported event-free survival, and Baldini reported overall survival

suggestive of a lymphoproliferative disorder; bone marrow examination was performed in 13 % and showed <10 % plasma cell infiltration in all cases. IgM burden was low (median M-protein, 1.2 g/dL; median quantitative IgM level, 675 mg/dL). Quantitative immunoglobulins were determined in 129 patients, and one-third had suppression of uninvolved immunoglobulins. During a median follow-up of 6.3 years, 29 progressed to a lymphoid malignancy ($n = 26$, 12 %) or amyloidosis ($n = 3$, 1.4 %). The highest RR was for patients to develop WM, and the most common malignant outcome was WM or lymphoplasmacytic lymphoma. The overall risk of progression was 1.5 % per year (Fig. 11.1), and patients continued to be at risk more than 20 years after diagnosis. A competitive model in these patients showed that rates of death from non-hematologic causes were substantially higher than rates of progression to lymphoid disorders (Fig. 11.2). Conversely, the monoclonal protein disappeared in nine patients, all but one of

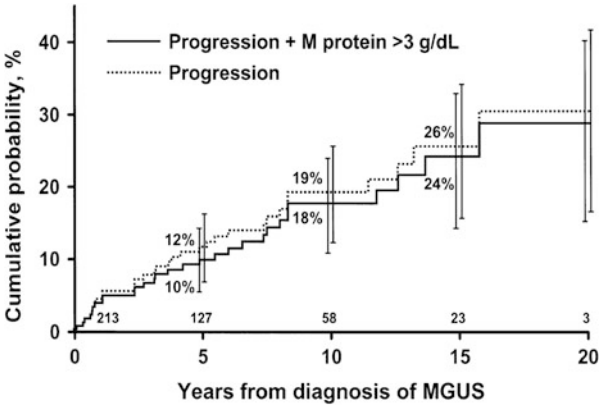

Fig. 11.1 Probability of progression in 213 monoclonal gammopathy of undetermined significance of IgM class (IgM-MGUS) patients. Patients were residents of southeastern Minnesota in whom IgM-MGUS was diagnosed from 1960 through 1994. *Curve* shows probability of progression of MGUS to lymphoma, Waldenström macroglobulinemia, primary amyloidosis, or chronic lymphocytic leukemia. Bars show 95 % confidence intervals. Numbers at bottom of the horizontal axis are numbers of patients at risk at each interval. *This research was originally published in Blood. Kyle RA, Therneau TM, Rajkumar SV, Remstein ED, Offord JR, Larson DR, Plevak MF, Melton LJ 3rd. Long-term follow-up of IgM monoclonal gammopathy of undetermined significance. Blood. 2003;102(10):3759–3764.* © *The American Society of Hematology*

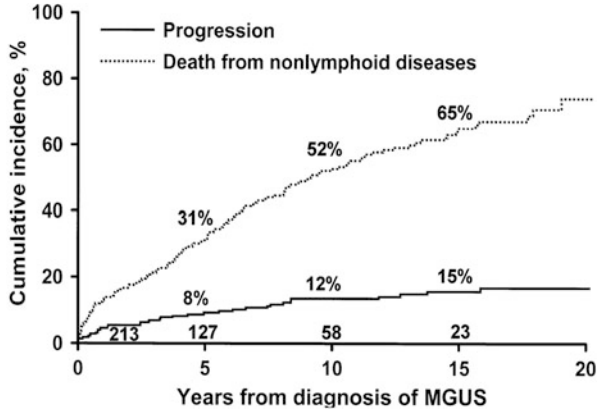

Fig. 11.2 Competitive model in 213 monoclonal gammopathy of undetermined significance of IgM class (IgM-MGUS) patients. Patients were residents of southeastern Minnesota in whom IgM-MGUS was diagnosed from 1960 through 1994. *Upper curve* shows probability of dying of nonlymphoid diseases. *Lower curve* shows probability of progression to lymphoma or a related disorder. *This research was originally published in Blood. Kyle RA, Therneau TM, Rajkumar SV, Remstein ED, Offord JR, Larson DR, Plevak MF, Melton LJ 3rd. Long-term follow-up of IgM monoclonal gammopathy of undetermined significance. Blood. 2003;102(10):3759–3764.* © *The American Society of Hematology*

whom had an initial M-protein concentration $\leq$1.0 g/dL. Multivariate analysis identified the concentrations of the serum monoclonal protein ($P = 0.03$) and serum albumin ($P = 0.01$) as prognostic predictors of progression.

Montoto [62] analyzed 52 patients with IgM-MGUS ascertained between 1970 and 2001 and diagnosed on the basis of an M-protein size <3 g/dL and absence of signs or symptoms suggestive of WM. Bone marrow examination was not required; however, in the subset having bone marrow available for review, median percentage of bone marrow plasma cells and bone marrow lymphocytes was 2.6 % and 16.1 %, respectively. The cases were unusual in having nearly a 1:1 gender distribution. During a median follow-up of 5 years, six patients developed a lymphoid malignancy, most commonly WM. Median time to WM diagnosis was 3.6 years. Multivariate analysis identified percentage of bone marrow plasma cells and percentage of bone marrow lymphocytes to be independently associated with risk of progression, which was 27.7 % at 20 years.

Morra [63, 65] studied 384 Italian patients with asymptomatic IgM monoclonal gammopathy. Of these, 138 had IgM-MGUS defined according to the 2003 WM Consensus Panel recommendations [1]. Events during the first 12 months of follow-up were excluded from analysis. Among the IgM-MGUS cohorts, there were 14 who progressed to either WM ($n = 13$) or IgM multiple myeloma ($n = 1$). For the entire group (IgM-MGUS and asymptomatic WM), cumulative probability of progression at 5 and 10 years was 8 % and 29 %, respectively, similar to the observations of Kyle et al. The M-protein disappeared in 12 patients (9 %), all of whom had a low concentration of paraprotein (median, 410 mg/dL; range 139–573). Univariate analysis in the IgM-MGUS subset identified low hemoglobin ($P = 0.02$) as significantly associated with progression, whereas M-protein size ($P = 0.06$), detectable Bence Jones proteinuria ($P = 0.06$), erythrocyte sedimentation rate ($P = 0.07$), and lymphocytosis ($P = 0.1$) were interpreted as showing a trend toward increased progression risk. A proposed risk stratification model incorporating these variables identified three risk groups among the entire series, and event-free survival in the high-risk group differed significantly from the other two groups combined. The utility of this prognostic index in the IgM-MGUS subgroup remains to be confirmed.

Baldini [64] investigated a multi-institution series of 217 IgM-MGUS patients in Italy and France. In their study, IgM-MGUS was defined as serum M-protein and bone marrow infiltration less than 3 g/dL and 10 %, respectively, and the absence of signs or symptoms of lymphoproliferative malignancy. The endpoint was chemotherapy for symptomatic lymphoproliferative malignancy. At a median of 56.1 months, 15 patients progressed to requiring chemotherapy for either WM ($n = 13$) or lymphoplasmacytic lymphoma ($n = 2$). A prognostic scoring model was devised using serum M-protein concentration, hemoglobin level, and sex—the three variables associated with risk of progression in multivariate analysis. Application of the scoring system stratified patients into three risk groups that differed significantly in time to evolution. The difference was most pronounced for the high-risk group, while the curves for the low- and intermediate-risk groups began to diverge after 96 months of follow-up.

Historically, it has not always been possible to separate IgM-MGUS from asymptomatic WM, and the two conditions are sometimes combined. However, data have emerged in the past few years showing substantially higher risk of progression for asymptomatic (or 'smoldering') WM [66], demonstrating the need for carefully designed eligibility criteria to reduce misclassification.

Using a retrospective approach, Steingrimsdottir [67] examined 10 patients with WM identified through the Icelandic Cancer Registry since 1991 who had frozen serum samples collected 3–14 years (median, 5.3 years) prior to diagnosis. Immunofixation electrophoresis identified a pre-diagnosis IgM M-protein in 3 of 10 WM patients. These results are unexpected based on other natural history data and require confirmation in larger studies. They may be due to chance, sample size, assay interval, or some other cause. It has been shown that non-IgM multiple myeloma is consistently preceded by MGUS [68, 69].

Investigations of familial WM have shown that a proportion of relatives of WM patients will be found to have IgM-MGUS if screened [70]. It is known that all first-degree relatives of WM patients are at increased risk of developing WM [71] and that familial WM may have poorer survival than sporadic [72]. It is not clear whether this increased familial WM risk reflects a higher prevalence of IgM-MGUS in relatives or is independent of it.

Based on these cumulative data, IgM-MGUS confers increased RR for subsequent development of WM, non-Hodgkin lymphoma, or chronic lymphocytic leukemia. The only consistent predictor of risk of progression is the size of the IgM M-protein at diagnosis. However, the absolute risk for an individual patient remains small, and most IgM-MGUS patients will never develop a lymphoproliferative malignancy.

11.5.2 Survival

MGUS patients overall appear to have a reduced life expectancy compared with the general population [50, 54, 73]. Three groups considered the impact of Ig subtype on survival. Gregersen [74] compared 1164 Danish MGUS patients with population data, demonstrating that deaths from malignant progression to multiple myeloma or lymphoproliferative malignancy (standardized mortality ratio [SMR] = 20.0; 95% CI, 16.2–24.4), non-hematologic malignancy (SMR = 2.3; 95%CI, 2.0–2.7), and nonmalignant causes (SMR = 1.8; 95%CI, 1.7–1.9) were more frequent in the MGUS cohort. The investigators went on to show that although malignant transformation was a major cause of death, it explained only one-fifth of the excess mortality among MGUS patients. Schaar [75] later conducted a prospective single-center study in the Netherlands that included 1464 patients with MGUS and confirmed that all MGUS patients had inferior survival compared to matched controls. Kristinsson [76] followed these studies with a population-based investigation of 4259 MGUS patients and 16,151 matched controls and concluded that MGUS patients had decreased life expectancy due to both malignant transformation and nonmalignant causes. These data therefore strongly suggest that MGUS

patients are at increased risk of dying from any cause compared to the general population. Questions remain regarding mortality related to IgM-MGUS, however. The Danish and Dutch studies found no difference in survival by Ig isotype, whereas the Swedish study found IgM-MGUS to be associated with superior survival compared to non-IgM-MGUS.

None of the previously mentioned specific studies of IgM-MGUS had survival as their primary endpoint (Table 11.1), and they varied in outcome definition. Morra [65] reported significantly increased event-free survival for IgM-MGUS compared to asymptomatic WM but concluded that there was no difference in overall survival between groups. Conversely, Baldini [64] reported a significantly better overall survival for IgM-MGUS patients compared to asymptomatic WM, noting that the median survival had not been reached for either group. The Minnesota study [61] took a yet different approach, calculating the rates of death due to causes other than plasmacytic or lymphoid malignancies (31 % at 5 years, 52 % at 10 years, and 65 % at 15 years). Median survival was 7 years, which was significantly shorter than expected among age- and sex-matched Minnesota residents (10.8 years; $P < 0.001$). In contrast, in a separate analysis of the Italian series, Gobbi [77] found a lower mortality (SMR = 0.42; 95%CI, 0.19–0.80) for 207 IgM-MGUS patients compared with that expected for the northern Italian population. The authors hypothesized that IgM-MGUS patients might enjoy a survival benefit due to increased medical surveillance following their diagnosis, permitting early diagnosis and treatment of cardiovascular risk factors (e.g., hypertension), second cancers, or infections. They further suggested that their results might differ from the Minnesota study because the Italian patients were younger (median age, 65 years vs. 74 years) and had lower M-protein (0.88 g/dL vs. 1.2 g/dL) at diagnosis.

11.6 Nonmalignant Clinical Correlates of IgM-MGUS

Although most patients with IgM-MGUS do not progress to malignancy, they are at risk for other comorbidities. Apart from the well-characterized IgM-mediated phenomena that are classified as IgM-related disorders, such as IgM-related peripheral neuropathy, there are numerous reports detailing co-occurrence of MGUS overall with a variety of other conditions. Given the high prevalence of MGUS in the general population, it is possible that many of these observed associations have occurred by chance [78]. Conversely, MGUS patients may have evidence of other disease manifestations prior to, or even in the absence of, progression to malignancy. Investigators have begun to address whether there are reproducible disease associations with MGUS overall, and some have included data for IgM-MGUS. For example, patients with IgM-MGUS may be at increased risk of infection. MGUS overall was found to be associated with increased risk of bacteremia in Denmark; nine cases of bacteremia were reported in IgM-MGUS patients, but these patients were not analyzed separately [79]. In a Swedish population registry study of 5326 MGUS patients and 20,161 matched controls, the 530 patients with IgM-MGUS were found to have significantly increased risk of any infection (hazard ratio

[HR] = 1.7; 95%CI, 1.4–2.1), bacterial infections (HR = 1.7; 95%CI, 1.4–2.1), and viral infections (HR = 2.7; 95%CI, 1.3–5.7) at 10 years of follow-up [80]. Overall, the risk for infection was similar for patients with IgM- and non-IgM-MGUS, as well as for patients with an M-protein concentration less than 1 g/dL compared to greater than 1 g/dL. Furthermore, the risk did not appear to be related to patients' risk of progression to WM, suggesting that subclinical malignancy did not account for the results. Mechanistic studies suggest that susceptibility to infection, as well as poor vaccination responses, might be mediated by effects of the monoclonal protein on B-cell repertoire diversity and response [81]. Together, these data support the hypothesis that MGUS is associated with an underlying immunodeficiency.

Interestingly, while the skeletal complications of multiple myeloma are well known, it appears MGUS patients may also be at increased risk for fracture. Early studies found abnormal bone osteoclastic resorption in histomorphometric studies of bone biopsies from patients with MGUS, including IgM-MGUS, in progression to B-cell malignancy [82]. Although not replicated in all studies, these results were supported by investigations of serum markers of bone resorption in MGUS patients, including IgM-MGUS with and without progression to WM [83]. A follow-up study combined densitometric and morphometric indexes together with biochemical parameters of bone remodeling and confirmed that excessive bone resorption contributes to fractures in patients with MGUS, although only one patient with IgM-MGUS was included [84]. These results suggested that abnormal bone metabolism could occur in MGUS cases in the absence of malignancy. However, because of the small number of IgM-MGUS patients in these studies, questions remained regarding whether IgM-MGUS patients shared the fracture risk attributed to non-IgM-MGUS. These questions are important since bone manifestations are not a prominent feature of WM, chronic lymphocytic leukemia, or non-Hodgkin lymphoma, the most common malignant outcomes associated with IgM-MGUS. Melton [85] reported one of the first studies to analyze a substantial number of IgM-MGUS patients. In this population-based study of 488 MGUS patients (17 % IgM), IgM-MGUS patients had an increased fracture risk compared to expected rates in the community (HR = 1.5; 95%CI, 1.02–2.1), exceeding that of patients with IgG-MGUS. Subsequently, Gregersen [86] conducted a case-control study of 1535 MGUS patients (299 IgM) and over 15,000 controls in Denmark to examine fracture risk. Because of concerns that some fractures observed in prior studies might have occurred in patients with smoldering malignancy misclassified as MGUS, the investigators excluded cases diagnosed with a relevant malignancy within a year following their MGUS diagnosis. Patients with IgM-MGUS were again noted to have significantly increased risk of fractures compared to controls (RR = 1.6; 95%CI, 1.1–2.2), despite adjustment for age, gender, comorbidity, and alcohol-related disease. Kristinsson [87] performed a similar investigation using Swedish registry data. In that study, risk of any fracture among 530 patients with IgM-MGUS was increased at 10 years (HR = 1.3; 95%CI, 1.0–1.6) but not at 5 years following diagnosis. Axial fractures accounted for most of the increased risk, and there was no difference in risk compared to non-IgM subtypes. All of the population-based studies were limited to some degree by lack of information on

potential confounders related to other conditions that might predispose to fracture risk and inability to perform assays of biochemical markers of bone metabolism or bone densitometry. A recent prospective clinically-based study also found no difference in fracture risk according to isotype [88]. It remains unclear whether the propensity to fracture is mediated in part by the monoclonal protein, disruptions in cytokine homeostasis, or some other mechanism.

In contrast to the findings for increased infection and fracture risks, another study in the same Swedish population showed that, unlike patients with non-IgM-MGUS, patients with IgM-MGUS did not have an increased risk for venous (HR = 1.0; 95%CI, 0.5–2.2) or arterial (HR = 0.8; 95%CI, 0.6–1.1) thrombosis compared with controls at 5 years of follow-up [89]. This null result was noted to be consistent with a series of WM patients treated with immunomodulatory agents, in whom no increased risk of venous thrombosis was observed [90]. However, in a population-based study from Sweden, an increased risk for venous but not arterial thrombosis was observed in patients with WM [91]. From these combined studies, it appears that IgM-MGUS patients are at risk for comorbidities in addition to cancer that may justify ongoing medical surveillance following diagnosis.

11.7 Conclusion

IgM-MGUS is an important premalignant condition. It shares many features common to all MGUS, but there is growing evidence that it differs in many important respects as well, including its racial distribution, clinical manifestations, genetics, spectrum of malignant outcomes, and mortality. It is worth repeating, however, that not all nonmalignant IgM monoclonal gammopathy is MGUS. Aside from syndromes such as hyper-IgM and POEMS, there is growing consensus that the category of IgM-related disorders is expanding to include, for example, skeletal and vascular outcomes. Furthermore, IgM-MGUS may indeed have clinical significance in some instances. To move the field forward, it will be important to harmonize definitions and to reach agreement about what nonmalignant phenotypes are truly attributable to IgM and should not be considered MGUS. For example, a recent Chinese survey of the clinical spectrum of IgM gammopathy found that only one-quarter of patients with nonmalignant IgM monoclonal gammopathy could be categorized as ‘healthy’, but not all identified comorbidities may be pathogenically related to IgM [92]. In the future, it would be highly desirable to target IgM-MGUS specifically in focused research studies. Moreover, because of institutional differences in case selection, population demographics, and detection methodology, large, well-coordinated consortial approaches are needed to harmonize approaches and to amass the numbers of cases required to conduct definitive analyses. Registry studies have provided valuable data, but many cancer registries do not routinely capture MGUS diagnoses and those that do often do not record immunoglobulin subtype data. Therefore, whenever possible, patients diagnosed with IgM-MGUS should be referred to an institution having an active research interest in it.

Familial relative risks for a patient with IgM-MGUS to develop WM are high. However, because of the rarity of WM in the general population, the absolute risk to an individual family member with IgM-MGUS is likely to be low. While valuable in the research context, routine screening for IgM-MGUS in relatives of WM patients is not recommended in clinical practice at present because of (1) the low rate of progression; (2) the inability to predict with precision which patients will progress; (3) the current lack of preventative or curative treatments for WM; and (4) the risk of inducing unwarranted psychosocial stress in both patients and their family members [93]. However, given the mounting evidence that MGUS patients may be at risk for both neoplastic and non-neoplastic complications, we recommend that, when discovered, IgM-MGUS patients should be followed for life. A few clinical trials have assessed possible prevention strategies, but to date there are no prevention or treatment strategies that have been proven effective. Future epidemiologic, genetic, and functional analyses should help to delineate the biological basis for the diverse outcomes associated with IgM-MGUS and may also provide insights into risk assessment, prognosis, and management.

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Part III

Clinical Features

Hyperviscosity Syndrome, Cold Agglutinin Hemolytic Anemia, and Cryoglobulinemia

12

Marvin J. Stone and Sigbjorn Berentsen

Morbidity in Waldenström's macroglobulinemia (WM) is caused by the bone marrow lymphoma infiltration itself and/or by effects of the IgM production. This chapter describes some specific clinicopathological manifestations mediated by the physicochemical or immunological properties of monoclonal IgM.

12.1 Hyperviscosity Syndrome

Hyperviscosity syndrome (HVS) is a clinical feature in 10–30 % of patients with Waldenström's macroglobulinemia (WM), sometimes as its presenting manifestation [1]. HVS occasionally accompanies other conditions, such as multiple myeloma, rheumatoid disease, polycythemia, sickle cell disease, leukemia, and spherocytosis. The latter four cellular causes of HVS are beyond the scope of this discussion. The purpose of this section is to discuss the characteristic features of HVS secondary to elevated plasma or serum viscosity and evaluate evidence supporting various diagnostic and treatment approaches.

In searching the Medline database on hyperviscosity syndrome, 594 English language references were listed back to 1965. Observational studies, systemic reviews, or case studies were included. No randomized trials on management of HVS were identified.

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_12

12.1.1 Methods to Measure Viscosity

Viscosity refers to resistance to flow or stickiness, from the Latin word "viscum alba" for mistletoe [2]. (Mistletoe berries were once used to make viscous glue.) Viscosity is classically measured in one of two ways: by determining the rate of fluid flow as a result of applying a predefined force and by measuring the amount of force required to achieve a predefined rate of fluid flow.

Although HVS is caused by hyperviscous blood, clinical laboratories generally only measure the serum or plasma component. Serum or plasma display Newtonian properties in that viscosity is independent of pressure drop or velocity gradient. Consequently, the method of measurement will not dramatically affect the test result. By contrast, whole blood viscosity is complex because of the suspension of red cells in plasma, resulting in non-Newtonian behavior. Different methods of measurement can yield various results.

In WM, serum or plasma viscosity measurements reflect the amount and properties of the IgM paraprotein. Monoclonal IgMs display a wide range of intrinsic viscosity values (0.106–0.162 dL/g), each protein having individual properties [3, 4]. However, relative viscosity values are highly reproducible for any individual protein. IgM is a star-shaped pentamer with a molecular size of 925 kDa (IgG is 150 kDa and albumin is 65 kDa). Thus it is not surprising that this giant IgM molecule, which is 80 % intravascular, exerts profound effects on blood flow and cells, especially when present in the high concentrations found in WM patients.

In measuring serum or plasma viscosity, we suggest using the Ostwald method because of its simplicity, reproducibility, and clinical correlation [5].

12.1.2 Clinical Presentation

HVS was described by Jan Waldenström in his original 1944 report of two patients with macroglobulinemia [6]. Bleeding, usually skin and mucosal, is the most common manifestation of HVS. Blurred vision, headache, vertigo, dizziness, nystagmus, deafness, and ataxia also occur in HVS [1, 6–8]. Patients with severe HVS may have confusion, dementia, stroke, or coma. Heart failure and other cardiovascular signs are less common. Patients with WM have an increased blood volume because of an expanded plasma volume [9]. Thus a component of the anemia in WM is dilutional. Plasma volume expansion correlates with the rise in relative serum viscosity. HVS may be suspected because of abnormal results in antibody screening in the blood bank.

Most patients with HVS have WM. Normal viscosity measured with an Ostwald tube is 1.4–1.8 relative to water. HVS is unlikely unless the serum viscosity is greater than 4 [1, 5, 7–10]. For patients with an IgG paraprotein, such as in multiple myeloma, the increase in serum viscosity is approximately proportional to the concentration of the paraprotein. For IgM paraproteins above a concentration of 3 g/dL, relative viscosity can rise exponentially.

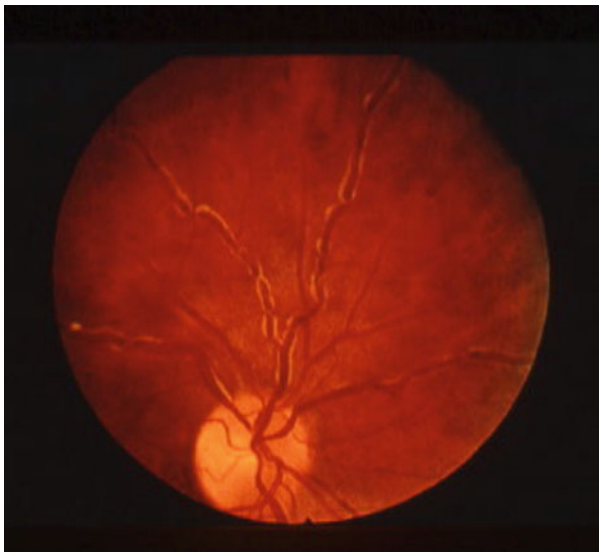

Fig. 12.1 Funduscopic appearance of patient with WM and mixed cryoglobulinemia. Note the marked retinal venous engorgement and "sausaging." The *white material* at the edge of the veins may be cryoglobulins. Serum from this patient is shown in Fig. 12.3

Viscosity levels in HVS vary significantly between patients. Such variation is the result, in part, of the previously mentioned wide range of intrinsic viscosity values noted in monoclonal macroglobulins. However, viscosity values correlate closely with signs and symptoms in the same patient ("symptomatic threshold") [1, 5, 7, 8, 11]. HVS can be diagnosed from the physical examination by the funduscopic finding of marked retinal venous engorgement resembling hot dogs on a string (i.e., "sausaging" Fig. 12.1) [5, 7, 8, 11]. Hemorrhages, exudates, microaneurysms, papilledema, and an appearance indistinguishable from central retinal vein occlusion may be seen in later stages. Prompt diagnosis of HVS from the eye examination enables the institution of appropriate therapy, i.e., plasmapheresis. In addition to raising plasma viscosity, macroglobulin coats red cells, leading to the characteristic stacking appearance (rouleaux) on peripheral blood smear in WM patients. Protein coating also contributes to a platelet functional defect that further accentuates the bleeding tendency.

The presence of cryoglobulinemia can result in a strikingly temperature-dependent elevation of serum viscosity in WM patients [12] (see Sect. 12.3). Most cryoglobulins are mixed monoclonal IgM-polyclonal IgG immune complexes with rheumatoid factor activity (type II). These antigen-antibody complexes have high thermal amplitude and precipitate at lower concentrations than single-component (type I) monoclonal cryoglobulins.

12.1.3 Treatment of HVS

Plasmapheresis, first carried out manually for macroglobulinemia in the late 1950s, was demonstrated to reverse retinopathy and other clinical manifestations in most

patients with HVS [14, 15]. In common usage, the terms plasmapheresis and plasma exchange are used interchangeably. Plasmapheresis remains effective short-term treatment for HVS in WM because of the demonstrated correlation between IgM levels and serum viscosity and the 80 % intravascular location of IgM. A relatively small reduction in IgM concentration has a significant effect on lowering serum viscosity. Because bleeding is the most common sign of HVS, urgent plasmapheresis using a cell separator should be carried out for patients experiencing visual symptoms to reduce the likelihood of blindness from retinal hemorrhages/retinal detachment [16]. Plasmapheresis can reverse HVS-induced retinal changes promptly, by reducing retinal venous diameter and increased venous blood viscosity [17]. Retinal examination findings correlate with symptomatic threshold for HVS in WM patients. Some WM patients can be managed predominantly with plasmapheresis [1, 5, 7, 18, 19]. Because plasma exchange does not affect the underlying disease process, systemic or pharmacologic therapy is often begun concomitantly. Initially, plasmapheresis can be carried out daily and then spaced out at longer intervals to keep the viscosity below the symptomatic threshold for that particular patient. Plasma exchange reduces plasma viscosity approximately 20–30 % per session [20]. Serial serum viscosity can be monitored daily to decide about further plasmapheresis. Generally, 1–1.5 plasma volumes are exchanged per session. Fluid replacement usually consists of albumin and saline in various proportions. Plasmapheresis is generally a safe and well-tolerated procedure. Various modifications of the apheresis procedure have been used for removal of paraprotein in patients with cryoglobulinemia (see Sect. 12.3 later in this chapter).

It is usually not necessary to plasmapherese patients down to normal viscosity to relieve symptoms. One potential exception is illustrated by a female patient with documented WM, who developed peripheral neuropathy associated with monoclonal IgM anti-myelin-associated glycoprotein antibody [8, 19]. Because her neurologic symptoms reproducibly recurred above a serum viscosity of 2.5–3, we sought to maintain the viscosity below 2.5 with frequent plasmapheresis. During a 23-year period, this patient underwent approximately 400 plasmapheresis procedures with little chemotherapy other than corticosteroids. Her prolonged course raises the possibility that patients with monoclonal IgM antibodies that produce neuropathy or other target organ dysfunction may benefit from a more aggressive effort to maintain serum viscosity near normal. Prospective clinical trials will be necessary to confirm this anecdotal observation.

Transient increases in IgM levels after single-agent rituximab therapy (“flares”) occur in 30–70 % of WM patients [21, 22]. It has been recommended that plasmapheresis be carried out in advance of rituximab therapy if serum viscosity is more than 3.5 cp or IgM level is greater than 5 g/dL. The mechanism of the rituximab flare may involve release of IL-6 after stimulation of monocytes. The flare phenomenon may become less of a problem with combination regimens that administer bortezomib or cytotoxic chemotherapy before rituximab.

Although randomized trials of this procedure in HVS are lacking, plasmapheresis remains a valuable adjunct to the treatment of some patients with WM. It should be carried out urgently in high-risk HVS patients.

HVS occurs uncommonly in myeloma [5]. Patients with the unusual IgG3 subclass are more likely to develop HVS than other myeloma patients because of concentration-dependent aggregation. Although IgG is only 40 % intravascular, plasmapheresis should be instituted in myeloma-associated HVS. HVS also occurs occasionally in IgA and light chain myeloma because of formation of polymers. Rheumatoid HVS is rare and may develop from aggregates of rheumatoid factor or intermediate IgG complexes.

Observational studies have consistently demonstrated that plasmapheresis can promptly reverse most clinical manifestations of serum HVS [5, 23]. Thus early diagnosis is crucial. The concept of a symptomatic threshold in individual patients seems valid. Maintaining serum viscosity below each patient's symptomatic threshold effectively prevents recurrent HVS. Plasmapheresis is sometimes necessary as an emergency procedure and is a useful maintenance therapy in selected patients. Vigorous plasmapheresis in WM patients with autoreactive IgM antibodies requires further study.

12.1.4 Conclusion

Although controlled trials for treatment of serum HVS are lacking, experience with management of patients by plasmapheresis has consistently demonstrated efficacy. Most signs and symptoms are reversible with prompt diagnosis and treatment. HVS is readily diagnosed on funduscopic examination, treatable with plasmapheresis, and monitored with serum or plasma viscosity measurements [5]. Plasmapheresis is usually well tolerated and safe. When patients are maintained at a level below their symptomatic threshold, clinical manifestations of the syndrome are usually prevented [23]. Whether patients having IgM proteins with autoantibody activity and consequent immune-mediated organ damage should be more aggressively pheresed is unknown, but this approach warrants a prospective therapeutic trial.

12.2 Cold Agglutinin-Mediated Hemolytic Anemia

In general, approximately 15 % of autoimmune hemolytic anemias (AIHA) are classified as primary chronic cold agglutinin disease (CAD). This well-defined entity should be distinguished from secondary cold agglutinin syndrome (CAS), which occasionally complicates specific infections and malignancies [24]. The cold agglutinins (CA) are monoclonal IgM-kappa in more than 90 % of patients with CAD, nearly always with specificity for the carbohydrate antigen I on the erythrocyte surface.

12.2.1 Diagnosis

In some patients, CAD can be suspected from the history; in others, the presence of CA is demonstrated after agglutination in blood samples in the hematology laboratory or observation of red cell agglutinates in the peripheral blood smear. The diagnostic criteria for CAD are chronic hemolytic anemia, direct antiglobulin test (DAT) positivity for complement protein fragment 3d (C3d), CA titer $\geq$64 at 4 °C, and no malignant disease other than low-grade B-cell disorders [24]. Evidence of clonality and the presence of cold-induced circulatory symptoms are considered confirmatory but not required for diagnosis. Blood specimens for CA titration and serum immunoglobulin analysis (including electrophoresis and, if required, immunofixation) must be kept at 37–38 °C from sampling until serum is removed from the clot.

12.2.2 Relationship Between CAD and WM

Although anemia is a major clinical manifestation in WM, the anemia is usually not hemolytic. When AIHA does occur in WM, the hemolysis is CA mediated in most cases. In a series of 122 untreated patients with WM, 3 % had CA-mediated hemolytic anemia [25]. Among the 172 individuals with monoclonal IgM in serum, CA with anti-I-specificity was found in titers between 512 and 65,536 in 8.5 % of the patients [13].

The frequencies of specific lymphoproliferative disorders in CAD have been studied in two series of more than 85 patients each [26, 27]. In one series, lymphoplasmacytic lymphoma (LPL) was reported in 50 % of the patients. Only 10 % had been diagnosed with WM in the other study; this retrospective series which went back to 1970, however, reported IgM-MGUS in 61 % of the patients. Thus, the difference in frequency of WM/LPL between the two series may be a matter of method, classification, and sensitivity. Within each series, there was a striking heterogeneity in hematological and pathological classification of the underlying lymphoproliferative disorder [26, 27].

The explanation for this perceived heterogeneity has probably been revealed by a recent study in which bone marrow biopsy and aspirate samples from 54 patients with CAD were systematically reexamined by a group of lymphoma pathologists, using a standardized panel of morphological, immunohistochemical, flow cytometric, and molecular methods [28]. The findings were consistent with a surprisingly homogeneous disorder termed “primary cold agglutinin-associated lymphoproliferative disease” by the authors and distinct from LPL, marginal zone lymphoma, and other previously recognized lymphoma entities. Intertrabecular nodular infiltration of clonal B-cell aggregates was seen in 74 % of the biopsy specimens, while the remaining 26 % showed only few and scattered clonal B cells. In those with aggregates, infiltration varied between 5 % and 80 % of the intertrabecular surface, with a median of 10 %. The *MYD88* L265P mutation could not be identified in samples from CAD patients. Somatically mutated clonal

IGHV4-34 gene rearrangement was found in all of eight fresh-frozen samples; mean sequence homology was 95.4 %.

These findings may indicate that most patients diagnosed with "WM and CAD" do not have typical WM but a distinct type of IgM-producing clonal lymphoproliferation specific for CAD [28]. The presence or absence of the *MYD88* L265P mutation should be determined in such cases. In CAD-associated lymphoproliferative disease, the cumulative rate of transformation to aggressive lymphoma is very low (probably 3–4 %), and the survival of the patients does not differ significantly from that of an age-matched general population [26].

12.2.3 Role of Complement

The CA-initiated destruction of red blood cells in CAD is entirely complement dependent [29, 30]. Cooling of blood during passage through acral parts of the circulation allows CA to bind to erythrocytes and cause agglutination. The antigen-antibody complex binds complement protein 1 (C1) and thereby triggers the classical complement pathway. C1 esterase activates C4 and C2, generating C3 convertase which cleaves C3 into C3a and C3b. Upon rewarming in the central parts of the circulation, CA detaches from the cell surface, allowing agglutinated cells to separate from each other, while C3b remains bound. C3b-opsonized erythrocytes are sequestered by macrophages of the reticuloendothelial system in the liver (extravascular hemolysis). On the surface of the surviving red blood cells, C3b is cleaved, generating C3d. These mechanisms explain why the monospecific DAT is strongly positive for C3d and, in a majority, negative for IgM and IgG (Fig. 12.2).

Complement activation may proceed beyond the C3b formation step, resulting in C5 cleavage, formation of the membrane attack complex, and intravascular hemolysis. Classical isotope studies as well as a recent in vitro study have shown, however, that clinically significant activation of the terminal complement pathway does not occur in most patients [29, 30]. The major mechanism of hemolysis in stable disease, therefore, is the extravascular destruction of C3b-coated erythrocytes. Despite this, C5-mediated intravascular hemolysis does occur in severe acute exacerbations and in some profoundly hemolytic patients, as evidenced by the frequent finding of hemosiderinuria, hemoglobinuria in 15 % of the patients, and a beneficial effect of C5 inhibition by eculizumab in at least occasional patients (Fig. 12.2) [26, 27, 32].

12.2.4 Clinical Manifestations

In some patients, hemolytic anemia is mild; occasional patients have a fully compensated hemolysis. Median hemoglobin level, however, was 8.9 g/dL in a series of 86 unselected patients, with a lower tertile at 8.0 g/dL and lower range at 4.5 g/dL [26]. Hemolytic anemia often shows characteristic seasonal variations. At

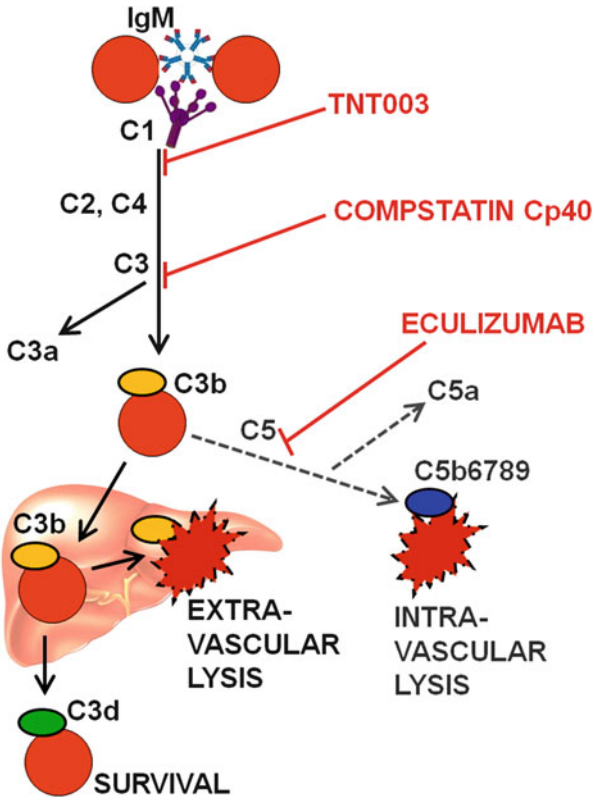

Fig. 12.2 Complement-mediated hemolysis in cold agglutinin disease and possible targets for complement inhibitors. *Black arrows*, major pathway of complement activation; *gray/dotted arrows*, minor pathway. *C* complement protein. First published in *Blood* [31], re-used with permission. Copyright: *Blood*, the Journal of the American Society of Hematology

least in a cool climate, 90 % of the patients have cold-induced acrocyanosis or Raynaud's phenomenon which can vary between slight and disabling. Approximately 75 % of the patients experience exacerbation of hemolytic anemia in febrile diseases or following major trauma or surgery. The probable explanation for such "paradoxical" exacerbation is that in stable, chronic CAD, most patients are complement depleted with low levels of C3 and very low levels of C4. During acute phase reaction, complement levels are repleted and exacerbation of hemolysis ensues [24].

12.2.5 Therapy

Patients with very mild anemia and no or negligible cold-induced circulatory symptoms can be managed just by warm clothing and avoidance of cold. Descriptive studies from Norway and the United States have shown, however, that 73 % and 82 % of the patients, respectively, had received pharmacological therapy [26, 27]. Corticosteroids and other unspecific immunosuppression should not be used for the treatment of CAD. Less than 20 % respond to corticosteroids, and, in

the few responders, unacceptably high doses are often required to maintain the remission [24, 26].

The relative success in treatment of CAD during the last 10–12 years has been achieved by targeting the pathogenic B-cell clone. Rituximab monotherapy produces remission rates of about 50 % and approximately 12 months of median response duration [33, 34]. Fludarabine and rituximab combination therapy has resulted in 75 % remission rate (20 % complete remissions) and median response duration of more than 66 months, however with some toxicity [35]. Remission following bortezomib-based regimens or the combination of bendamustine and rituximab has been reported in single cases.

Given that hemolysis in CAD is entirely complement mediated, complement modulation would be an alternative therapeutic approach (Fig. 12.2) [30–32]. Improvement during therapy with the anti-C5 monoclonal antibody eculizumab has been observed in single cases [32]. In vitro experiments have shown that TNT003, a mouse monoclonal antibody targeting C1s serine protease, prevented CA-induced, complement-mediated lysis of human red blood cells. TNT003 completely inhibited CA-induced deposition of C3 fragments on erythrocytes at the same antibody concentration that stopped hemolysis. The monoclonal antibody also inhibited erythrophagocytosis by a phagocytic cell line and efficiently suppressed the classical pathway-driven generation of anaphylatoxins C4a, C3a, and C5a. Furthermore, compstatin Cp40, a low-molecular peptide that inhibits cleavage of C3, has been shown in vitro to prevent complement-mediated lysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria but has not been tested in the CAD setting [36].

Complement modulation is currently not an evidence-based therapeutic approach in CAD. If safe and efficacious inhibitors can be developed, however, such drugs would enable rapidly acting therapy for acute exacerbations following acute phase reaction as well as treatment of severely affected patients not responding to immunochemotherapy.

Erythrocyte transfusions can safely be given in CAD provided some specific precautions are observed [24]. Antibody screening and, if required, compatibility tests should be performed at 37 °C. The patient and, in particular, the extremity used for infusion should be kept warm, and most authors recommend the use of an in-line blood warmer.

12.3 Cryoglobulinemia

Cryoglobulins (cryos) are immunoglobulins (Ig) in plasma or serum that precipitate (ppt) or gel at <37 °C and dissolve on rewarming. The presence of a cryoglobulin indicates a monoclonal gammopathy, an immune complex, or both. Cryofibrinogen is a normal component of plasma and not present in serum. The role of cryofibrinogen in clinical disease is unclear.

Cryos were first described by Wintrobe and Buell in a 57-year-old female with cold sensitivity and multiple myeloma [37]. Cryos may be monoclonal single

component (type I) that are usually IgM or IgG. Type II cryos are mixed monoclonal IgM—polyclonal IgG. The IgM is an antibody (Ab) to the IgG antigen (Ag) [38, 39]. These are rheumatoid factor positive, often in high titer. Occasionally the Ab is IgG. Type III cryos are mixed polyclonal Ab-Ag (90 % IgM-IgG). In Dallas, 70 % of cryos are mixed type II and 20 % single monoclonal component type I [40]. Complement components and viral RNA may be present in the mixed type II cryoprecipitate.

The incidence of cryos varies considerably in different series and locales. The frequency is especially high in Italy [41]. Some patients with cryos are asymptomatic but others have a variety of manifestations including purpura, arthralgias, weakness, acrocyanosis, Raynaud's phenomenon, peripheral neuropathy, leg ulcers, gangrene, and renal functional impairment [41]. Kidney involvement is most frequently manifested as membranoproliferative glomerulonephritis [42].

If a cryoglobulin is suspected, proper specimen collection is essential. The blood must be drawn and allowed to clot at 37 °C. The quantity of cryoprecipitate can be underestimated or totally lost if the blood sample is not collected and processed at 37 °C.

Formation of the cryoprecipitate or gel typically occurs within 48–72 h at 4 °C and often within minutes (Fig. 12.3). Single-component cryos (type I) are usually IgM or IgG and are often concentration dependent. Type II mixed cryos are composed of monoclonal IgM Ab bound to the Fc portion of polyclonal IgG Ag. Either isolated reactant is soluble but the cryoprecipitability is due to the immune *complex* [11, 12, 39, 40]. Thermal amplitude (temperature at which the cryo precipitates) is often quite high (near 37 °C) with mixed cryos. Therefore, careful collection and processing are even more important if accurate quantification is to be determined. Mixed IgM-IgG cryos have higher thermal amplitude and precipitate at lower concentrations than single-component cryos [40]. Cryocrit measurement is not an accurate method for quantifying either singly or serially.

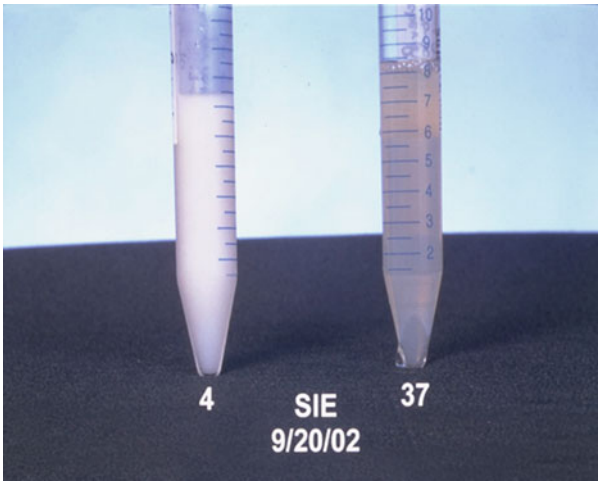

Fig. 12.3 Serum from patient Sie at 4 °C and 37 °C on September 20, 2002. This specimen was collected on March 30, 1970. The antibody activity of this Waldenström's macroglobulin was demonstrated in [8, 11, 12]. Stone et al., Semin Oncol 30: 318–324, 2003 [13]

After proper collection, measurement of the redissolved cryo is best achieved by protein concentration and immunologic methods.

Type II mixed cryos are associated with hepatitis C. Mixed cryos with hepatitis C may indicate a preneoplastic condition. Some patients with hepatitis C develop non-Hodgkin's lymphoma including Waldenström's macroglobulinemia (WM). It is possible that hepatitis C virus is involved in multistep lymphomagenesis in certain patients [41, 43, 44].

Chronic B-cell stimulation may result from HCV infection leading to antigen-driven expansion. B-cell clones producing monoclonal IgM rheumatoid factor can be found in the peripheral blood, bone marrow, and liver, which bind to anti-HCV antibodies. Intrahepatic B cells are able to synthesize rheumatoid factors with a major cross-reactive idiotype (WA). Thus HCV may have a role in driving B-cell expansion including rheumatoid factor-producing cells. It is possible that type III (polyclonal) cryos precede the development of type II mixed cryos with monoclonal IgM.

Either type I or II cryos can be associated with WM.

12.3.1 Treatment

Therapy of cryoglobulinemia is based on severity of clinical manifestations and etiology [41, 45]. In asymptomatic patients, treatment may not be necessary. Since type I single-component cryos are often concentration dependent, treatment to reduce the M-component—plasmapheresis and chemotherapy—is often effective. By contrast, mixed type II cryos are often associated with hepatitis C. Therefore, sequential or combination antiviral therapy may be employed [41, 45]. Biologic therapy to reduce B-cell proliferation is frequently added. Cryos can dramatically worsen hyperviscosity syndrome [5, 12]. Urgent plasmapheresis is indicated for HVS with cryoglobulinemia [5, 8, 11]. Monoclonal Ab (rituximab), plasmapheresis, and chemotherapy are often used in addition to antiviral agents. The possibility of adverse events due to complex formation between rituximab, which is IgG, and monoclonal IgM rheumatoid factor should be kept in mind [46]. Double filtration plasmapheresis has been reported to be effective in cryoglobulinemic vasculitis and HVS [45]. When viscosity is lowered, clinical signs and symptoms of cryoglobulinemia usually improve. Mixed cryos often respond to corticosteroids with or without cyclophosphamide. Newer antiviral drugs appear more effective for hepatitis C and thus favorably influence the incidence of malignancy, including non-Hodgkin lymphoma/WM [47]. It is likely that the incidence of mixed cryoglobulinemia and HCV-related lymphoma will decrease in the coming years.

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Neuropathy in Waldenström's Macroglobulinaemia

13

Karine Viala and Michael Lunn

13.1 Introduction

Waldenström's macroglobulinemia (WM) is the result of a malignant proliferation of lymphocytes that produce monoclonal immunoglobulin (Ig) M. The diagnostic criteria for WM require the presence of monoclonal IgM of any concentration in the serum and bone marrow infiltration by small lymphocytes, plasmacytoid cells and plasma cells immunophenotypically characterised as CD19+ CD20+ CD5− CD10− CD23− [1].

Neuropathy is the most common organ-specific complication of WM and is frequently the presenting feature. It is estimated that neuropathy occurs in 40 % of patients [2–4], but there is almost certainly acquisition bias in these estimates and true rates may be higher. Neurophysiological involvement of peripheral nerves, not evident without close clinical examination, may occur in the majority of patients with IgM paraproteins [5]. Clinically relevant peripheral neuropathy may be disabling and as a result can determine a decision to treat in otherwise nonprogressive disease; as such it is important to recognise. Furthermore accurate diagnosis of the type of peripheral nerve involvement is important as some neuropathies respond to non-WM treatment regimens or may not respond to therapy at all.

A typical neuropathy is distal and symmetrical, involving the legs more than the arms and both motor and sensory modalities. Typical neuropathy unrelated to WM (such as diabetic neuropathy or idiopathic axonal neuropathy) occurs by chance in this generally older group of patients. Waldenström's macroglobulinaemia however

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_13

also causes one of a number of neuropathies and by a variety of different mechanisms [6]. These result in a number of phenotypic presentations differing very greatly from 'typical' neuropathy.

Paraproteinaemic IgM in WM and MGUS can possess activity against peripheral nerve epitopes, most typically myelin-associated glycoprotein, but also GM1 and other gangliosides [7, 8]. Anti-MAG neuropathy is the commonest of the presentations, with a sensory ataxic neuropathy associated with tremor. IgM anti-GM1 activity can result in a patchy pure multifocal motor neuropathy with electrophysiological conduction block sometimes confused with motor neurone disease [9, 10]. Small-fibre neuropathies occur in which the presentation is sensory symptoms of prominent nocturnal burning discomfort and a 'normal' neurological examination. IgM can also act as a cryoglobulin and deposit in vessels resulting in vasculitis. Vasculitis is usually rapidly progressive and can be devastating. Vasculitis is usually easy to recognise from the other associated systemic features. Acquired AL amyloid can occur with deposition and polymerisation of free light chains in numerous tissues, with a painful progressive motor and sensory axonal neuropathy with autonomic involvement. Rarely tumourous deposits of amyloid can occur in individual nerves resulting in mononeuropathies. These may be less accessible to neurophysiology or biopsy but can be identified with targeted MRI in specialist centres where biopsy may also be possible. Even more rarely still WM cells invade the peripheral nerves sometimes without significant systemic load and can result in a presentation that looks like an inflammatory neuropathy but requires nervous system penetrating chemotherapy.

13.2 Neuropathy with Direct Antibody Targeting of Nerve Epitopes

13.2.1 IgM Anti-MAG Paraproteinaemic Peripheral Neuropathy

Anti-MAG neuropathy is the easiest of the WM syndromes to recognise. Patients present with progressive distal sensory loss in the feet. Although pain is described, IgM anti-MAG paraproteinaemic peripheral neuropathy is usually painless or with a tightness or aching. Early unsteadiness and frequently a tremor are reported in the majority [7, 11]. The tremor is postural and has cerebellar and peripheral components, frequently with a side-to-side finger tremor and some jerkiness [12]. Motor weakness occurs late and predominantly in the distal legs. As a result of the sensory loss, patients walk with a broad-based gait and are unsteady in the dark. On examination, a neuropathy with reduced or absent reflexes is found. The defining features are of a tremor of the upper limbs, little or no wasting of muscles and a rather typical sensory loss where pinprick is reduced distally in the lower limbs, joint position sense is only minimally impaired, but vibration sensation is reduced often to the hips or even the costal margin.

Neurophysiological findings are very typical. The neurophysiologist finds a patchy demyelinating neuropathy with very prolonged distal motor latencies in

comparison to the slowing of the main motor trunk; this results in a terminal latency index of <0.25 in tested nerves [13].

Anti-MAG activity is part of the innate antibody repertoire. IgM anti-MAG activity can be detected in the serum using one of a number of techniques, usually now ELISA based. Anti-MAG antibodies cross react with SGPG and SGLPG, gangliosides with the common HNK-1 epitope. Although there are various ways that these antibodies are reported, anti-MAG activity is much more likely to be relevant when the levels of activity are reported as 'strongly positive'; simply finding an anti-MAG antibody does not make a diagnosis of an anti-MAG neuropathy, and the clinical and electrophysiological picture must also be correct.

A nerve biopsy may be performed in cases where there is diagnostic uncertainty. The finding of pathognomic widely spaced myelin by electron microscopy confirms the pathogenic potential of the anti-MAG antibodies [14, 15] (Fig. 13.1), but other pathologies such as vasculitis can exist in parallel which may be more relevant to the clinical presentation.

Some anti-MAG neuropathies are slowly progressive and non-disabling and some appear to become static, not requiring treatment. Those associated with WM rather than MGUS tend to be more aggressive and more frequently treated. In IgM paraproteinaemic neuropathies, the evidence to support effective treatments is not extensive [16]. Intravenous immunoglobulin is effective in some cases in the short term, but is not sustained for many weeks and frequently does not persist on repetition. Two randomised controlled trials of rituximab treatment for IgM paraproteinaemic neuropathies exist, neither reporting success in their primary outcome, but both reporting functional benefit from the patients in about half of the treated (and none of the placebo) patients [17, 18]. Small series of patients treated with plasma exchange, steroids, cyclophosphamide, chlorambucil, cladribine, fludarabine and combinations of these exist with varied but unconvincing evidence for consistent benefit. There is a theoretical benefit to extending

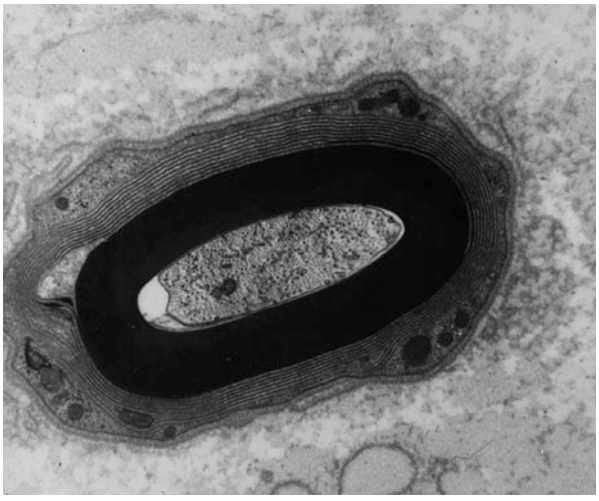

Fig. 13.1 Widely spaced myelin as a diagnostic pathognomic feature of anti-MAG neuropathy. The normally densely compacted intraperiod line is widened from 2–4 nm to between 20 and 30 nm, and an amorphous material is seen in the new space at high magnification, possibly immunoglobulin

single-agent rituximab to a combination therapy with a purine analogue or proteasome inhibitor [19]. If this can be justified from the WM point of view, then patients may stabilise.

13.2.2 Neuropathies with IgM Activity Directed to Other Peripheral Nerve Epitopes

Gangliosides are present on the surface of many cells, but are elaborated on nervous system cells with over 100 species detected [20]. They present a somewhat flexible carbohydrate antigenic target, rather like the glycoprotein MAG, and likewise can be targeted by the IgM of WM.

Antibodies to ganglioside GM1 result in a progressive pure motor phenotype often with cramping and fasciculation that can be mistaken for motor neurone disease [10]. There are never any upper motor neurone features other than those caused by a second pathology (e.g. cervical spondylosis). Neurophysiology, which needs to be performed by a skilled operator, demonstrates multiple conduction blocks away from typical sites of compression, although sometimes these are difficult to find on the first examination. The sensory examination is normal. High-titre anti-GM1 antibodies, sometimes with anti-GD1b antibodies, can be found by ELISA.

The disialosyl group of gangliosides (GD1b, GT1b, GQ1b, GD3) share a common epitope and hence activity against all is found in the serum. The rare CANOMAD syndrome (chronic ataxic neuropathy with ophthalmoplegia, M-protein, agglutinins and disialosyl antibodies) can be found in occasional patients [21]. Diplopia in combination with their ataxia is very disabling.

Other anti-ganglioside activities are frequently reported but are of uncertain significance. Anti-sulphatide antibodies are not uncommon, often of very high titre, but not associated with any particular phenotype of neuropathy except perhaps loosely sensory. Anti-GM2 antibodies are also associated with both motor and sensory phenotypes and are of limited use in planning or justifying treatment.

13.3 Neuropathies Linked to Physicochemical Properties of IgM

13.3.1 Cryoglobulinaemic Vasculitis

The IgM of WM can act as a cryoglobulin and deposit in vessels resulting in vasculitis [19]. Occasionally IgM anti-MAG activity can also be found in the serum; whether they are one and the same is unknown [22]. Patients with a cryoglobulinaemic vasculitis will present with painful distal sensory neuropathy with burning, shooting pains and deep aching. Motor weakness appears early and the neuropathic involvement is symmetrical and distal. Large- and small-fibre modalities are equally affected and the clinical picture usually confluent and length dependent. Systemic clues are livedo reticularis of the skin and lower limb

ulceration, the ulcers being multiple, often small in diameter and 'punched out' [23]. There is not necessarily any other systemic response.

Neurophysiology demonstrates a confluent length-dependent sensorimotor neuropathy. A biopsy is essential to make the diagnosis, and an affected sensory nerve can be selected from the neurophysiology, usually sural or superficial peroneal. This should be excised, processed and examined at a centre competent in nerve biopsy and analysis. Skin biopsies are not necessarily helpful [24].

Treatment is not supported by RCT evidence. Plasma exchange and steroids can be useful acutely. Cyclophosphamide and steroids can also be helpful with rituximab receiving support from multiple experts [25]. Aggressive treatment of the WM in this situation is however usually warranted as vasculitis is progressive and potentially fatal.

13.3.2 Amyloidosis

Light chains can deposit in any tissue and condense to the hard, insoluble and amyloid fibrils visible on electron microscopy [26]. Amyloidosis also presents with a progressive distal painful sensorimotor axonal neuropathy [6]. The clues to amyloidosis are the addition of coincident carpal tunnel syndrome and autonomic involvement with erectile failure, postural dizziness, diarrhoea and urinary difficulties.

Amyloid can deposit in all tissues and thus systemic screening with a SAP (serum amyloid protein) scan can be very useful [26, 27]. Rectal biopsies or fat aspirates can be useful as can a bone marrow aspirate. Detection of amyloid in peripheral nerves is very feasible with Congo red staining with appropriate protocols and may be the only site where it is detected.

Preventing further accumulation of amyloid is the key to treatment, but the depth of remission needed to achieve this is unknown [26]. Patients may have poor performance status because of co-existing autonomic failure.

Autonomic failure can, in its own right, be very disabling, and fludrocortisone, ephedrine and midodrine are useful in maintaining blood pressure, alongside fluid and electrolyte management and physical manoeuvres. Bladder overactivity may respond to anticholinergics or even intravesical botulinum toxin, and bowel involvement can be managed with codeine, loperamide or octreotide.

Pain is the most disabling aspect of amyloidosis in the long term. Combinations of gabapentinoids, tricyclic antidepressants or duloxetine and opiates may be necessary.

13.3.3 Deposition of Light Chains in the Endoneurium

Light chain deposition disease is only rarely described in peripheral nerve disease [28]. Light chains probably deposit in vessel walls before condensing into more recognisable amyloid deposits, with the symptoms and signs above.

The aetiology of small-fibre neuropathies in WM is not known but might be postulated to be related to light chain deposition. Small-fibre neuropathies are distal, symmetrical, purely sensory and painful [29]. The characteristic pain is burning and very prominent at night, often disturbing sleep. Patients will expose their feet outside the bed covers, spray them with or immerse them in cold water or seek out cold ceramic surfaces on which to stand. No signs are found on examination as the tendon reflexes are preserved and joint position, vibration and sometimes pinprick sensation are preserved. Most usually distal pinprick and temperature sensation are impaired. Standard neurophysiology testing is also normal, but thermal thresholding (quantitative sensory testing) is able to demonstrate abnormalities. Dermal and epidermal skin biopsies stained for the axonal marker PGP9.5 can quantitate epidermal fibre loss against normal values for confirmation [30].

There is no definitive treatment for small-fibre neuropathies; pain can be managed as above. Occasionally plasma exchange or IVIG can be effective in resistant patients.

13.3.4 Tumefactive Amyloid Deposition

Deposition of amyloid locally within individual nerve trunks can be the most difficult WM complication to diagnose. Mononeuropathy or multiple neuropathies can occur, with motor and sensory involvement often without pain. A competent neurological examination in combination with directed high-field MRI neurography can locate intraneural deposits (Fig. 13.2) and direct a diagnostic biopsy [31]. PET of the deposits is cold. Often it is difficult to localise proximal neural deposits initially and they may be MRI invisible. Persistence and repetition of investigations can be rewarding.

Treatment is directed at inducing remission from WM if possible; deposits do not tend to regress even with the most aggressive treatment. External orthotic devices can be helpful for lower limb instability. For stable and nonprogressive disease, tendon transfer can facilitate active movement in muscles in the affected nerve territories.

13.4 Direct Intraneural Invasion by WM

Bing-Neel syndrome is a disease without a clear definition, but involves central nervous system invasion by WM cells or the deposit and activity in the CNS of immunoglobulin released by them [32]. Certainly cord involvement in Bing-Neel syndrome can present with symptoms of distal sensory loss that may superficially resemble peripheral neuropathy; spastic tone in the limbs with preserved or brisk reflexes and a central sensory loss soon redirect the clinician.

Invasion of the peripheral nervous system can also occur [33] and perhaps should come within the definition of Bing-Neel. Invasion can present with almost any of the symptoms or signs above; mono- or multiple neuropathies, confluent

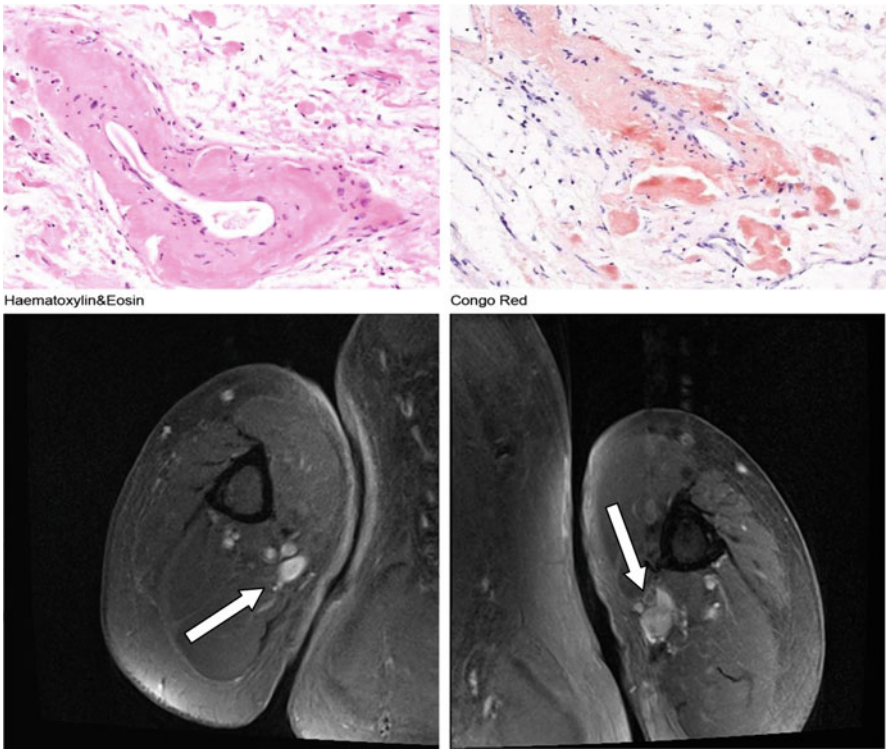

Fig. 13.2 Light chain amyloid, staining positively with Congo red (with birefringence in polarised light), can deposit in peripheral nerves just as in other tissues. Biopsy of the proximal right radial nerve (*top panels*). Tumefactive deposits in nerves can be seen (*arrowed*) on the fat saturated T2 MRI scans of the right and left proximal upper limb. The most affected side was less notable by MRI

motor and sensory distal neuropathies or proximal and distal weakness of a polyradiculoneuropathy reminiscent of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) can all be presenting features. Electrophysiology can also be confluent or patchy and axonal or demyelinating. All are progressive over weeks and can be aggressive and disabling. PET scanning can identify affected nerves and sometimes can correlate with high-field MRI neurography images to direct biopsy if peripheral sites for biopsy cannot be identified clinically or with electrophysiology. The biopsy will show clonal lymphoid cells, sometimes with endoneurial immunoglobulin deposition [34].

Treatment decisions should be made with the knowledge that both CNS and PNS tissues are hidden behind the blood nerve and blood-brain barriers, immunologically restrictive structures preventing ingress and egress of materials from the endoneurium CNS tissues. Standard chemotherapy regimens without CNS penetration will not be effective and relapse is not uncommon if response is achieved at all; outcomes and survival are universally poor. CNS-penetrating chemotherapy should

be selected as a matter of course with high-dose methotrexate as a minimum and other agents (e.g. idarubicin and Adriamycin) considered in addition.

13.5 Conclusions

The true extent of the peripheral neurological complications of WM has not ever been accurately studied but they are common and result in substantial disability. The different types of peripheral neurological involvement are easily distinguished clinically and investigations are available for confirmation. Some are more treatable than others, but advances in standard systemic WM chemotherapy and regimens for neural penetration will improve outcomes and survival.

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IgM Amyloidosis

14

Morie A. Gertz, Taimur Sher, Angela Dispenzieri, and Francis K. Buadi

14.1 History

The association of amyloidosis and Waldenström macroglobulinemia predates the publication by Drs. Isobe and Osserman in 1973 that recognized that the amyloid deposits of patients with "primary amyloidosis" were composed of immunoglobulin light chains [1]. Amyloid arthropathy associated with Waldenström macroglobulinemia was first recognized as early as 1969 [2]. Earlier publications from 1966 recognized the association between amyloidosis and an IgM monoclonal protein [3] as well as with full-blown Waldenström macroglobulinemia [4]. Amyloid neuropathy associated with Waldenström was first characterized as early as 1973 [5]. A publication in 1976 claimed to be the 14th reported case of Waldenström macroglobulinemia associated with amyloidosis with primary lymph nodal involvement [6]. In this era, it was not possible to determine what form of amyloidosis these patients had consistently because of the lack of uniformly accepted criteria for classifying the subunit protein.

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_14

14.2 Prevalence

In an effort to understand the association between Waldenström and amyloidosis, a study of non-hereditary amyloidosis from the Cancer Registry in Sweden was undertaken for a 13-year period from 1997 to 2010. Among 1400 identified amyloidosis patients, cancer risk was increased for myeloma, non-Hodgkin lymphoma, and squamous cell skin cancer. In looking at patients with light chain amyloidosis, the relative risk of myeloma was 204. Presumably, this was all non-IgM amyloid. Remarkably, in patients with amyloidosis, the relative risk of lymphoplasmacytic lymphoma and Waldenström macroglobulinemia was 51.4, demonstrating the strong association between amyloidosis and the recognition of Waldenström macroglobulinemia [7].

14.3 Diagnosis of Waldenström Macroglobulinemia with Amyloidosis

Failure to recognize amyloidosis as being a significant risk factor in patients with IgM monoclonal proteins often leads to clinically important delays in diagnosis. This failure to recognize is particularly prevalent in patients who present with a peripheral neuropathy and IgM monoclonal protein. These patients are frequently diagnosed as having chronic inflammatory demyelinating polyneuropathy (CIDP) and are often treated empirically with plasma exchange or intravenous immunoglobulin without taking sufficient biopsy samples to exclude the possibility that the IgM-associated neuropathy is due to light chain amyloidosis. The same is true of the kidney. There is a large body of literature on Waldenström and the kidney, suggesting immune complex deposition in the kidney directly related to Waldenström, often leading to empiric trials of corticosteroid therapy without histologic exclusion of amyloid fibrillar deposition in the kidney. One patient reported had amyloidosis that was erroneously diagnosed as benign follicular lymphoma. This patient suffered from hypotension and was found to have minimal enlargement of retroperitoneal lymph nodes with an IgM >5 g/dL. This patient had multiple hospitalizations for infection and progressive disability and ultimately died of progressive heart failure. At postmortem exam, extensive systemic amyloidosis was found with significant cardiac involvement, which was unrecognized premortem. This points out that when a patient with Waldenström macroglobulinemia has heart failure or hypotension, amyloid must be kept in the differential diagnosis and appropriate stains obtained [8].

Attempts to recognize amyloidosis by imaging are ongoing. In a study of ten patients with AL amyloidosis, two were recognized to have associated Waldenström macroglobulinemia. PET scanning showed abnormal results in seven of ten patients, and the FDG update on PET was concordant with the known amyloid organ impairment in six of the seven in which it was positive. The recognition that high FDG update can be present in amyloidosis is important in the differential diagnosis [9].

14.4 Forms of Amyloidosis Associated with Waldenström

One unique feature of Waldenström macroglobulinemia is that it can be associated with both localized as well as systemic amyloidosis. Moreover, the systemic amyloidosis may be of AL (immunoglobulin light chain) type or AA (secondary) type. The syndrome of pulmonary Waldenström is widely recognized. However, when lymphoplasmacytic lymphoma involves the lung, secreted monoclonal IgM can form amyloid in the lung, which appears as nodular deposits, which are not a threat to the patient and do not require intervention or resection. The presence of pulmonary amyloid deposits in the presence of Waldenström macroglobulinemia may not reflect a systemic disorder [10].

Multiple reports of secondary systemic amyloidosis associated with Waldenström exist in the literature. Over 30 years ago, a patient with known Waldenström macroglobulinemia had the amyloid fibril protein sequenced and was proven to be of AA type, confirming it was secondary [11]. A patient with malabsorption, nephrotic syndrome, orthostatic hypotension and Waldenström macroglobulinemia had AA amyloidosis detected immunohistochemically in a rectal biopsy [12]. Although the prevalence of secondary amyloidosis has been falling dramatically over the past 30 years, cases of Waldenström and secondary amyloidosis continue to be reported as recently as 2010 [13] and again in 2013. Remarkably, in this patient, the patient underwent a melphalan-based autologous stem cell transplantation to treat the underlying Waldenström and thereby control the secondary amyloid [14].

Even though localized and secondary amyloidosis must be considered in the differential diagnosis of patients with amyloidosis and Waldenström, the overwhelming majority of patients described have light chain or heavy chain amyloidosis. The first recognition that the immunoglobulin heavy chain M can be amyloidogenic was reported in 2003 in a patient with Waldenström macroglobulinemia with nephrotic syndrome whose renal biopsy only stained for mu heavy chain and no immunoglobulin light chains [15]. Among 17 patients who were found by mass spectroscopy to have heavy chain amyloid or heavy combined with light chain amyloid, 1 had an IgM λ monoclonal protein with M in the amyloid deposits. Laser microdissection and mass spectrometry were invaluable in detecting heavy chain deposits in the amyloid. Interestingly, these patients had less frequent cardiac involvement than light chain amyloidosis. Occasionally, patients with heavy chain amyloid will have a completely normal immunoglobulin free light chain level and ratio, further adding confusion to the diagnosis [16]. In a follow-up study from the same institution, combined light and heavy chain amyloidosis was recognized in 15 patients, 2 of whom had IgM monoclonal proteins. It appears that combined light and heavy chain amyloidosis comprises 6 % of patients who have immunoglobulin-derived amyloid [17].

14.5 Amyloid Evolving from an IgM Monoclonal Gammopathy of Undetermined Significance

Many physicians recognize that patients with a monoclonal gammopathy of undetermined significance must be followed indefinitely because of the development of multiple myeloma. What is much less frequently recognized is that these patients can go on to develop amyloidosis with their IgM monoclonal protein, and there are a number of series that have examined this. In a long-term follow-up study of 430 patients in whom a monoclonal IgM serum protein had been identified, 6 (1 %) developed primary amyloidosis. The median time from the recognition of the M protein until the development of AL ranged from 4 to 9 years. Changes in the size of the IgM or the bone marrow were not reliable predictors of progression [18]. An Italian series investigating 452 patients seen over 26 years with a median follow-up of 49 months recognized one patient to develop immunoglobulin amyloidosis. Patients with asymptomatic IgM monoclonal proteins need indefinite follow-up for the rare possibility that amyloidosis will develop [19]. The Mayo Clinic experience has been regularly updated. Looking at a population-based study of 213 southeast Minnesota patients with IgM monoclonal proteins, amyloidosis was diagnosed in 3, indicating that the relative risk of amyloidosis in patients with an IgM monoclonal protein was 16 times baseline [20]. In patients with strictly defined smoldering Waldenström macroglobulinemia, 48 patients were identified; and during follow-up, 1 patient developed immunoglobulin amyloidosis [21, 22].

14.6 Clinical Characteristics of IgM Amyloidosis

The kidney is a common target of amyloid deposits, but the IgM monoclonal protein can also produce immune complex deposition in the kidney. One patient was reported who had both renal amyloidosis and crescentic glomerulonephritis. At autopsy, crescent formation was present in 80 % of the glomeruli and breaks in the glomerular basement membrane coincided with amyloid deposits [23]. A 67-year-old woman with a monoclonal IgM protein and nephrotic syndrome was found on kidney biopsy to have amyloidosis. Chemotherapy with cyclophosphamide, vincristine, and prednisolone reduced the IgM but did not impact the renal amyloidosis [24]. A 61-year-old patient with Waldenström macroglobulinemia and an IgM of 3244 mg/dL had a renal biopsy that showed diffuse amyloid deposition in the mesangium with classic changes of membranous nephropathy, suggesting that the lambda light chain deposited as amyloid and served as an antigenic stimulus to immune complex formation [25]. A case series of 14 patients with a monoclonal IgM protein and a renal disorder were identified retrospectively. Etiologies included light chain deposition disease, membranoproliferative glomerulonephritis, and amyloidosis in three, two of whom were associated with heavy chain deposits only and one with a lambda light chain deposit. This reflects a wide spectrum of renal lesions and the need for renal biopsy before assuming a direct connection between Waldenström macroglobulinemia and a renal process [26]. In a large series

of patients with Waldenström macroglobulinemia and amyloidosis, the kidney was identified as being the most commonly involved organ and failure to recognize was a major cause of irreversible organ damage. It was suggested that patients with IgM MGUS be serially monitored with urine assessments for albumin and measurements of natriuretic peptides to diagnose early development of cardiac failure [27].

14.7 Nerve

There is often confusion when a patient presents with Waldenström macroglobulinemia and a peripheral neuropathy. It may represent amyloid or CIDP. In a case series where nerve biopsies of amyloid were reviewed, 100 patients were seen and 47 were proven to be AL. Thirty-two of these patients had immunoglobulin subtyping, and nine had an IgM monoclonal protein. All the patients with IgM amyloidosis demonstrated significant inflammation. In most instances, moderate to large collections of inflammatory cells were directly contiguous with the amyloid deposits. Inflammation was more often seen in IgM amyloid than other amyloid subtypes [28, 29].

The presence of anti-myelin-associated glycoprotein (anti-MAG) is considered a diagnostic of CIDP. However, in a case series of 46 patients with IgM amyloidosis in whom 21 had polyneuropathy, 7 of the 20 with known amyloidosis had elevation of the anti-MAG by ELISA and 2 additional had antibodies by Western blot. One of the patients with proven amyloid had the highest anti-MAG titer. It was important to recognize that finding serum anti-MAG antibodies does not exclude the diagnosis of amyloid neuropathy [30]. Two case series on amyloid polyneuropathy with Waldenström macroglobulinemia exist. In the first, 24 neuropathy patients were diagnosed with an M protein over a 27-year period. Three were found to have an IgM monoclonal protein, and it was concluded that light chain amyloid polyneuropathy should be suspected when there is painful neuropathy and weight loss. Approximately one in eight of these patients will have an IgM monoclonal protein or Waldenström macroglobulinemia [31]. The Waldenström Macroglobulinemia Center at the Dana-Farber Cancer Institute reported on the clinical characteristics of IgM peripheral neuropathy with amyloidosis. Among 61 patients tested for amyloid, 13 were positive, 1 of whom was also positive for MAG antibody. Only one patient with amyloid-related neuropathy received intravenous immunoglobulin, and this was without benefit. Improvements in symptomatic neuropathy were more likely with IgM non-amyloid neuropathy vs. IgM amyloid-related neuropathy. Improvement was only reported in 15.4 % of patients [32].

14.8 Lung

The lung is also a common target organ, both in Waldenström macroglobulinemia and in amyloidosis. Macroglobulinemia-associated amyloidosis was reported in both the pulmonary parenchyma and hilar and mediastinal lymph nodes

[33]. Three patients with Waldenström macroglobulinemia and extensive deposition in the lung were reported. The deposits were intraalveolar and extensive [34].

14.9 Soft Tissue

Amyloid arthropathy has also been reported in Waldenström macroglobulinemia. Arthropathy is most commonly seen in dialysis-associated amyloid, but a patient with proven Waldenström macroglobulinemia presented with bilateral symmetric polyarthritis who had a synovial membrane biopsy that was Congo red positive. Chemotherapy effectively alleviated the joint manifestations [35]. Secondly, a patient with IgM kappa monoclonal gammopathy who had large lymph nodes and received two courses of vincristine, doxorubicin, and dexamethasone followed by high-dose chemotherapy and stem cell transplantation was in hematologic remission 4 years posttransplant with marked reduction in the size of the amyloid-laden lymph nodes. This was proof that amyloid deposits can regress from the tissue if chemotherapy succeeds in reducing the precursor amyloidogenic light chain [36].

14.10 Case Series of IgM-Associated Amyloidosis (Table 14.1)

We first reported on 50 patients with an IgM monoclonal gammopathy and biopsy-proven amyloidosis. The ratio of men to women was 64:36, and the age range was 43–93 years. The percentages of patients presenting with cardiac, renal, hepatic, and pulmonary amyloid were 44 %, 32 %, 14 %, and 10 %, respectively. A 10 % prevalence of pulmonary amyloid is remarkably high. Forty-two percent of the patients had an M protein >1.5 g/dL, and only 12 % had an M component >3 g/dL. The diagnosis was generally established noninvasively with deposits found in subcutaneous fat, the rectum, and the bone marrow in 84 %, 72 %, and 50 %, respectively. The bone marrow biopsy was diagnostic of Waldenström macroglobulinemia in only 10. An additional 11 showed a clonal lymphoproliferative disorder. The median survival of this group was 24.6 months. Fifty-three percent of deaths were due to cardiac amyloid, 12 % due to respiratory failure, and only 7 % due to macroglobulinemia. Of the patients presenting with cardiomyopathy, the median survival was 11.1 months with only two surviving over 5 years. There were only eight 5-year survivors in the group [37, 38].

A multicenter French study identified 72 patients with IgM-associated amyloidosis. AL was found in 64, localized was found in 5, and AA in 3. This group also noted the unusually high pattern of lymph node amyloid (31 %) and lung amyloid (17 %). The hematologic response rate in this group was only 37 % and organ response only 21 %. The hematologic response rate to stem cell transplantation was 100 %, complete in 75 %, and 75 % organ responses. Purine analogs with rituximab produced hematologic response rates in 73 %. The prognostic factors for survival were serum albumin and the presence of cardiac involvement [39]. The National Amyloidosis Centre of Great Britain published 103 consecutive IgM-associated

Table 14.1 Case series of IgM amyloidosis

Institution	#	M/F	Cardiac	Renal	Hepatic	Pulmonary	Nodal	Survival	Reference
Mayo	50	32/18	44	32	14	10		246	[38]
French	72	47/25	33	50	25	15	31	47	[39]
British	103	65/38	35	53	14	3	21	49	[40]
Pavia	60	36/24	53	70	17	10	25	76	[42]
Bari	4	3/1	y	y	y	y	y	10 months to 2 years	Abstract
Boston U.	50	31/19	37	59					Abstract

amyloidosis patients seen over an 18-year period. They found renal, cardiac, and lymph node amyloid in 53 %, 35 %, and 21 % of patients, respectively, and two or more organs involved in 54 %. Eighty-seven percent showed lymphoplasmacytic lymphoma in the marrow. The median IgM monoclonal protein level was 0.8 g/dL, and the free light chain ratio was abnormal in 77 of 87 (88 %). An involved free light chain >10 mg/dL was seen in only 31 % of patients. Thirty-two percent achieved a partial hematologic response to therapy with a greater response to combination rather than single-agent alkylator. Four achieved organ responses. None of the patients with lymph node involvement showed nodal improvement, and median overall survival was 49 months [40].

The National Italian Amyloidosis Center found 60 patients representing 7 % of all amyloidosis patients with an IgM monoclonal protein. These patients had a significantly older age (67 years vs. 62 years) and had a high frequency of lymph node involvement (25 %). The extent of renal involvement was also less with a median urinary protein of only 1.2 g. There was also less prevalent cardiac involvement. Involved free light chain concentrations were lower than in non-IgM amyloid (6.3 vs. 18.2 mg/dL). Survival was predicted by serum albumin and NT-proBNP. Fourteen patients who received melphalan and dexamethasone showed a 64 % hematologic (29 % complete) and a 43 % organ response rate. In this cohort, organ dysfunction was less advanced, and melphalan and dexamethasone were effective [41, 42]. In an Italian series of 121 patients with Waldenström macroglobulinemia, 4 were found to have amyloidosis (3.3 %). There were three men and one woman, age ranging 46–82 years with heart, kidney, liver, lymph node, and pulmonary involvement. Two patients were treated with chlorambucil and prednisone, two others melphalan and prednisone for a median of six cycles. Survival ranged from 10 months to 2 years [43].

The Boston amyloidosis research center reported their results and found 94 patients with IgM-related amyloidosis constituting 6 % of their total population of amyloidosis patients. Complete treatment data was available in 50. The median age was 65 years, 62 % men. The kidney, heart, and GI tract involvement was seen in 59 %, 37 %, and 22 %, respectively. The mean IgM was 1049 mg/dL. Sixty-eight percent were lambda light chain amyloid. The highest hematologic response rate was seen with high-dose melphalan and stem cell transplantation 100 % ($n = 10$). Alkylating agents produced responses in 73 % (16/22), bortezomib 70 % (7/10), rituximab 69 % (9/13), and immunomodulatory drugs 50 % (2/4). There was no association between a response to treatment and types of organs involved, gender, or age at diagnosis [44]. A summary of the case series is given in Table 14.1.

14.11 Therapy

14.11.1 Stem Cell Transplantation

We reviewed our results in the transplantation of patients with IgM-associated amyloidosis. In our first report, we reported on 12 patients, 1 of whom died related to therapy. The overall hematologic response rate was 89 %, and the organ response rate was 67 %. Our results were similar to those of other forms of immunoglobulin-derived amyloidosis [45].

We updated our results 3 years later on 17 patients. These patients were older, had a higher incidence of amyloid neuropathy, and a lower instance of cardiac involvement based on cardiac biomarkers. Median survival had not been reached [46]. By 2011, we had transplanted 22 such patients. Overall survival was not different from non-IgM-amyloidosis, and we confirmed the lower level of involved immunoglobulin free light chains previously reported. A Japanese group reported a patient with IgM amyloidosis who received high-dose melphalan and autologous stem cell support with a complete hematologic remission and improvement in nephrotic syndrome. No studies exist to help define the appropriate conditioning regimen for these patients, whether it be melphalan or carmustine, etoposide, cytarabine, and melphalan [47].

14.11.2 Conventional Dose Chemotherapy

Given the high response rate using rituximab, bortezomib, and dexamethasone in the treatment of Waldenström macroglobulinemia, it was logical to use this same triplet combination in patients with IgM-associated amyloidosis. Ten patients with IgM amyloidosis received this regimen. A hematologic response was achieved in 78 %, including three refractory to prior rituximab alone. Grade 3 toxicity or greater was seen in three of the patients. This is a regimen that warrants further exploration [48]. Bendamustine has been used in the treatment of amyloidosis. Thirty-six patients from two European referral centers were treated with bendamustine and prednisone. The patients received 28-day cycles of bendamustine ranging from 60 to 100 mg/day, day 1 and 2 of each cycle. Ten of the 36 patients (28 %) had IgM monoclonal protein. Of these ten, eight received rituximab with bendamustine and prednisone. By intention to treat, 47 % achieved a hematologic response, complete in 3 %. Among the eight subjects with IgM amyloidosis receiving bendamustine and prednisone with rituximab, six (75 %) responded with one complete response. The 3-year survival was 65 %. Response to bendamustine conferred a significant survival advantage [49]. A series comprising 267 patients with IgM-related amyloidosis was reported in 2012. Sixty-four percent of patients fulfilled criteria for lymphoplasmacytic lymphoma (Waldenström). Lymph nodal amyloid was found in 18 %, renal involvement in 64 %, and cardiac involvement in 42 %. The free light chain ratio was abnormal in 72 %. One hundred thirty-five patients were treated, 124 evaluable for response. The overall hematologic response rate was 28 % with a

better outcome noted for frontline high-dose melphalan, cytoxan, vincristine, prednisone and cyclophosphamide, fludarabine, and rituximab. The median overall survival was 48 months. A difference between involved and uninvolved free light chain >18 mg/dL was predictive of a poor outcome (48 vs. 29 months, respectively). Mayo cardiac stage predicted outcomes with the three stages having survivals of 74, 24, and 10 months, respectively. A survival advantage was seen for hematologic responders. A survival advantage was also seen for high-dose melphalan, bortezomib combinations, and fludarabine, cyclophosphamide, and rituximab [50].

A subset of patients over the age of 75 years was reported. Two patients had IgM amyloidosis and were treated with rituximab, cyclophosphamide, vincristine, and prednisone. A third patient received rituximab, cyclophosphamide, and dexamethasone for IgM-associated amyloid. The median overall survival for the entire cohort was 10.7 months. Hematologic response VGPR or better resulted in an improved overall survival [51].

14.12 Conclusion

IgM amyloidosis represents approximately 6 % of all amyloidosis patients and is a rare complication of Waldenström macroglobulinemia. Failure to recognize this complication of Waldenström is a major cause of morbidity. These patients appear to have an unusually high incidence of lymph node and lung involvement. High-dose therapy with stem cell reconstitution-, bortezomib-, and alkylator-based therapies all appear to have activity in this disease.

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The Bing-Neel Syndrome

15

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15.1 Introduction

The care of patients with extranodal non-Hodgkin's lymphomas (NHL) and Waldenström's macroglobulinemia (WM) falls to the hematologist/oncologist who is seldom prepared for the central nervous system (CNS) complications of these diseases. The risk factors for CNS involvement in WM are unknown, but insights may be gleaned from those of diffuse large B-cell lymphoma (DLBCL). CNS involvement in DLBCL correlates with elevated serum levels of LDH, involvement of more than one extranodal site of disease, and possibly retroperitoneal lymph node involvement, hypoalbuminemia, and advanced age [1]. When lymphomatous invasion occurs in the subarachnoid space, it usually coexists with relapse in the brain or systemic sites [2]. WM is characterized by elevated levels of circulating pentameric monoclonal immunoglobulin (Ig) M with resulting CNS complications mediated by hyperviscosity and direct malignant cell invasion [3]. In WM, IgM antibody activity against peripheral nerve constituents, such as

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_15

myelin-associated glycoprotein (MAG), can produce a demyelinating peripheral neuropathy [3]. Other peripheral nervous system (PNS) complications include IgM deposition without antibody activity, causing an axonal neuropathy; accumulation of IgM cryoglobulin; amyloid deposition; and direct malignant cell infiltration of nerve structures [3]. While up to one-third of WM patients can have PNS involvement [3], WM lymphoplasmacytic (LPC) cell invasion of dura, leptomeninges, subarachnoid space, and brain parenchyma is rare [4]. Involvement of these CNS structures constitutes the *Bing-Neel syndrome* (BNS) [5]. Although BNS was first reported over 70 years ago [5], half a decade before Jan Waldenström's seminal paper, there remains no consensus as to diagnostic criteria, staging evaluation, or therapy. To aid the International Waldenstrom's Macroglobulinemia Foundation (IWMF) and hematologists, we have drawn from our experience and 26 examples in the literature. We separate WM patients with CNS involvement into two groups: those with LPC cells in cerebrospinal fluid (CSF) ($\geq$5 LPC cells/mm^3) and/or LPC cells on biopsy of brain parenchyma, leptomeninges, or dura and those without apparent CSF LPC cells ($<$5 LPC cells/mm^3) [6, 7]. Literature cases were accrued from published reports as previously described [6], excluding cases of cerebrovascular accidents, transformation events, infections, disorders of peripheral nerve or root, and those who lacked MRI data.

15.2 Background

This chapter aims to provide WM caregivers with a base for identification of the BNS. The International Prognostic Scoring System (IPSS) for WM [8] provides no insight into PNS and CNS complications of WM, and yet these complications often necessitate immediate therapy. As IPSS scores do not presage BNS, these CNS complications should be appended to this prognostic system to provide additional indications for therapy. Medical oncologists tend to apply the term "Bing-Neel syndrome" to all neurologic conditions in WM patients, even in the absence of evidence of direct LPC infiltration or paraneoplastic effects in the CNS. We thus recommend the *exclusion* of cases with masses of brain parenchyma [4, 9], WM with malignant transformation ([10–13], Case IV [9], Cases I–II [14]), hyperviscosity or brain ischemia from pentameric IgM deposits in blood vessels [15], and those presenting as isolated IgM-related peripheral neuropathies [16].

15.3 History

In 1936, Jens Bing and Axel Neel reported two patients with CNS difficulties, assumed to be related to a "universal toxic effect (...) and not to a local effect" in the absence of "signs of myeloma (...) found either by clinical examination, including X-ray photography (...) or by autopsy" [5]. The index case was a 36 year-old woman who presented with fatigue and loss of appetite, followed by pain affecting her back, ankle, and shoulders. Sedimentation rate was as high as

168 mm/h and she had proteinuria. Serum protein was 10.8 % and globulin 7.1 %. Three months after symptom onset, she "noticed great loss of power in arms, legs and whole body, (. . .) vomiting and severe headaches, (. . .) the voice has been a whisper." She then became aphonic. On neurologic exam, tone was increased but reflexes were normal and sensation preserved. CSF contained one third lymphocytes and was notable for elevated protein (150–160 mg/dl). She succumbed to pneumonia. Postmortem examination revealed "no sign of myeloma" but evidence of a polyradiculitis, myelitis, and encephalitis. In the cervical spinal cord, "a number of anterior horn cells seemed to be missing" and there was "proliferation of (. . .) macro- and microglia." Plasma cells were found along the capillaries and small vessels of the medulla, spinal cord, and cauda equina. In addition, Gram-positive bacteria were identified penetrating the walls of blood vessels.

The second patient, a 59 year-old woman, developed dizziness, tingling, and loss of finger sensation after 6 months of weight loss, fatigue, and vomiting. She was anemic, sedimentation rate was 156 mm/h, and serum protein and globulin were 6.7 % and 4.6 %, respectively. X-rays were not suggestive of myeloma. The lymph glands and spleen were hyperplastic. A polyradiculitis and toxi-infectious "myeloencephalopathia" were diagnosed on the basis of degenerative changes in the spinal cord and roots, anterior horn, and ganglion cells, medulla, and pons. One single medullary vessel contained a round cell infiltrate. In neither case was bone marrow examined extensively.

Eleven months later, the authors reported three additional patients [17]. Cases 1 and 2 had multiple myeloma. Case 3 had "fatigue of the extremities"; neurologic exam revealed upper and lower extremity weakness, reduced reflexes, and upgoing toes. ESR was 137 mm/h. CSF contained "30/3 cells" and elevated protein of 180–200 mg/dl. On postmortem examination, LPC infiltrates were seen in the perivascular space of nerve roots and meninges as well as within and around the lumen of cortical blood vessels. In addition, degenerative changes were found in the spinal cord tracts, medulla, and nerve roots.

15.4 Manifestations of BNS

We identified 26 cases of BNS in the literature from 1992 and 2010, supplemented by 10 cases ("Cases 1–10") from our experience (Table 15.1). Males predominated (23 males vs. 13 females) with median age of symptom onset at 65 years (range 51–82).

15.4.1 The Setting of BNS

Twenty-three (64 %) patients had received chemotherapy (CTH) with rituximab (RTX) (22 %), either alone or with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) or COP (cyclophosphamide, vincristine, prednisone) as treatment for WM. No CTH combination appeared to predispose to or prevent

Table 15.1 Summary of BNS cases at our institution

Case number	Age at BNS, sex	WM: prior therapy	Interval: WM to BNS (months)	WM stable at BNS onset?[a]	BNS features	Serum IgM (g/l) at BNS onset	CSF or pathology[b]	MRI	BNS treatment	BNS response
LPC cells present in CSF and/or on biopsy										
Case 1	62, M	RTX, Bor + RTX	101	No	Apneic episode, quadriplegia	IgM: 2.3	CSF: 0 cells Biopsy: focal LPC cells, possibly monoclonal IgM λ+	Enhancing dural masses: brain and C2–C3 level	XRT	CR
Case 2	70, M	Chlor, RTX; RTX+flud; CPA, Bor, Dex; XRT	138	No	Headache	IgM: 7.1	CSF: 0 cells Biopsy: small lymphoid cells, monoclonal IgM κ+, CD79a+	Diffuse cranial dural enhancement	R-CHOP, Dex; XRT	PR
Case 3	58, M	Ph, RTX	0[c]	No	Paresthesia, pain hands/feet	IgM: 14.8	CSF: pleocytosis	Normal MRI of cord	RTX, Ph	PR
Case 4	79, M	Chlor, COP, RTX, R-COP	84	No	Personality and memory changes, confusion	IgM: 0.8	CSF: LPC pleocytosis, CD19+/ monoclonal IgM κ+	White matter lesions	XRT, steroids, IT MTX	PD: died in 5 months
Case 5	67, F	None	0[c]	No	Diplopia, visual field deficit, gait ataxia	IgM: 8	CSF: 94 l/mm^3; normal protein, glucose	Leptomeningeal enhancement of the brain/cord, sulcal and optic nerve T2/FLAIR	XRT, MTX; RTX, Clad, ARA-C	PR: died in 2 months

Case 6	72, M	ARA-C, RTX, R-CHOP	96	No	Memory problems, leg pain, urinary incontinence	N/a	CSF: suspicious LPC cells Biopsy: LPC cells	Enhancement of meninges (T10 to cauda equina); brain and CNs	XRT, IT ARA-C	PR
LPC cells absent in CSF and/or on biopsy										
Case 7	62, M	RTX, Ph	0[c]	No	Seizure, depression, visual hallucinations, gait ataxia	IgM: 6.1	CSF: 0 cells	T2/FLAIR in the brain/cerebellum/ midbrain white matter	RTX, flud, Ph	PR
Case 8	65, F	Sild	Between 137 and 148	No	Homonymous hemianopia	IgM: 19.9	CSF: 0 cells	Large enhanced optic nerves, chiasm and tracts; T2/FLAIR in globus pallidus/ midbrain	Steroids, MTX, RTX	PR
Case 9	59, M	None	0[c]	No	CN XI palsy, facial numbness	IgM: 1.3	CSF: 0 cells	Normal brain	RTX, steroids, R-COP	None
Case 10	55, F	Clad; RTX +Ph; COP	72	N/a	Gait ataxia, behavior, sleep change	N/a	CSF: 0 cells Biopsy: no LPC cells, perivascular IgM+ cells	Enhancement: dura/ leptomeninges of cervical cord; T2/FLAIR: cord/ medulla/brain	Steroids, Ph	N/a

Bor bortezomib; *Chlor* chlorambucil; *Clad* cladribine; *CN* cranial nerve; *ARA-C* cytarabine; *CPA* cyclophosphamide; *Dex* dexamethasone; *Flud* fludarabine; *IT* intrathecal; *MTX* methotrexate; *L* lymphocytes; *Ph* plasmapheresis; *XRT* radiation therapy; *RTX* rituximab; *R-COP* rituximab + cyclophosphamide, vincristine, prednisone; *R-CHOP* rituximab + cyclophosphamide, doxorubicin, vincristine, prednisone; *Sild* sildenafil

[a]Defined as: normal or stable IgM levels, regression of WM on BM biopsy, and/or explicit statement by treating physicians

[b]Includes flow cytometry of CSF and/or immunohistochemistry of CNS tissue

[c]0 months: denotes BNS as initial presenting feature of systemic WM

progression to BNS. Nine patients, including six with BNS diagnosed prior to WM, received either no treatment or had insufficient therapy data. Six patients underwent plasmapheresis in addition to CTH (Cases 3, 7, 10, [18–20]). Twenty-eight percent ($n = 10$) of patients had stable WM at BNS onset but almost two-thirds of BNS ($n = 23$) occurred in the setting of rising or elevated levels of plasma IgM, clinical and/or radiographic deterioration, and/or increased LPC marrow infiltrates (Table 15.2). Most commonly, serum viscosity was normal. The diagnosis of WM anteceded neurologic symptoms by a median of 57 months (range 0–180). Although most cases of BNS appeared at least 1 year after WM diagnosis, one-fifth of patients had BNS onset prior to WM.

15.4.2 Clinical, Imaging, and Histopathologic Features (Table 15.2)

The most common symptom of BNS was cognitive problems (39 %; $n = 14$), including memory deficits, personality changes, confusion, hallucinations, and depression. Notably, these changes occurred in the absence of hyperviscosity, thrombocytopenia, or cryoglobulinemia. For example, Case 4 became confused and exhibited memory difficulties and belligerent personality in the setting of progressive WM, and Case 7's BNS was heralded by depression, visual hallucinations, and micrographia. Progression was often rapid over months. Case 10 developed cognitive decline that progressed to hypersomnolence and a nearly persistent vegetative state over 3 months. Twenty-eight percent ($n = 10$) of symptoms were related to spinal cord, nerve root, or cranial nerve problems. Less common were headaches (19 %), seizures (14 %), cerebellar syndromes, and basal ganglia difficulties [18, 20]. Concomitant PNS involvement was rare (11 %; $n = 4$).

MRI findings of BNS included foci of T2/FLAIR hyperintensity and/or abnormal contrast enhancement in 30 of 36 cases. Most patients had areas of abnormal gadolinium enhancement in the brain, spinal cord, or both, usually in the presence of concomitant CSF pleocytosis. In half of patients, there was no correlation between location of MRI abnormalities and clinical findings. MRI was reportedly normal in 17 % ($n = 6$) of patients but this included four who lacked contrast-enhanced studies [20–23].

The diagnosis of BNS was based on biopsy in 14 patients (39 %), which targeted brain parenchyma (Case 10, [18, 24–28]), dura (Cases 1–2, [29]), meninges (Case 6, [30]), or orbit [31, 32]. Biopsies corresponded to areas of either contrast enhancement or T2/FLAIR hyperintensity when no enhancement was seen on MRI. Histologic exam revealed the presence of LPC cells staining for IgM, with light chain restriction in seven subjects (Cases 1–2, [24, 27, 28, 30, 32]).

CSF studies were available in 31 patients (86 %). Twenty-one of these contained $\geq$5 leukocytes/mm^3, with immunophenotypic studies performed in 9 and monoclonal kappa light chain restriction confirmed in 5 (Case 4, [21, 23, 33, 34]). IgH gene rearrangement was not studied in any patient. Ten patients' CSF contained fewer than 5 leukocytes/mm^3. Four of these had LPC infiltrates on biopsy or at autopsy (Cases 1, 2, [26, 28]).

Table 15.2 Characteristics of BNS

		$n = 36$	%	Our institution (case number)	Literature
WM stability at BNS onset	Yes	10	28		[18, 21, 22, 25, 28, 34, 35 (Patients 1–3), 36]
	No	23	64	1–9	[19, 20, 23, 24, 26, 27, 29–31, 33, 37, 45–47]
	N/a	3	8	10	[32, 40]
Clinical presentation	Seizure	5	14	7	[22, 25, 30, 36]
	Headache	7	19	2	[21, 25, 29, 34, 40, 45]
	Cognitive	14	39	1, 4, 6–7, 10	[19, 21–24, 27, 28, 45, 46]
	Cranial nerve	10	28	5, 7–9	[21, 26, 31, 32, 34, 35 (Patient 3)]
	Spinal cord/nerve root	10	28	1, 3, 5–6, 10	[33, 35 (Patients 1–2), 37, 47]
	Cerebellar	6	17	7	[19, 26, 36, 45, 46]
	Basal ganglia	2	6		[18, 20]
Biopsy from dura, leptomeninges, and/or parenchyma	LPC cells present	13	36	1–2, 6	[18, 24–32]
	LPC cells absent	1	3	10	
	N/a	22	61	3–5, 7–9	[19–23, 33, 34, 35 (Patients 1–3), 37, 40, 45–47]
CSF studies	Leukocytosis	21	58	3–6	[19, 21–23, 25, 29, 32–34, 35 (Patients 1–3), 37, 45–47]
	Flow cytometry	9	25	4	[21, 23, 25, 32–34, 36, 47]
	Confirmed Ig light chain restriction	5	14	4	[21, 23, 33, 34]
	No leukocytosis	10	28	1–2, 7–10	[20, 26, 28, 40]
	N/a	5	14		[18, 24, 27, 30, 31]
MRI	Enhancing lesions	23	64	1–2, 5–6, 8	[18, 24, 25, 27–32, 33–35 (Patients 1–3), 37, 40, 46, 47]
	Brain	12	33	2	[18, 24, 25, 27–31, 35 (Patient 3), 40, 46]
	Spinal cord	7	19	6	[33–35 (Patients 1–2), 37, 47]
	Brain and cord	4	11	1, 5, 8	[32]
	Normal MRI	6	17	3, 9	[20–23]

15.4.3 Treatment of BNS (Table 15.3)

No WM therapy heralded the appearance of BNS. Three quarters of WM patients had received systemic CTH and one-third intrathecal (IT) therapy. Neuraxis irradiation was given to 42 % ($n = 15$) and plasmapheresis to 14 % ($n = 5$). Two patients underwent stem cell transplantation after failing three cycles of systemic CHOP and

Table 15.3 Treatment of BNS

		$n = 36$	%	Our institution (case number)	Literature
Treatment regimen	XRT	15	42	1–2, 4–6	[18, 24, 27, 29, 31, 32, 34, 35 (Patients 1–2), 45]
	Systemic CTH	27	75	2–5, 7–10	[18, 19, 23, 25, 26, 29–35 (Patients 1–3), 36, 37, 45–47]
	IT CTH	12	33	4, 6	[21, 23, 32, 33, 35 (Patients 1–3), 37, 47]
	Plasmapheresis	5	14	3, 7, 10	[30, 37]
	BM Tx	2	6		[23, 32]
	Other	5	14	9	[20, 25, 32, 40]
	No Rx; n/a	2	6		[22, 28]
Treatment response	CR	7	19	1	[18, 21, 24, 25, 30, 32]
	PR	20	56	2–3, 5–8	[20, 23, 27, 29, 31, 33–35 (Patients 1–3), 36, 37, 45, 47]
	None/ progressive	5	14	4, 9	[26, 28, 46]
	Awaiting; n/a	4	11	10	[19, 22, 40]
	CR on imaging	9	25	1	[18, 24, 25, 30, 32, 35 (Patient 2), 37]
	Death	10	28	2, 5	[21, 26, 28, 34, 35 (Patients 1, 3), 45, 46]

IT methotrexate (MTX) [23] and to consolidate a major response to steroids, MTX, and focal XRT [32]. Hematologists had a tendency to treat both the neuraxis as well as systemic WM when BNS presented in the setting of stable or progressive WM. Treatment included systemic CTH (in 70 % and 78 % with stable and progressive WM, respectively) and neuraxis radiation (in 40 % and 43 % with stable and progressive WM, respectively). There was a predilection for rituximab (RTX) administration to those with progressive WM (35 % vs. 10 % in stable patients). In the setting of BNS and progressive WM, therapies included CHOP ($n = 3$), COP ($n = 2$), chlorambucil ($n = 2$), vindesine ($n = 1$), IT CHT ($n = 6$), plasmapheresis ($n = 4$), and stem cell transplantation ($n = 1$). BNS patients with stable WM received ifosfamide ($n = 3$), vepeside ($n = 3$), procarbazine ($n = 1$), vincristine ($n = 1$), and IT CTH ($n = 5$).

Patients with LPC infiltrates of the neuraxis based on $\geq$5 leukocytes/mm^3 in CSF received XRT (50 %, $n = 15$), IT CTH (40 %, $n = 12$), bone marrow transplantation (7 %, $n = 2$), and systemic high-dose MTX (23 %, $n = 7$). In this group, two-thirds ($n = 14$) achieved a partial response (PR) and seven a complete response (CR) after XRT, systemic MTX, IT MTX, or a combination thereof. Patients lacking cells in CSF were treated with systemic CTH, plasmapheresis, and symptomatic therapies such as carbidopa/levodopa for parkinsonian features [20].

Neurologic symptoms and CSF cell counts improved in 27 BNS patients, including 7 with CR. Six had resolution of MRI abnormalities after XRT, systemic CTH, IT MTX and cytarabine, and stem cell transplantation (Cases 1, [18, 24, 25, 30, 32]). Three patients with MRI resolution had residual clinical difficulties and were classified as partial responders (Patient 2 [35–37]). Response to treatment lasted a median of 18 months (range 2–120 months). Duration of response for complete responders was 21 months vs. 18 months for partial responders. Nine patients succumbed after a median of 2.5 months (range 1–26 months) following PR or progressive disease (PD).

Recently, two retrospective studies analyzed 34 and 44 BNS patients; response rate was respectively 68 % and 70 %, with heterogeneous treatment regimens [38, 39]. Overall survival rate was 59 % at 3 years and 71 % at 5 years, respectively.

15.4.4 Specialized Forms of BNS That Suggest Antibody-Mediated Disease

Although the majority (83 %, $n = 30$) of BNS appeared to be mediated by direct malignant cell invasion of the CNS, a small subset of patients did not have the evidence of LPC cells in CSF (Cases 7–10, [20, 40]). Whether these cases reflected covert infiltrates of LPC cells, which were not sampled or suppressed by therapy, is unclear. It is conceivable that WM-associated IgM antibody binds to CNS myelin in a fashion analogous to selective binding to myelin of peripheral nerves [41]. Similar selective binding of WM IgM underlies Schnitzler syndrome, WM protein-losing enteropathy, and POEMS syndrome [3]. We have identified two patients with optic nerve involvement by WM in a fashion that would be antibody-mediated rather than cell-mediated (unpublished data). In addition, two cases in the literature support a possible antibody-mediated CNS disease process [20, 42]. One patient with known WM developed a demyelinating sensorimotor polyneuropathy in the setting of elevated titers to MAG, followed by emergence of a Parkinsonian syndrome 3 years later [20]. CSF did not show pleocytosis. MRI brain was normal but PET imaging revealed decreased FDOPA uptake in the bilateral caudate and putamen and glucose hypometabolism in the lentiform nuclei, arguing against idiopathic Parkinson disease. Scheithauer et al. [42] reported a case of WM with progressive left-sided tremors, in the absence of CSF pleocytosis. Postmortem brain examination revealed focal areas of acellular myelin loss, with concomitant infiltration by focal LPC cells and positive staining for IgM kappa light chains.

15.5 A View to the Future of BNS

Hematologists and oncologists should be knowledgeable to the clinical features of BNS. Although BNS occurred in the setting of progressive WM in most patients (64 %, $n = 23$), a subset (28 %, $n = 10$) developed neuraxis involvement during stable or remitting WM. As with DLBCL prognostic indices, further analyses will be needed to identify BNS risk factors. Thirty of 36 patients had areas of enhancement and/or T2/FLAIR hyperintensity on MRI. Contrast-enhancing lesions were seen in the vast majority (21 of 23) of patients with ≥ 5 LMP cells/mm^3 in CSF, suggesting breakdown of the blood-brain barrier secondary to direct invasion of malignant cells. None of these lesions had signal characteristics consistent with ischemia. Clinicians may benefit from use of diffusion-weighted imaging (DWI) sequences, which are sensitive for acute and subacute brain ischemia, to differentiate ischemia/infarction from true LPC cellular invasion. Similarly, there is future utility to use of advanced MRI techniques such as measurement of cerebral blood volume, cerebral blood flow, and dynamic contrast-enhanced MR imaging to assess changes in permeability of the blood-brain barrier. Imaging findings of patients without CSF pleocytosis, on the other hand, were most remarkable for focal areas of T2/FLAIR hyperintensity. MRI may thus be helpful in distinguishing between cell- and antibody-mediated forms of BNS.

Therapy with XRT, systemic MTX, and IT MTX provided complete MRI response in nine patients with LPC CSF cells, with corresponding clinical remission and/or normalization of serum IgM levels in six subjects. Given our experience with primary CNS lymphoma, there is a predilection to provision of systemic MTX to BNS patients with LPC cells. However, BNS patients without LPC cells might benefit from reduction of circulating IgM with plasmapheresis and systemic cytoreductive WM therapy as in Case 7 in whom plasmapheresis and RTX led to a PR. The activity of ibrutinib in WM suggests a possible role in the cellular variant of BNS although the penetration of this agent into the neuraxis is unknown.

We suggest neurologic additions to the IPSS for WM, particularly for patients with new-onset altered mental status, memory and behavioral changes, headache, seizures, or focal neurologic deficits [7]. These additions should include CSF studies (flow cytometry, cytology, immunohistochemistry) along with contrast-enhanced MR imaging of the brain and entire spine. Excellent examples exist in the literature of patients with careful evaluation of LPC cells in the CNS [21, 25, 29]. In the setting of CSF LPC cells, clinicians should obtain CSF for polymerase chain reaction (PCR) analysis for clonal IgH gene rearrangement or in situ hybridization for kappa and lambda Ig light chains on CNS tissue. Without LPC cells, CSF studies should include molecular studies for clonal B cells and consideration given to a brain biopsy. Special attention is warranted to advanced MRI studies and demonstration of IgM deposits in the brain [42]. On a molecular level, use of allele-specific PCR for the L265P mutation in the MYD88 gene may be a potential tool for detection of WM [43], and the diagnostic of BNS could be improved by the detection of this mutation in CSF specimens, as recently published [44]. To these staging criteria should be added standardized assays of performance

status and response to treatment, including sequential blood tests, CSF studies, and contrast-enhanced MRI [7]. Assessment of these parameters in future large-scale studies will improve risk stratification of patients.

Disclosure RF is a consultant radiologist, shareholder, and member of the medical and quality assurance committee for Real Time Medical Inc. RF has also acted as a consultant for Biogen Idec in the past and as a speaker at a lunch and learn session titled "Dual-Energy CT Applications in Neuroradiology and Head and Neck Imaging" sponsored by GE Healthcare at the 27th Annual Meeting of the Eastern Neuroradiological Society in September 2015.

EPH is a consultant for Flatiron and Intervention Insights. FHH has declared financial interests in Cascent and Tavec Pharmaceuticals. All remaining authors have declared no conflicts of interest.

Authorship and Acknowledgments All authors reviewed and approved the manuscript. The authors thank Dr. Russell J. H. Ryan for hematopathologic expertise and Julien Colbert for his help in organizing the clinical data.

Funding No funding to be declared.

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Unusual Manifestations of IgM Monoclonal Gammopathies

16

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In addition to symptoms directly caused by the clonal cells, such as bone marrow infiltration, lymphadenopathy, and splenomegaly, immunoglobulin M (IgM)-secreting monoclonal B-cell proliferations can be featured by symptoms due to the monoclonal IgM, either by deposition of all or part of its molecule or because of a symptomatic autoantibody activity. Some of these IgM-related manifestations such as hyperviscosity can only occur in high tumor mass proliferation. The others can also complicate an overt IgM-secreting proliferation but most often occur in the context of an indolent proliferation or even of a MGUS. This situation has led to the introduction of the concept of "dangerous" small B-cell clone [1] and of monoclonal gammopathy of renal significance (MGRS) [2].

Autoantibody activities of the monoclonal IgM are involved in several manifestations of IgM gammopathies such as type II cryoglobulinemia (CG), cold agglutinin disease, and peripheral neuropathies which are described in other chapters of this book. The deposition mechanism is mainly illustrated by AL amyloidosis that is treated in the first part of this chapter. The two other parts are devoted to kidney and skin manifestations of IgM gammopathies, which are not uncommon and could in some cases involve other mechanisms than deposition or autoantibody activity.

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_16

16.1 AL Amyloidosis in IgM Gammopathies

In AL amyloidosis, a monoclonal light chain (LC) misfolds, aggregates, and deposits in tissues, which causes organ dysfunction and ultimately death if left untreated [3]. An IgM clone is responsible for the disease in approximately 4 % to 7 % of cases [4–8]. Immunoglobulin M (IgM)-related amyloidosis remains a rare and little-known complication of monoclonal IgM-associated disorders, and it may be easily missed. Older patients with IgM-AL amyloidosis are being increasingly recognized posing particular challenges in management [9]. In a large European collaborative study comprising 263 patients with IgM-related AL amyloidosis, the underlying lymphoplasmacytic proliferative disorder was more frequently the Waldenström macroglobulinemia (WM)/lymphoplasmacytic lymphoma (LPL) (40 %) followed by other non-Hodgkin lymphomas (NHLs) (23 %), IgM MGUS (20 %), and patients with predominant plasma cell bone marrow infiltration (8 %). In 27 % of patients, the underlying lymphoplasmacytic disorder was uncertain [10]. It is important to note that although infrequently (4 %), WM and other IgM-related NHL can be associated with reactive AA amyloidosis, not AL amyloidosis, with important practical implications [7]. Although patients with IgM-AL amyloidosis share many of the major characteristics of other patients with this disease, this condition presents distinct features. Amyloid deposition affects more frequently the lungs (up to 22 %) and lymph nodes (20–31 %) as well as peripheral or autonomic nervous system (up to 38 %) [4–8]. Moreover, patients with IgM-AL amyloidosis have a lower concentration of circulating amyloidogenic free LCs (FLCs), and only two-thirds had evaluable dFLC $>$ 50 mg/L [4, 6, 8, 9], which may render difficult the evaluation of response to therapy and the participation in trials. The heart is less frequently and less severely involved than in non-IgM-AL amyloidosis, with the concentration of the cardiac biomarkers N-terminal pro-B-type natriuretic peptide (NT-proBNP) and troponins significantly lower in IgM-AL amyloidosis [4, 6].

In recent series, the median survival of patients with AL amyloidosis and an underlying IgM clone ranged from 48 to 78 months, with no significant differences compared with patients without IgM [4, 6, 8, 9]. Risk stratification may be performed also in these patients using the Mayo cardiac staging system [11], as recently validated in the large European cohort [10].

The diagnostic criteria for IgM-related amyloidosis are:

(a) Presence of an IgM monoclonal component (with LC of the same isotype of that identified in the amyloid deposits).
(b) Biopsy-proven amyloidosis (the preferred biopsy site is the periumbilical fat pad; if negative, the biopsy of the minor labial salivary glands may be helpful. The biopsy of the affected organ, particularly the kidney, is safe, while for the liver, the transjugular route is suggested due to the severe bleeding risk.) [3].

(c) Demonstration of AL type by immunohistochemistry or immuno-electron microscopy or mass spectrometry, if AA amyloidosis is suspected.
(d) Organ involvement (the heart, kidney, liver, peripheral nervous system, or soft tissue including LN). No amyloid-specific syndrome, such as carpal tunnel syndrome or skin purpura as the only evidence of disease.

Additional adverse prognostic factors have been validated in different series, such as performance status >1 [8], differential FLC concentration >180 mg/L [10], and serum albumin < 35 g/L [12]. A recent European collaborative study, including 250 patients, has reported that cardiac involvement/advanced Mayo disease stage, peripheral neuropathic involvement, and low albumin<30 g/L were independent factors at multivariate analysis, impacting survival. A novel staging system combining abnormal NT-proBNP and troponin-T with low albumin (<30 g/L), and the presence of peripheral neuropathy gives a better risk model: median survival of patients with none, one, or two/more abnormal factors was 73, 55, and 17 months, respectively [9]. In non-IgM-AL amyloidosis, hematologic and cardiac response criteria have been defined and validated throughout an international effort [13]. These criteria have been validated also in the large European population of patients with IgM-related amyloidosis [9, 10]. However, only 2/3rd of patients had evaluable dFLC > 50 mg/L. There is consensus among the experts that in patients with low dFLC, response can be defined by M-protein changes (i.e., partial response: decrease by >50 %) evaluated either by serum electrophoresis or by nephelometry/turbidimetry assays. The results of the recent European study indicate that in IgM AL both light chains and the monoclonal IgM should be used for response assessment, contrary to emerging literature in non-IgM-AL amyloidosis [9]. Organ response can be defined using the same criteria used in non-IgM-AL amyloidosis for cardiac [13] and renal response [14], which have been validated in large populations.

Treatment should be aimed at the underlying IgM-related condition in order to reduce the concentration of the LC rapidly and deeply, since it has been reported that hematological response to treatment improves the survival [10]. As in non-IgM AL, the goal of VGPR or better remains the therapeutic end point in patients with IgM-related AL amyloidosis. There is no consensus on the best treatment of IgM-related amyloidosis, and evidence is limited to small, noncontrolled trials. Bortezomib, bendamustine, cyclophosphamide, and purine analogs in combination with rituximab and dexamethasone are increasingly favored, but hematological responses remain poor with very few complete responses [9, 10, 15]. The striking paucity of complete responses and few VGPRs observed in a large European study [9] highlights the difficulties of deep clonal eradication in low-grade NHL.

In patients with peripheral neuropathy, bortezomib should be avoided. Stem cell-sparing regimens should be used in patients potentially eligible for autologous stem cell transplantation (ASCT), and patients achieving less than very good partial response should be considered for ASCT [16]. Ibrutinib has been recently approved in the States for the treatment of WM, but no data are available regarding the efficacy and tolerability (especially cardiac and bleeding adverse events) in these

patients. Overall, current evidence indicates that ASCT and bortezomib-based regimes are associated with best responses [9]. For patients with other NHL, therapy should be tailored to the specific lymphoma. New and novel combinations need to be studied into prospective studies to improve responses and survival.

16.2 Renal Manifestations of IgM Gammopathies

Renal disease is a frequent complication of all B-cell lymphoid proliferations. Indirect mechanisms, such as infection, metabolic disorders, or treatment-related renal lesions, are often involved. In addition, clonal cells may cause renal damage by various mechanisms, including urinary tract obstruction by lymph nodes, tumor infiltration of the renal parenchyma, or deposition of a monoclonal immunoglobulin (Ig). Regarding IgM-secreting proliferations, renal manifestations are usually considered as less frequent and less diverse than in other B-cell lymphoid disorders. However, recent data have shown their previously unexpected heterogeneity and their clinical, therapeutic, and prognostic importance in many cases [17].

16.2.1 Tumor Mass-Related Renal Manifestations

Some renal manifestations of IgM-secreting monoclonal B-cell proliferations only occur in the course of a high tumor mass proliferation. They include glomerular monoclonal IgM intracapillary deposits, light chain (LC) cast nephropathy (CN), and interstitial infiltration of the kidney.

Glomerular monoclonal IgM intracapillary deposits occluding capillary lumens have been recognized for decades as the hallmark of renal disease in WM [18, 19]. Lesions are typically featured by isolated monotypic IgM thrombi, which occurred in patients with advanced disease and high serum IgM levels, usually with hyperviscosity syndrome and sometimes with detectable cryoglobulinemia. The frequency of this complication has decreased over time, thanks to the improvement in the management of WM [18].

LC cast nephropathy (CN) is not exceptional in patients with high tumor burden IgM-secreting monoclonal B-cell proliferations [17, 20]. Although usually relatively low in all IgM gammopathies, urinary excretion of LC may be elevated in some patients with symptomatic WM. In addition, cast formation, which is the trademark of CN, is determined by both urine LC concentration and peculiarities of LC variable sequence that determine interaction with uromodulin [21]. Moreover, local conditions in the tubule milieu, such as a decrease in urine flow and an acid pH, are key favoring factors. Accordingly, as in multiple myeloma, symptomatic measures to prevent LC intratubular precipitation should be considered in patients with overt WM, particularly with high FLC levels or Bence Jones proteinuria. These preventive measures are important since CN appears to be associated with poor renal outcome in WM [17, 20].

The infiltration of the interstitium of the kidney by lymphoplasmacytic cells is the more frequent tumor mass-related renal complication. The renal biopsy shows a lymphoplasmacytic patchy infiltration of the renal interstitium predominantly made of CD20 positive lymphocytes which also stained positive for μ and k or λ light chains. Associated interstitial fibrosis is frequent. When dense, the tumor parenchymal infiltration may cause acute kidney injury, usually in association with acute tubular necrosis. It may be associated with any other renal diseases occurring in the setting of a high tumor mass IgM-secreting proliferation and, in this case, is likely to contribute significantly to the severity of renal disease [17, 22].

16.2.2 Tumor Mass-Unrelated Renal Manifestations: Monoclonal Gammopathy of Renal Significance (MGRS) of IgM Type

In addition to the renal complications which occur only during the course of an overt B-cell proliferation, others are tumor mass-unrelated. They may complicate another symptomatic disease, but much more often, they feature either an indolent and asymptomatic proliferation or even a MGUS. The concept of monoclonal gammopathy of renal significance, the so-called MGRS, was recently introduced to highlight the causal relationship between the gammopathy and renal damage and the need for early hematological treatment to preserve renal prognosis [2]. As in all MGRS, the main mechanism of MGRS of IgM type is Ig deposition, and all or part of the monoclonal IgM can depose in the glomerular or in the tubular structure of the kidney [23].

With regard to glomerulopathies, IgM deposits can be either organized or non-organized according to their ultrastructural pattern. As mentioned above, *amyloidosis* belongs to the organized type, in a fibrillar structure. IgM deposits complicating *type I or type II cryoglobulinemia* also have an organized structure, which is microtubular. *The so-called GOMMID or immunotactoid GN* is also due to Ig deposits with a tubular structure. It is usually associated with a silent chronic lymphocytic leukemia but rarely, if ever, features an IgM-secreting monoclonal B-cell proliferation such as WM [17, 23].

Monoclonal Ig deposition diseases, which are sometimes called Randall's diseases, have also rarely been described in the course of WM and other IgM-secreting monoclonal B-cell proliferations. Another type of glomerulopathy characterized by non-organized granular Ig deposits is more frequent, namely, *proliferative glomerulonephritis with monoclonal Ig deposits (PGMID)*. This nephropathy is characterized by cellular proliferation associated with granular subendothelial deposits in the absence of intracapillary thrombi. On the renal biopsy, endocapillary hypercellularity and mesangial matrix increase are key features, and immunofluorescence studies revealed a bright μ (and either k or λ) staining of capillary walls with a granular pattern [23].

Regarding tubulopathy, *Fanconi's syndrome* is a rare but possible complication of IgM monoclonal gammopathies. It is invariably related to the proximal tubule

reabsorption of LC of kappa isotype and may occur alone or in the setting of extrarenal manifestations of a crystal-storing histiocytosis (CSH) [17].

16.2.3 Diagnostic Approach

The incidence of the different renal complications of IgM gammopathies is not well known. A recently published short retrospective study ($n = 35$) pointed out an under-recognized frequency of tubulointerstitial disorders, including CN (5/35), LC-Fanconi syndrome (3/35), and isolated severe tumor infiltration (1/35) [17]. Otherwise, glomerular symptoms, usually with nephrotic syndrome, featured about ¾ of cases, AL amyloidosis, and membranoproliferative glomerulonephritis, including PGMID and cryoglobulinemic kidney, accounting for most cases. Only one-third of patients had symptomatic hematological disease, usually WM, and the two other thirds had MGRS [17].

From a clinical point of view, the distinction between the different renal diseases mainly relies upon analysis of proteinuria. When detected during the course of an overt IgM-secreting monoclonal proliferation, a low-grade proteinuria mixing small amounts of albumin and LC will suggest a lymphoid infiltration of the kidney. This is usually observed in the presence of a moderate renal failure, sometimes with kidney enlargement. Less frequently, a proteinuria predominantly made of LC will indicate cast nephropathy, usually in the context of precipitating factors such as dehydration, infection, and use of toxic drugs. The Fanconi syndrome should be suspected when a monoclonal IgM always associated with a k free LC is detected in a patient with metabolic disorders such as hypouricemia and hypophosphatemia and normoglycemic glycosuria which should prompt further explorations for proximal tubular dysfunction.

Proteinuria mainly composed of albumin indicates a glomerular process. Its nature may be suspected by the assessment of other renal symptoms, by careful search for extrarenal manifestations and by the characteristics of monoclonal gammopathy, particularly its LC isotype and whether it corresponds to an overt gammopathy or not. Very rarely, in the context of a high tumor mass WM, usually with symptoms of hyperviscosity, the diagnosis will be glomerulonephritis with IgM intracapillary thrombi. Much more often, the issue is determining the nature of the nephropathy featuring an IgM MGRS. Thus, a lambda IgM MGUS revealed by nephrotic syndrome in a patient presenting with suggestive extrarenal symptoms, including, for example, heart failure or, less frequently, signs such as macroglossia or purpura of the eyelids, will reveal AL amyloidosis [3]. Alternatively, nephrotic syndrome associated with vascular purpura lesions in the skin mostly suggests cryoglobulinemic glomerulonephritis. Other glomerular disorders also mostly manifest as nephrotic syndrome. In all cases, the diagnosis should be confirmed by an appropriate biopsy, of an extrarenal site when possible, or of the kidney. If AL amyloidosis is suspected, the suggested biopsy site is the abdominal fat; if negative, a renal biopsy is always diagnostic and can be performed relatively safely [3, 24].

The diagnosis is not always so easy since clinicopathological correlations may be misleading, and past medical history potentially responsible for renal disease, such as hypertension or diabetes, may be confusing. In addition, association between the different renal lesions is not rare as well as overlapping between the different forms. For example, the concomitant discovery of interstitial diffuse infiltration with clonal B cells and a non-amyloid glomerular disorder is not rare, particularly in patients with severe renal disease at presentation [17, 18]. Accordingly, the indication of a kidney biopsy should be discussed in most patients, including detailed immunofluorescence (and electron microscopic) studies in most cases. This will impact both treatment decisions and prognosis, not only in patients with overt WM, but more importantly in patients with MGRS. Indeed, in these patients, starting a treatment as for WM is often justified for renal reasons with the aim of preserving the kidney, even if the IgM gammopathy is an indolent one or even a MGUS [25].

16.3 Cutaneous Manifestations of IgM Gammopathies

IgM monoclonal gammopathies can be featured by various cutaneous manifestations that can be due to the clonal cells, to the monoclonal IgM, or to as yet unknown mechanisms.

16.3.1 Cutaneous Manifestations Due to Clonal Cells

Although rare, cutaneous nodules and plaques due to *tumor infiltration* made of lymphoplasmacytic IgM-producing clonal cells may be observed in WM, usually in the course of an overt disease lasting for several years [26]. The clonal cells can also provoke skin manifestations indirectly by absorbing biologically active molecules, such as the von Willebrand factor (VWF) or C1q inhibitor (see below). Another mechanism can be the secretion, by the clonal cells themselves or by their environment, of cytokine such as vascular endothelial growth factor. This is likely to explain the angiomas of the so-called POEMS syndrome, which was exceptionally reported as associated with an IgM-producing monoclonal proliferation [27].

16.3.2 Monoclonal IgM-Related Cutaneous Manifestations

Cutaneous and mucosal hemorrhagic manifestations are common features of the *hyperviscosity syndrome* that can complicate the monoclonal IgM when present in the serum at high concentration.

As in the kidney, all or part of the monoclonal Ig can deposit in various structures of the skin. Non-organized deposits are rare and mostly represented by the so-called *cutaneous macroglobulinosis*, characterized by localized or

coalescent papules, caused by amorphous intradermal deposits made of monoclonal IgM molecules [28].

Organized deposits are more frequent. Cutaneous signs of *AL amyloidosis* of IgM type are classical, including purpuric lesions and waxy papules or nodules, often with purpuric appearance and located on flexural areas. The most suggestive skin manifestations of *type I cryoglobulinemia* (CG) are cold-triggered ischemic symptoms located on the extremities including Raynaud's phenomenon, retiform or stellar purpura, and skin necrosis. Painful leg ulcers and cold urticarial are also frequent and suggestive [29]. Of note, palpable purpura related to leukocytoclastic vasculitis, which is the hallmark of mixed type II CG, is rarely observed if ever. Indeed, cutaneous symptoms of type I CG are due to cold-induced intravascular precipitation of the monoclonal Ig, thrombotic obstruction of the vessels, and ischemia of the underlying skin [29, 30].

Conversely, palpable purpura, ischemic and ulcerous lesions, and other cutaneous manifestations of *type II-mixed CG* are consecutive to vasculitis. Here, the rheumatoid autoantibody activity of the monoclonal IgM results in immune complexes, made of the monoclonal IgM and polyclonal IgG, which depose in the vascular walls and trigger consumption of the main complement pathway and chemoattraction of cells forming the perivascular infiltrate characteristic of true leukocytoclastic vasculitis.

Other autoantibody activity of monoclonal Ig can be symptomatic at the cutaneous level because of the precipitation of immune complexes not in peripheral vessels, but in macrophages. This is exemplified by *xanthomatosis*, a condition characterized by the deposition of cholesterol-rich material in large foam cells accumulating in the skin and the tendons. Particularly in its normolipemic form, it is associated with monoclonal Ig which can be an IgM, although very rarely [31]. Complement abnormalities (with, most often, a low C4 serum level) are nearly constant, indicating an activation of the classical complement pathway by immune complexes that may result from antigen-antibody interactions between the monoclonal immunoglobulin and various lipoproteins. Such interaction, which has been documented in some cases, seems to result in enhanced lipid accumulation in the macrophages [32].

IgM gammopathies can be associated with *autoimmune bullous skin diseases*, involving autoantibodies either recognizing antigens of the intercellular substance of the epidermis (pemphigus group) or directed against antigens of the dermoepidermal junction [bullous and cicatricial pemphigoid and epidermolysis bullosa acquisita (EBA)]. In the first group, the so-called paraneoplastic pemphigus, which is clinically featured by usually severe polymorphous blistering eruption with prominent mucosal and acral involvement, has been rarely described, mostly in patients with B-cell lymphoproliferation who were treated by fludarabine, and a potential favoring role of the drug has been suggested [33]. Cutaneous manifestations associated with linear Ig deposits at the dermoepidermal junction are frequently atypical and limited to few discrete blisters or vesicles. In most cases, immunoprecipitation and immunoblotting studies demonstrate autoantibodies targeting type VII collagen, which are a characteristic of EBA [34].

Immunofluorescence studies most often show polytypic cutaneous Ig deposits, and a restriction of Ig LCs to the one expressed by the monoclonal IgM is rarely observed. In addition, the autoimmune and the lymphoid processes usually do not display a parallel course, reinforcing the hypothesis that the monoclonal IgM does not bear the skin autoantibody activity which is likely to be produced by bystander B cells not related to the B-cell clone. So, the pathogenesis of these immunobullous diseases appears similar to that of the autoimmune disorders that target hematopoietic cell antigens and explain the frequency of autoimmune thrombocytopenic purpura and hemolytic anemia in chronic IgM-secreting B-cell lymphoproliferation [34].

16.3.3 Unknown Mechanisms: The Schnitzler Syndrome

Several skin diseases that are frequently or constantly associated with a monoclonal Ig have a still largely unknown physiopathology. Some are exceptionally, if ever, associated with a monoclonal IgM, such as *neutrophilic dermatosis*, *cutaneous mucinosis*, and *acquired cutis laxa* [35].

In contrast, the *Schnitzler syndrome* is defined by the combination of a chronic/recurrent urticarial rash with a monoclonal IgM gammopathy, most often with the characteristics of a MGUS. The skin rash consists of rose pale macules or slightly raised papules and plaques, clearly distinct from common urticaria, since the eruption is fixed and usually not or only moderately itchy. Lesions, which can occur on every body part, though involvement of face and extremities is rare, resolve within hours without any sequel. The frequency of flares is variable from patient to patient and in the same patient from unknown factors (Fig. 16.1). In addition to urticarial outbreaks, intermittent spiking fever and fatigue are frequent. Musculoskeletal involvement is another cardinal feature of the disease, affecting about ¾ of patients. Bone pain, mostly of iliac bone and tibia, is the most characteristic finding. Almost 50 % of the patients showed bone lesions on imaging. The most frequent radiological finding is osteocondensation with cortical hyperostosis, particularly of distal femora and proximal tibiae. Bone technetium scanning reveals hyperfixation in the areas of radiological involvement, and magnetic resonance imaging confirms thickening of cortices.

At examination, lymphadenopathy, liver, and spleen enlargement are not rare. In addition to the monoclonal IgM, laboratory investigations usually show a major increase in sedimentation rate, C-reactive protein (CRP), and fibrin serum levels. On the blood count, neutrophilic leukocytosis is very common (2/3), inflammatory anemia and elevated platelet count are frequent. Pathological findings are most often featured by a mild dermal perivascular neutrophilic infiltrate without detection of any Ig or complement deposition. In the absence of any biological or other confirmative examination, diagnosis of Schnitzler's syndrome is considered definite in any patient with current urticarial rash and a monoclonal IgM gammopathy (obligate criteria) and two minor criteria (recurrent fever, objective signs of abnormal bone remodeling, elevated CRP level or leukocytosis, and neutrophilic

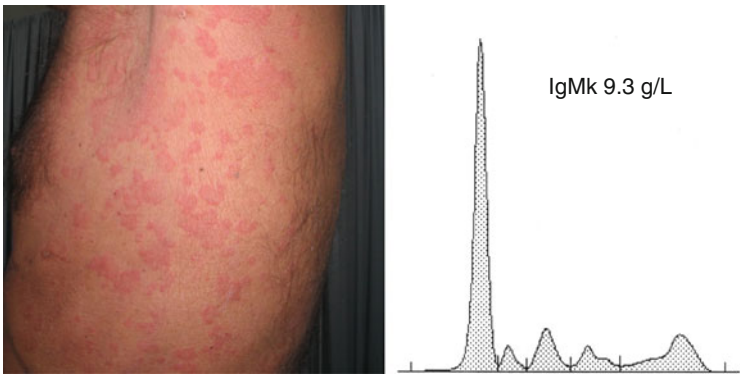

Fig. 16.1 Schnitzler's syndrome. The patient presented with a recurrent urticarial rash and monoclonal IgMk with recurrent fever

infiltrate on skin biopsy). It is considered probable, if only one minor criterion is present [36].

Until recently, treatment of the Schnitzler syndrome was only symptomatic and unsatisfactory, as assessed by the long list of treatments that were proposed. In fact, steroids were nearly the only effective drug, usually within a precise daily threshold dosage which is often relatively high. Thalidomide, α-interferon, and antibiotic, pefloxacin, are also symptomatically effective. This was changed with the introduction, in 2005, of the IL-1 receptor antagonist anakinra [37]. Indeed, within hours after the first injection, anakinra produces a dramatic improvement of all clinical and biological manifestations of the disease, including fatigue and pain, inflammatory abnormalities, and hyperleukocytosis. Patients recover a health state that they often did not know for years. Anakinra does not modify the level of the monoclonal IgM, and similarly to steroids, it does not cure the disease, but only has a symptomatic effect. Because of the short half-life of the drug (about 6 h), daily subcutaneous injections are usually required. Anakinra can be maintained over several months and even years, with sustained efficacy and good tolerability ([38]; Asli and Fermand, personal data). So, most investigators consider that any patient with symptoms, significant alteration in the quality of life, and/or regularly elevated inflammation markers such as CRP needs to be treated with anakinra 100 mg/day [36].

The course of the disease is long-lasting. The overall prognosis of the syndrome depends on the possible evolution into a symptomatic lymphoproliferative disorder, with a similar risk as compared to isolated IgM gammopathy. Before the era of anakinra, reactive AA amyloidosis complicated the course of at least four patients ([39, 40]; Merlini personal data). Importantly, the hypothesis of a potential causal relationship between the gammopathy and the manifestations of the syndrome is documented by the resolution of all symptoms of the disease observed in one patient in whom the monoclonal IgM spontaneously disappeared and by the parallel dual complete remission that was obtained in another patient who was treated by a

rituximab, cyclophosphamide, and dexamethasone (RCD) regimen because of an overt WM [41, 42].

NOD-like receptor mutations, particularly gain-of-function mutations within the NOD domain of cryopyrin, are associated with genetic autoinflammatory disorders [43]. Importantly, these are characterized by skin rashes and periodic fever, and they mimic the Schnitzler syndrome. Accordingly, this syndrome might be an acquired autoinflammatory disorder, due to an unregulated secretion of IL-1 via interaction of a clonal product (may be the IgM) with a key component of the IL-1 pathway. However, the precise physiopathology of this syndrome remains to be established.

16.4 Miscellaneous

16.4.1 Acquired C1 Inhibitor Deficiency

Angioedema, due to the acquired deficiency of the inhibitor of the first component of human complement (C1-INH), the so-called acquired angioedema (AAE), is rare. Its clinico-biological characteristics are similar to those of hereditary angioedema, except for the later onset of symptoms, the absence of a family history, and the association with MGUS, non-Hodgkin B-cell lymphoma (NHL), and/or anti-C1-inhibitor autoantibodies. Various types of NHL can be observed, but indolent forms, particularly lymphoplasmacytic, including WM and marginal zone lymphoma, are the most frequent. Patients usually have advanced stage disease, with bone marrow infiltration. For MGUS, the IgM isotype accounts for about 25 % of cases [44, 45].

The physiopathology of AAE is most often related to an autoantibody activity directed against C1-INH, leading to enhanced clearance or inhibition of function, increased proteolytic degradation, and/or adsorption by neoplastic cells when present [45]. The only consumption of C1-INH by neoplastic cells, independently of associated autoantibodies, is sometimes identified as type I AAE but is likely to be rare [46]. In patients with MGUS or NHL, AAE may be consecutive to an autoantibody activity of the monoclonal Ig, whether or not secreted. Alternatively, it may be caused by polyclonal Ig produced by bystander B cells not related to the B-cell clone, particularly in WM and other indolent lymphomas in which autoimmune manifestations are frequent.

AAE patients are usually poorly sensitive to attenuated androgens and antifibrinolytic agents which are normally used in hereditary angioedema. Acute attacks can be treated by high dose of plasma-derived C1-inhibitor or by the bradykinin B2 receptor antagonist icatibant. In all cases, treatment targeting the underlying B-cell proliferation should be discussed, including in patients with IgM-secreting MGUS who may receive rituximab alone which can improve angioedema symptoms and complement parameters [45].

16.4.2 Acquired Von Willebrand Syndrome

Disturbance with coagulation pathway is a well-known complication of IgM gammopathy. It comprises procoagulant activity with thrombosis [47] and bleeding disorders involving various interactions between the monoclonal component and primary hemostasis or plasma coagulation factors [48].

Acquired von Willebrand syndrome (AVWS) is uncommon and usually associated with an underlying disorder. Most frequently, this is a lymphoproliferative disease, often an IgM-secreting monoclonal gammopathy including an IgM MGUS [49, 50]. As in AAE, the AVWS associated with a lymphoid disease is usually due to autoantibodies which either interfere with platelet or collagen-binding or increase von Willebrand factor (VWF) clearance from the plasma. Nonimmune mechanisms such as adsorption to malignant cells (or platelets) or increased proteolytic degradation are rarer [51, 52]. Treatment of the underlying lymphoproliferative disorder, when present, should always be considered. Successful chemotherapy with rituximab usually results in remission of the AVWS which are associated with IgM gammopathy. Otherwise, plasmapheresis combined with VWF-containing concentrates may be proposed for controlling and preventing bleeding. Desmopressin usually results in short-lived improvement of VWF, and IVIG are considered to be effective only in patients with monoclonal IgG and not in patients with monoclonal Ig of IgM class [49].

Acknowledgments GM is supported by grant from "Associazione Italiana per la Ricerca sul Cancro – Special Program Molecular Clinical Oncology 5 per mille n. 9965" and from CARIPLO "Structure-function relation of amyloid: understanding the molecular bases of protein misfolding diseases to design new treatments n. 2013-0964."

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Part IV

Laboratory Investigations

Laboratory Investigations and Findings: Hematological Abnormalities, Biochemical Investigations, Free Light and Heavy Chains

17

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17.1 Introduction

Waldenström macroglobulinemia (WM) was first described by Jan Gosta Waldenström in 1944 when he reported the cases of two patients presenting with lymphadenopathy, infiltration of the bone marrow by lymphoid cells resulting in anemia and thrombocytopenia, and various symptoms (such as oronasal bleeding, elevated erythrocyte sedimentation rate, and high serum viscosity) which he attributed to the presence of an abnormally elevated high-molecular-weight serum protein; later to be demonstrated as a monoclonal pentameric IgM.

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_17

Interestingly, decades later, the finding that IgM is a hallmark of WM remains in its diagnosis criteria [1]. The IgM monoclonal protein is also still used for prognostication [2], monitoring of response to therapy, and so on, despite many flaws in its measurement [3, 4].

Herein, we discuss the diagnostic workup of WM, along with the various attempts to either replace IgM or develop new tools to improve IgM serum measurement.

17.2 Hematological Abnormalities

As a result of bone marrow infiltration and the presence of large quantity of serum IgM, patients are often diagnosed with cytopenias, essentially anemia in WM. A complete blood count is, thus, mandatory at diagnosis.

Anemia Anemia is observed in more than half of WM patients. Anemia is usually normocytic, normochromic, nonregenerative. The mean corpuscular volume can be falsely macrocytic related to pseudoagglutination due to the excess of IgM coating the erythrocyte surfaces, also described as Rouleaux formation on blood smear.

The presence of anemia and importantly its relation to WM is important to recognize, as it is the most common indication for initiation of treatment. As treatment decision may rely on it, exclusion of other causes of anemia is critical; indeed, one is expecting to treat WM based on anemia when secondary to the presence of tumor cells in the bone marrow.

Anemia is often multifactorial; the most common causes being apoptosis of erythroid precursors mediated by Fas/FasL interactions, along with bone marrow involvement resulting in inadequate erythropoiesis, and anemia of chronic disease. The erythroid precursors indeed express the Fas receptor as a physiological mechanism of regulation of the erythroblast population in the bone marrow, but this death signal is exacerbated in WM due to the expression of FasL on WM tumor cells. An increased plasma viscosity has also been shown responsible for inappropriate erythropoietin formation by renal peritubular cells, resulting in lack of maturation and differentiation of erythroid colonies and erythroid progenitors in the bone marrow [5]. The level of hemoglobin is also partly decreased related to dilution induced by the presence of high concentrations of IgM monoclonal immunoglobulin in the serum—especially above 30 g/L. Interestingly, a falsely elevated hemoglobin can be found as well, due to abnormal reactions between reagents and monoclonal Ig present in excess [6].

Considering that the median age of WM is above 65 years old, other causes of anemia are frequently encountered in patients with WM, which may require specific therapy but rarely treatment of WM per se. For example, many patients display iron or vitamin deficiency. Interestingly, iron deficiency anemia if often refractory to oral treatment in WM, but not to intravenous iron repletion [7]. Furthermore, WM patients may present with occult hyperviscosity-related gastrointestinal bleeding, which should be considered and eventually explored in these patients who may

develop second malignancies. It is though worth noting that serum levels of ferritin can be falsely low due to interference related to the IgM monoclonal protein [8]. It has also been demonstrated that lymphoplasmatic cells produce hepcidin, which could contribute to anemia in WM [9]. Hepcidin indeed regulates iron metabolism by inhibiting ferroportin, which leads to sequestration of iron in enterocytes, monocytes, and macrophages. Therefore, since absorbed iron cannot be released (unless given intravenously), it is not available for erythroblasts; this situation can also be found in chronic inflammation.

Anemia can, as well, be a consequence of myelosuppressive chemotherapy. Late development of anemia may indicate development of myelodysplasia after treatment [10].

Hemolytic anemia can also occur in some patients with WM, either warm or cold agglutinin hemolytic anemia. If the reticulocytes count shows that anemia is regenerative, a test for cold agglutinins and an erythrocyte Coombs test should, thus, be performed. The Coombs test is positive in about 10 % of patients, but less than 5 % of patients develop significant hemolysis [11]. Warm autoimmune hemolytic anemia is mediated by IgG autoantibodies, resulting from an activation of autoreactive T cells stimulated by the tumor cells and is, thus, not directly related to tumor cells or the monoclonal IgM component. Warm autoimmune hemolytic anemia is likely to be primarily extravascular [7], mainly of splenic type. However, the monoclonal IgM may present with cold agglutinin activity, resulting in chronic immune hemolytic anemia with acute phases of intravascular hemolytic anemia [12]. This disorder occurs in less than 10 % of WM patients. In most cases, it is associated with cold agglutinin titers $> 1{:}1000$; the monoclonal component is usually an IgMk and it reacts most commonly with I/i antigens, with complement fixation and activation. Mild chronic hemolytic anemia can be exacerbated after cold exposure but is rarely responsible for a hemoglobin decrease below 70 g/L. Clinical manifestations can include Raynaud syndrome, acrocyanosis, and livedo reticularis. It is important to identify this hemolytic syndrome, as patients may benefit from a treatment of hemolytic syndrome and only get treated for WM later on if the primary proposed treatment failed.

Thrombocytopenia Thrombocytopenia $< 100 \times 10^9$/L is not infrequent, seen in about 15 % of patients and mainly related to bone marrow involvement; while a thrombocytopenia $< 50 \times 10^9$/L is observed in less than 5 % of patients [11]. Of note, immune thrombocytopenia (ITP) is a frequent etiology in patients with WM, an important condition to diagnose since these patients may also benefit from a specific treatment prior to be treated for the underlying WM [8]. Other causes of thrombocytopenia include hypersplenism or myelosuppressive chemotherapy. Similarly to anemia, late development of thrombocytopenia may be related to development of myelodysplasia secondary to treatment [10]. Severe thrombocytopenia may increase the risk of bleeding associated with hyperviscosity, particularly when hyperviscosity presents with acquired von Willebrand syndrome [8].

White Blood Cells In most cases, the white blood cell count is normal. Neutropenia is rare, with less than 5 % of patients presenting with neutropenia $< 1 \times 10^9$/L. However, a lymphocytosis can be observed that rarely exceed 15×10^9/L in about 10 % of patients [11]. Even if not detected in standard white blood cell count, a circulating clonal population can be detected by flow cytometry immunophenotyping in many more patients. This is the source of several research projects to try and understand the significance and utility of such identification. Performing a flow cytometry immunophenotyping to seek for circulating WM tumor cells is not part of the recommended diagnosis check up so far.

Recommendation

To perform a CBC count with smear, plus reticulocyte count and hemolytic workup at diagnosis of WM.

If hemolysis is suspected (cold or warm hemolysis or ITP), a workup, including peripheral smear, reticulocyte count, LDH, bilirubin, haptoglobin, and Coombs test, should be performed. Hemolysis is, however, most often extravascular. Testing for cold agglutinins, and direct and indirect Coombs antibody testing if cold agglutinins are negative, is advised as part of the workup of hemolytic anemia.

17.3 Bone Marrow Investigations

Characterization of the bone marrow infiltration is essential in WM and should be performed in all symptomatic patients to confirm the diagnosis of WM prior to start treatment. In asymptomatic patients, its value is not clearly established but an arbitrary threshold of 10 g/L of serum IgM has been proposed to consider performing a bone marrow assessment [13].

A bone marrow biopsy is the recommended exam, as it allows better quantification of the infiltration and reveals the classically intratrabecular pattern of bone marrow infiltration, which may be diffuse, interstitial, or nodular. In contrast, a solely paratrabecular pattern is rare in WM and should raise suspicion of other low-grade B-cell lymphoma subtypes. A myelofibrosis with reticulin fibers can be detected in some cases. Intranuclear and cytoplasmic immunoglobulin inclusions, termed as Dutcher and Russell bodies, respectively, are frequently observed in the WM tumor lymphoplasmacytic cells. Interestingly, an increased number of mast cells close to lymphoid aggregates are also a common finding in WM, although the exact meaning of this histologic finding remains to be fully unraveled [12]. Besides the demonstration that mast cells and WM tumor cells cross talk, it is not yet known whether the tumor cells stimulate the production and aggregation of the mast cells or whether the mast cells are part of the modified bone marrow microenvironment which helps provide survival and proliferation advantage to the WM tumor cells.

If the marrow biopsy cannot be performed, a bone marrow aspiration along with bone marrow smear and flow cytometry immunophenotyping can be proposed as an adequate alternative.

Concerning the amount of infiltration, the Second International Workshop on Waldenstrom's Macroglobulinemia criteria does not require a minimum amount of bone marrow involvement to make the diagnosis of WM or recommend to start WM treatment [1]; however, the Mayo Clinic criteria require at least 10 % bone marrow involvement to differentiate IgM MGUS from WM [14].

The bone marrow infiltration is characterized by a lymphoplasmacytic clone constituted of a mixture (also called a continuum) of clonal B lymphocytes, plasma cells, and plasmacytoid/lymphoplasmacytic lymphocytes [15].

Immunophenotypic studies (flow cytometry and/or immunohistochemistry) should always be performed to confirm the diagnosis, showing the following profile: sIgM+, CD19+, CD20+, CD22+, and CD79+. The lymphoid component typically lacks CD10 (unlike follicular lymphoma) and can show some degree of CD5 expression, however, weak, clearly not the kind of strong CD5 expression observed in chronic lymphocytic leukemia/small lymphocytic lymphoma or mantle cell lymphoma, similar to CD23. The plasmacytic component expresses the same Ig light chain as the lymphocytic component, is positive for CD38 or CD138, and shows normal expression of B-cell-associated antigens (CD19, CD20, PAX5, etc.), which allows differential diagnosis from IgM secreting myeloma plasma cells. WM cells have also been shown to be $CD25^+$, $CD27^+$, $CD75^-$, $FMC7^+$, $Bcl2^+$, and $Bcl6^+$ [1, 16, 17], although the latter panel is not required in routine practice for the diagnosis of WM. Characteristics of the immunophenotype in WM is summarized in Table 17.1.

To note, tissue biopsy is not recommended in WM since the primary recommended biopsy for the diagnosis is the bone marrow biopsy; however, a

Table 17.1 Characteristic immunophenotype in WM

Markers	Proteins	%	Remarks
Clonality	Surface IgM	100	High level of expression
	Light chain	100	Kappa > Lambda
B cell phenotype	CD19/CD20	100	
	CD22	100	Low intensity
	CD79	100	
	FMC7	>85	Low intensity
	CD5/CD19	10–27	Low intensity
	CD23	14–33	Low intensity
	CD10	<10	
Plasma cell phenotype	CD38	0–60	Important variability
	CD138	<10	
Memory lymphocytes	CD27	42	
Other	CD25	90–100	Homogeneous expression
	CD103	0	Always negative
	CD43	<20	Low level of expression
	CD11c	0–31	Low level of expression
	CD52	100	Always positive

tissue biopsy is recommended in all patients with suspected histological transformation. Detailed pathological assessment should include assessment of Epstein–Barr virus (EBV) [16].

Cytogenetics and Molecular Biology The normal equivalent of the WM malignant cell is believed to be a post-germinal center B cell that has undergone somatic hypermutation but transforms before isotype class switching and terminal differentiation to plasma cells.

The molecular study of IgVH sequence, thus, reveals somatic hypermutation without intraclonal diversity (no switch). There is a biased use of the heavy chain gene repertoire with an overrepresentation of VH3 and JH4 families.

Cytogenetic analysis is required for the routine diagnostic assessment of WM patients [16]. Conventional karyotyping without specific stimulation has limited applicability in WM, as it is difficult to obtain tumor metaphases because of the low rate of cell proliferation. The absence of IgH involving translocation can be used in difficult cases for differential diagnosis; indeed, it was shown that although WM never harbors IgH translocations, multiple myeloma producing IgM frequently has this abnormality, particularly the t(11;14)(q13;q32) [18].

Several frequent cytogenetic abnormalities, such as del(6q) and trisomy 4, can be explored but are better detected by FISH analysis than conventional karyotyping.

Fluorescence in situ hybridization (FISH) improved cytogenetic studies, even though it only looks at prespecified probes. Although not completely specific for WM, deletion of the long arm of chromosome 6 encompassing del(6)(q21) is the most common genetic abnormality detected by FISH, being detected in 40–50 % of patients [19, 20]. This genetic abnormality is rarely seen in other lymphoproliferative or plasmaproliferative malignancies, but can be found in IgM gammopathy of unknown significance (MGUS) [21]. Several candidate tumor suppressor genes in this region are under study, in order to provide an explanation for the frequency of this abnormality. Among these genes is BLIMP-1, which regulates the transition of mature B cells to terminally differentiated plasma cells and is, thus, a major candidate [22]. Deletion of TP53 occurs in a minority of patients and appears to confer a poor outcome [23]. The deleted region of TP53 varies a lot between patients; a minimal deleted region has been described by Poulain et al. [24].

Other cytogenetic abnormalities can be found in WM: trisomy 4 is reported in up to 20 % of cases, and trisomy 5, monosomy 8, and deletion of the long arm of chromosome 20 have also been reported, along with del17p and del13q [20].

MYD88 L265P Mutation, A Molecular Signature The recent finding of MYD88 L265P mutation in about 90 % of cases of WM made it a major marker of the disease [25].

Our understanding of the pathogenesis of WM has improved considerably since this discovery. MYD88 (myeloid differentiation primary response 88) indeed plays a significant role in Toll-Like Receptor (TLR) and interleukin-1 receptor signaling.

The acquired gain-of-function mutation of MYD88 gene on the short arm of chromosome 3, resulting in the change in the amino acid leucine to proline at position 265 (L265P), activates downstream signaling of the transcription protein complex NF-kB (nuclear factor kappa-light chain-enhancer of activated B cells), thereby promoting WM cell growth and survival [26]. MYD88L265P also activates Bruton's tyrosine kinase (BTK), another crucial enzyme involved in NF-kB regulation [27].

This single point somatic mutation, detected by PCR, is also present in 87 % of IgM MGUS, suggesting that this may be an early oncogenic event [28]. It remains, however, unclear whether MYD88 is a driver mutation for the transformation of MGUS to WM or solely a marker of MGUS with predisposition to progress to WM [29, 30].

The MYD88 mutation can also be useful in distinguishing WM from splenic marginal-zone lymphoma and IgM MM, as it is present in more than 90 % of WM patients but is uncommon in IgM MM and found in only 5–7 % of patients with marginal-zone lymphoma [25, 31, 32].

Its presence has been associated with early onset of WM presentation and a greater bone marrow involvement compared to MYD88 WT [28]. It has been described that WM patients with the absence of MYD88 mutation were characterized with a female predominance, a splenomegaly, a gain of chromosome 3, and CD27 expression [32].

Inhibition of MYD88 signaling has been shown to induce cytotoxicity and inhibit cell growth of cell lines issued from patients with WM [32].

All of these recent data confirmed the value of screening for the MYD8 L265P mutation in the routine diagnostic setting, and promising novel treatments resulting from its discovery are under study [28, 33].

Mutations of CXCR4 at the carboxyl (C) terminal domain have also been observed in approximately 30 % of patients with WM and 20 % of IgM MGUS patients [28].

WM cells are known to express high levels of chemokine and adhesion receptors. The C–X–C chemokine receptor type 4 (CXCR4), via its interaction with its ligand, stromal derived factor-1 (SDF-1/CXCL-12), is believed to play a critical role, particularly in modulating clonal lymphoplasmacytic cell homing to the bone marrow niches [26].

CXCR4 mutations in WM are the first reported acquired somatic mutations of CXCR4 in any human cancer. They have been described in patients with WHIM syndrome—a rare, congenital immunodeficiency disease characterized by Warts, Hypogammaglobulinemia, recurrent Infections and bone marrow Myelokathexis resulting in chronic neutropenia. In WM, two types of CXCR4 mutations have been observed in equal proportions: the frame shift mutations ($CXCR4^{WHIM/FS}$) and nonsense mutations ($CXCR4^{WHIM/NS}$) [26].

Ninety-eight percent of CXCR4 mutations coexist with MYD88 L265P mutations. While the MYD88 L265P mutation has a role in WM cell proliferation and survival, CXCR4 mutation has been implicated in tumor progression and drug

resistance. CXCR4 mutation indeed mediates drug resistance to mTOR, PI3K, and BTK inhibitors by blocking CXCR4 receptor internalization and leading to a persistently activated CXCR4, which prolongs and enhances activation of AKT1 and MAPK1 [34]. $CXCR4^{WHIM/NS}$ mutations have been reported to be associated with more aggressive disease features such as greater marrow involvement and higher serum IgM levels with increased risk of hyperviscosity syndrome, but without impact on overall survival [26].

Anti-CXCR4 drug, plerixafor, has been shown to successfully inhibit this pathway.

As multiple CXCR4 mutations can be found, the development of a PCR-based assay is difficult, and routine testing for these mutations is not recommended yet.

Recommendation
To perform a bone marrow biopsy accompanied by immunophenotyping. Cytogenetic is recommended, particularly to identify whether del6q is present; however, to date the identification of MYD88 L265P mutation has clearly became a hallmark of the diagnosis of WM along with histology. It seems CXCR4 is not yet to be recommended in routine check up for WM identification, as its role may be more into understanding the risk of chemoresistance.

17.4 Hemostasis

Viscosity Hyperviscosity syndrome is a classical feature of WM but occurs in less than 15 % of patients. It is related to an increased vascular resistance and blood viscosity secondary to the presence of elevated serum IgM levels. Common symptoms include headache, blurred vision, vertigo, oronasal bleeding, or hearing defects. Other manifestations can be observed, such as neurological symptoms or aggravation of congestive heart failure due to the increased blood viscosity and expanded plasma volume arising from increased osmotic pressure. Thromboembolic events can also be observed. Symptoms typically occur when the serum viscosity reaches 4–5 cP, which usually correlates with a serum IgM level > 30 g/L [12]. Although it can prove a very useful adjunct to the clinical assessment of patients, measurement of plasma viscosity is not routinely available. A high sedimentation rate is another marker for hyperviscosity.

In symptomatic patients or if IgM levels are > 30 g/L, a fundoscopic examination is recommended in order to screen for indicators of hyperviscosity, such as retinal venous engorgement with dilated and tortuous veins and focal constrictions ("venous sausaging") or flame-shaped retinal hemorrhages in the macular area [7].

If hyperviscosity syndrome is suspected, plasmapheresis and treatment of WM should be started quickly to rapidly remove IgM.

Thromboembolism Patients with WM present an increased risk of venous thromboembolism, especially during the first year following diagnosis but also up to 10 years after [35]. Unlike patients with multiple myeloma, no excess risk of

arterial thrombosis was described. There is no consensual approach concerning venous thromboembolism prophylaxis for WM patients yet; however, this prophylaxis should be considered in periods of additional risk, such as surgery [16].

Bleeding Syndrome Monoclonal IgM component may interact with circulating proteins or with proteins expressed on cell membranes. Interaction with platelets, fibrinogen, and coagulation factors (especially factors V, VII, and VIII) may cause thrombopathy and coagulation disorders, such as acquired hemophilia, acquired von Willebrand disease, or defects in fibrin formation.

Clinically, these alterations are responsible for abnormalities in bleeding and clotting times [12].

Acquired von Willebrand Syndrome (AVWS) [36] Among manifestations caused by IgM in WM, the occurrence of acquired von Willebrand syndrome (AVWS) is a rare event, characterized by bleeding related to the decrease of von Willebrand factor (VWF) activity [37]. By contrast with congenital von Willebrand disease, patients do not have any personal or family history of bleeding.

VWF is an adhesive glycoprotein produced by the vascular endothelium and megakaryocytes, which plays a key role in primary hemostasis. Multiple pathogenic mechanisms are described in AVWS, including selective VWF adsorption on tumoral cells, increased VWF proteolysis, and the presence of both neutralizing and non-neutralizing anti-VWF antibodies [38].

In a recent study including 72 patients with WM, 13 % fulfilled criteria of AVWS [37]. Patients with AVWS had higher serum IgM concentration, higher serum viscosity, and more frequent cryoglobulinemia than unaffected patients; it appeared to be unrelated to the bone marrow infiltration.

AVWS could offer protection against venous thromboembolism and can lead to sometimes severe hemorrhagic events, even though patients often present biological features without clinical manifestation [37].

Systematic screening of AVWS should be performed in patients with WM as it may have important clinical implications. If an AVWS is detected, treatment includes plasma exchanges which by reducing hyperviscosity allow a quick resolution of the VWF defect, desmopressin, or replacement therapy to cover invasive procedures [37].

Recommendation

It is recommended to identify patients at risk for hyperviscosity and, in parallel, patients that will present with bleeding disorders, particularly prior to performing biopsies.

17.5 Biochemical Investigations

Diagnostic biological workup for WM is summarized in Table 17.2.

Table 17.2 Diagnostic biological workup recommended for WM, adapted from [12]

Hematological workup	
Cell blood count Peripheral smear	Normocytic or macrocytic anemia, normochromic, reticulocyte count, thrombocytopenia Rouleaux on smear
Hemolytic workup	Elevated reticulocyte count, bilirubin, haptoglobin, LDH, Coombs, cold agglutinin
Hemostasis workup	Acquired VWD
Bone marrow	
Histological analysis	Lymphoplasmacytic cells (small lymphocytes with plasmacytoid differentiation and plasma cells)
Immunophenotype	sIg^+, $CD19^+$, $CD20^+$, $CD22^+$, $CD79^+$, $CD5^{+/-}$, $CD23^{+/-}$, $CD138^+$, $CD10^-$, $CD103^-$
Cytogenetic	6q deletion by FISH in 30–50 % of cases
Molecular biology	MYD88 L265P mutation in 90 % of cases Discussion on CXCR4 mutation to identify chemoresistance and decide for specific treatment
IgM immunoglobulin measurement	
SPEP, IFS	Monoclonal Ig difficult to detect if cryoglobulin, sampling to be done, maintained, and performed at 37°. Lower sensitivity in low level, migration defect. Monitor hypogammaglobulinemia
Proteinuria	It is possibly more important to check for albuminuria (glomerulopathy of cryoglobulinemia type, AL amyloidosis type, renal infiltration by tumor cells) and that can simply be done with urine bandelette UPEP and IFU: Bence-Jones proteinuria frequently positive but exceptionally associated to occurrence of CAST nephropathy
sFLC	Serum free light chain is increased similar to IgM to perform possibly in patients informative to sFLC and pauci informative on IgM measurement
Plasma viscosity	Elevated viscosity > 3cp: indication of treatment and plasmapheresis
Beta-2 microglobulin	Prognostic marker

LDH lactate dehydrogenase, *SPEP* serum protein electrophoresis, *IF* immunofixation, *sFLC* serum free light chain, *UPEP* urine protein electrophoresis

Serum IgM Monoclonal Protein Biochemically, IgM monoclonal protein remains the hallmark of WM; indeed, the definition of WM [1] requires the presence of a monoclonal IgM protein, irrespective of its serum level—although most of the time, this level is > 5 g/L.

The first clue to diagnosis of WM is often the identification of the presence of an excess of serum protein (elevated serum sedimentation rate and/or elevated serum protein level) suggesting an IgM monoclonal component. The presence of a monoclonal component is often characterized using serum protein electrophoresis (SPEP). An immunofixation is then recommended to demonstrate clonality and further characterize the isotype of the monoclonal component; this immunofixation

should be performed in all cases, since SPEP could miss small quantities of IgM. The IgM M-spike can sometimes migrate into the beta region rather than the gamma region. In rare cases, two M-spikes can be identified, which represent either the association of monomeric and pentameric forms of IgM or true biclonality. Class switching with corresponding IgG or IgA M-spikes is unusual.

An elevated IgM is usually associated with a reciprocal hypogammaglobulinemia in WM, made of a profound decrease in polyclonal IgM along with IgG and IgA. There is no correlation between the serum level of IgM and the percentage of bone marrow involvement or to the depth of hypogammaglobulinemia.

However, many limitations are associated with the use of this IgM marker, and development of more accurate markers is needed as discussed below.

Limitations to IgM Measurement in WM Even though IgM monoclonal protein remains the key marker in WM, many limitations can be argued against its use:

- The definition of WM [1] requires the presence of a monoclonal IgM protein, irrespective of its serum level, which means that serum IgM level is not sensitive enough to differentiate WM from Monoclonal Gammopathy of Undetermined Significance (MGUS).
- Although the presence of serum monoclonal IgM protein is the hallmark of WM, it is not a disease-specific finding. A study found that nearly 60 % of patients with IgM paraprotein-related lymphoid neoplasms had WM; other causes included CLL (20 %), marginal-zone lymphoma (7 %), follicular lymphoma (5 %), DLBCL (5 %), and MCL (3 %) [39]. Furthermore, serum IgM paraprotein level was not a reliable discriminator in the differential diagnosis.
- The measurement of IgM can be obtained by SPEP (serum protein electrophoresis) or by nephelometry, but none of these techniques allow measurement of the exact concentration of monoclonal IgM. Furthermore, a number of studies have identified discordance between the two methods, preventing direct comparison of results [40]. IgM measurement using nephelometry is not a valuable technique, not only does it not allow differentiation between clonal and non-clonal Ig but it is affected by technical problems.
- It is also difficult and unreliable to compare IgM-spike concentrations from different laboratories.
- Moreover, monoclonal Ig can be difficult to detect in some WM patients, due to the presence of cryoglobulins, because of the migration defect of the IgM protein or because low IgM concentrations can be obscured by the polyclonal background.
- In the IPSS WM score [2], IgM has an adverse prognostic impact only when >70 g/L, a rare occurrence at such a very high serum level: IgM serum level is, thus, not a sensitive prognostic marker.
- In addition, even a high IgM serum level is not a sufficient indication by itself to initiate therapy [41] because IgM does not correlate to tumor mass.

- However, although serum IgM level is not correlated to tumor mass, it is included in the response criteria for WM [42] but with considering the above limitations in its measurement.
- As IgM has a prolonged half-life (IgM clearance from the serum takes about 3 weeks) [43], it is not a sensitive marker for early response.
- Moreover, serum IgM can vary differently depending on the treatment. Serum IgM level may indeed increase following rituximab therapy, and distinction between progression and flare effect might be difficult to affirm [44], making response or progression assessment impossible in this situation. Following nucleoside analogues, response can be delayed by months, sometime a 1-year period of time, and, therefore, patients with delayed response can be miscategorized as non- or poor responders [42]. Conversely, bortezomib-containing regimens may demonstrate excellent IgM responses, while suboptimal bone marrow responses [45].

For all these reasons, there is a need to identify new surrogate markers for measurement of tumor burden in WM, especially for patients with low or high IgM levels, and extramedullary disease.

Study of Urine Proteinuria in WM There are numerous reasons for renal alteration in WM, and one must be aware of the various types of syndromes that can be encountered to properly guide the tests needed to optimally treat the renal alterations in WM, to avoid worsen the prognosis often associated to renal insufficiency.

Although Bence-Jones proteinuria [identified using urine electrophoresis (UPEP) with subsequent urine immunofixation (IFU)] is frequently present in WM patients (40–80 %), it rarely exceeds 1 g/24 h (about 3 % of cases) and is, therefore, not always routinely performed [12]. Furthermore, CAST nephropathy as a consequence of excess of Bence-Jones proteinuria is rarely seen in WM [46].

Glomerular nephropathy is more often seen in WM as a consequence of several kidney alterations, among which the most frequent are glomerulopathy of cryoglobulinemia type, AL amyloidosis type, and renal infiltration by tumor cells [47]. The diagnosis of these various types of renal alteration is performed on a kidney biopsy using histopathology tests, along with red congo staining, polarized birefringence, and immunofluorescence identification of light chain deposits for AL amyloidosis. Consequently, one would then recommend to seek for the presence of albuminuria and to quantify the importance of albuminuria, using, for example, a very simple test in routine practice, i.e., the urine bandelette.

Cryoglobulins Cryoglobulins (type I anti-IgM monoclonal component related or type II with immune complex of monoclonal IgM anti-IgG type) can occur in WM and can lead to underestimation of the serum IgM levels and increased blood viscosity. Diagnosis is confirmed by measurement of cryoglobulins using a warm

bath collection; this test should be repeated if necessary. The temperature range of cryoglobulins can be wide.

Type I cryoglobulinemia may be detected in 7–20 % of WM patients but is symptomatic in less than 5 % [12]. Due to impaired blood flow in small vessels, manifestations such as Raynaud phenomenon, acrocyanosis, arthralgias, purpura, and skin ulcers can occur [48, 49]. It is usually associated with lymphoproliferative disorders.

Type II cryoglobulinemia results from the autoantibody activity of the monoclonal IgM against the Fc portion of IgG, acting as a monoclonal rheumatoid factor [12, 48, 49]. Rheumatoid factor blood test is, thus, usually positive. The cryoprecipitating property results from the size and limited solubility of the IgM–IgG immune complex. Deposition of these immune complexes on the walls of small vessels and subsequent activation of the complement cascade can result in a systemic vasculitis—clinical features of which are purpura, arthralgias, weakness, liver involvement, renal involvement (cryoglobulinemic glomerulonephritis), peripheral neuropathy, and widespread vasculitis [12]. Type II cryoglobulinemia has been associated with the hepatitis C virus.

Dosage of antinuclear antibodies and syphilis serology can be falsely positive in case of the presence of cryoglobulins. Patients with cryoglobulinemia should be screened for hepatitis C infection [16].

If a symptomatic cryoglobulinemia is identified, plasmapheresis is indicated to rapidly remove cryoglobulins from the blood circulation.

Complement Exploration Among abnormalities in complement exploration, serum C4 levels can be found very low. Exceptionally, an acquired C1 esterase inhibitor deficiency can be observed, leading to angioedema [50].

Serum Free Light Chain Test The serum free light chain (sFLC) assay is a nephelometric measurement of serum kappa and lambda light chains that circulate as light chain monomers or dimers and that are not bound to immunoglobulin heavy chain. It can be analyzed whether using iFLC (involved FLC level), ratio kappa/lambda, or dFLC (difference between clonal and non-clonal sFLC); the most informative being iFLC, although the difference or the ratio are recommended for patients with some degree of renal insufficiency. This test has been widely used in the assessment of response in patients with multiple myeloma and other plasma cell dyscrasias and is now relatively widely available among laboratories worldwide.

It has been previously shown that the use of iFLC values accurately diagnosed patients with WM and differentiated them from patients with IgM MGUS (monoclonal gammopathy of undetermined significance) who presented with significantly lower values [51, 52]. sFLC assay was further demonstrated to be a useful and sensitive marker of tumor measurement in WM that correlates well with IgM and M-spike measurement [52]. Response according to iFLC was indeed comparable with the response obtained using SPEP or IgM measurement by nephelometry, indicating that it can be used in the future as a reliable measurement of disease response [52]. However, there are two particular interests in iFLC measurement:

first, it allowed a more rapid detection of response and progression than standard tests studying intact immunoglobulin, due to its significantly shorter half-life [53], and next, it has been proven a sensible marker to assess lower tumor burden compared with SPEP.

sFLC could also be useful in situations where SPEP is defective. For instance, a case of Waldenstrom's macroglobulinemia with type I cryoglobulinemia was reported where quantification of the paraprotein was not possible using conventional serum protein electrophoresis due to the high serum viscosity, but monitoring using serial sFLC measurements was proved successful [54].

Moreover, sFLC should be measured and followed if amyloidosis is suspected.

sFLC can also be used as a prognostic marker in WM, as elevated iFLC has been shown to correlate to shorter time to first treatment [55]. Interestingly, patients with early iFLC response seem more likely to have intermediate/high ISS-WM stage, elevated β-2 microglobulin or low hemoglobin levels [52, 56]. These results may be indicating that high iFLC and rapid reductions after therapy could reflect a more aggressive disease.

There are caveats to sFLC test in WM, such as the flare effect previously reported with IgM measurement and which has also been observed with sFLC test. An iFLC flare was observed following rituximab therapy, with a lack of correlation with IgM flare, which suggests that iFLC cannot help to differentiate progression from flare in this situation [52], similar to serum IgM measuring tests. No correlation between iFLC flare and response rate was observed.

Further studies should help determine whether sFLC assay will help in making decision of treatment modification in WM.

Hevylite Assay (HLC for Heavy/Light Chain) The serum IgM Hevylite immunoassay measures specifically serum IgM kappa and IgM lambda separately [57, 58]. It is based on specific polyclonal antibodies, which recognize unique epitopes spanning the junction of the heavy and light chains of the individual immunoglobulin isotypes. This test allows true quantitative measurement of the intact immunoglobulin involved in the clone serum secretion, e.g., the IgMk M-spike for IgM kappa WM. The test measures separately the involved and uninvolved immunoglobulin for all existing immunoglobulins. Involved IgM HLC measurements indeed reflect the clonal IgM production, and quantification of uninvolved IgM HLC assesses the tumor-induced polyclonal suppression, e.g., the hypogammaglobulinemia, both more precisely than available tests.

It has been shown that the IgM Hevylite ratio (involved monoclonal immunoglobulin/uninvolved polyclonal immunoglobulin) correlated well with the M-spike measured by SPEP [55, 59].

Hevylite levels were indeed higher in WM than in IgM MGUS and higher in symptomatic than asymptomatic WM. Interestingly, high Hevylite serum levels predicted a shorter time to treatment for patients with asymptomatic WM, traducing a shorter survival free of evolution towards symptomatic WM requiring treatment. Koulieris et al. also showed that Hevylite was a potential prognostic marker of high tumor burden and poor prognosis. They have proposed a prognosis score including

Hevylite level above median, beta-2 microglobulin > 5.5 mg/L, and elevated LDH at diagnosis, which distinguished four groups of patients with significantly different overall survival (the four groups were constituted according to the number of adverse factors: 0, 1, 2, or 3) [60].

Hevylite, thus, represents a promising technique to monitor the monoclonal IgM in WM.

These novel M-component-based biomarkers appear to be reliable markers for the diagnosis of WM, for the monitoring of WM, and potentially the prognosis of WM. They are easy to perform in routine practice, which make these tools of potential choice to replace IgM measurement. However, they do not seem to be really superior to IgM measurement, at least for sFLC. More studies are needed to demonstrate the impact of Hevylite in WM and whether the couple sFLC and Hevylite could replace the current tests for all patients with WM or solely a subset yet to identify.

Spurious Results Monoclonal proteins can interfere in certain measurements because they may form precipitates during the procedure. This can result in artifactually increased total bilirubin concentration, low high-density lipoprotein and cholesterol, and low ferreting and transferring [61].

Recommendation
It is recommended to use SPEP and IFS to monitor IgM M-component. In certain cases, sFLC could be of some help. Cryoglobulinemia should be looked at particularly in symptomatic WM patients to prevent complications and to optimize measurement of IgM M-component in these patients. Urine albuminuria is far more important to investigate compared to Bence-Jones proteinuria, given the greater risk of glomerulopathy over CAST tubular nephropathy.

17.6 Flare Effect

IgM flare is defined as any increase (relative to baseline) in IgM levels that occurred before the end of therapy and that would not be related to disease escape, e.g., progression of WM. As such, this phenomenon should be transient and decrease thereafter to the level of the depth of response.

This phenomenon was first suggested when it was reported that some patients treated with rituximab or cladribine for WM developed transient increases in monoclonal protein levels [62, 63], and its exact mechanism remains unraveled. It was then further characterized by Ghobrial et al. who found that an IgM flare could be observed in up to 54 % of patients following rituximab monotherapy, but that 73 % of these patients will have a decrease of their IgM levels within 4 months of therapy [44]. This phenomenon, thus, should not discourage physicians from continuing to administer this treatment. Response rates were, however, poorer for patients who experienced an initial IgM flare compared with those who did not (28 % versus 80 %). In patients with high IgM levels (>40 g/L), the use of

rituximab as single-agent therapy should be avoided, as considerably lower response rates were reported in those patients [64, 65]. Similarly, the IgM flare can occur in the maintenance phase of rituximab therapy and can be mistaken for progression [7]. A bone marrow biopsy should be performed if differentiating these two situations appears difficult.

Patients with increased IgM levels after treatment might develop hyperviscosity and require plasmapheresis, and IgM flare may also lead to worsening of IgM-related neuropathy, cryoglobulinemia, and other IgM-related complications [7]. Serum IgM levels should, therefore, be closely monitored (at least weekly) during the time of risk to develop flare effect, for example, early on during the first cycle on rituximab-based therapy, particularly if not combined to regular chemotherapy. The IgM flare may last for several weeks and even months [44, 66]. No factor has been identified for predicting the occurrence of this IgM flare.

In combination therapy, the occurrence of the rituximab-mediated IgM flare can vary considerably and seems to depend both on the associated drugs and the administration schedule of rituximab [67]. For instance, bortezomib appears to suppress IgM production independently of tumor cell killing [7].

In order to try and avoid the rituximab-related IgM flare and its possible effects on IgM-related morbidity, the introduction of rituximab can be deferred in patients considered at high risk, arbitrarily defined by an IgM level > 40 g/L and/or a plasma viscosity > 4 cP [16].

17.7 Other

Workup for Peripheral Neuropathy Peripheral neuropathy is frequent in IgM dyscrasia, including WM, as it has been reported in about 25 % of patients, with various etiology to consider [7]. One of the most frequent mechanisms involved in IgM-related peripheral neuropathy, including WM, is related to demyelinization and is the consequence of the anti-myelin autoactivity of the IgM monoclonal component.

Common etiologies related to WM are lymphoplasmacytic infiltration of the nerve fibers, IgM deposition, autoantibody deposition, cryoglobulinemia, and amyloidosis; differential diagnosis includes radiculopathy, diabetic neuropathy, cobalamin deficiency, thyroid dysfunction, HIV infection, Lyme disease, autoimmune processes such as systemic lupus erythematosus and other vasculitides, and chronic inflammatory demyelinating polyneuropathy.

If a peripheral neuropathy is suspected, the etiologic workup should include: measurement of serum anti-MAG (anti-myelin-associated glycoprotein) antibodies, anti-ganglioside M1 antibodies, anti-sulfatide IgM antibodies, along with electromyography signature. The nerve biopsy should be avoided when it comes to diagnosis of anti-MAG neuropathy as it affects sensitive nerves that are not comprised in the biopsy. If performed, one could visualize IgM deposits at the site of MAG localization or sulfatide moieties on nerve sheaths. However, this biopsy might be helpful for the differential diagnosis such as peripheral neuropathy

related to cryoglobulin deposits and AL amyloidosis [7, 12]. Amyloidosis can also be ruled out using other histology exams such as salivary glands and/or fat pad biopsy and congo red staining.

Hepatic and Renal Function Renal and hepatic functions are to be assessed before any therapy.

Renal failure is rarely a manifestation of WM. Proteinuria can be observed due to deposition of the monoclonal protein in the kidneys. CAST nephropathy as a consequence of excess of Bence-Jones proteinuria is, however, a rare event in WM [46]. Proteinuria still needs to be assessed, but mainly to detect albuminuria. If albuminuria is detected, suggesting a glomerulopathy, prompt investigation should indeed be performed to detect AL amyloidosis, cryoglobulinemia, or infiltration of the kidney by tumor cells [7].

Hypercalcemia is unusual in WM.

HIV, Hepatitis B and C Status Viral status should be assessed before starting therapy. Screening for hepatitis B and C viruses is specifically required prior to the introduction of rituximab-containing regimens, and hepatitis C should be screened in case of cryoglobulinemia [16]. The role of chronic antigenic stimulation was suspected in WM; however, the implication of hepatitis C or HHV8 viruses remains controversial [68].

17.8 Prognostic Markers

As for most cancer types, the prognosis of WM can be summarized based on three main entities: (i) the disease itself, e.g., the intrinsic agressivity of tumor cells, (ii) the patient characteristics, e.g., a mix of age and comorbidities, particularly given that WM is a disease of the elderly, and finally (iii) the sensitivity of tumor cells to treatment, e.g., a prolonged progression-free survival that translates into a prolonged overall survival, in patients that would not die from WM but from other age-related causes.

Interestingly, one may consider that the patient- and disease-based entities of the prognosis of WM are summarized into the International Staging System (ISS WM) [2]. This prognosis score of WM depends on five major factors: age > 65 years, anemia < 11.5 g/dL, thrombocytopenia $< 100 \times 10^9$/L, β-2 microglobulin > 3 mg/L, and IgM level > 70 g/L. Low risk disease is defined by 0–1 adverse features excluding age, intermediate risk by two adverse factors or age, while high-risk is defined by >2 adverse features. These risk categories each comprise approximately 1/3 of patients and are associated with 5-year survival rates of 87 %, 68 %, and 36 %, respectively.

This staging system appears robust and was validated in the relapse setting as well, with the main caveats that it was created in the era of "old" WM-based

treatments. Another limitation would be that there is no evidence yet that it should influence treatment decisions for individual patients.

Elevated LDH, low albumin, B symptoms, poor performance status, and increased C-reactive protein, but not transformation to high-grade B cell non-Hodgkin lymphoma (DLBCL), have also been associated to poor prognosis.

17.9 New Biomarkers to Measure the Activity of Tumor Cells in WM Independently of the M-Component

Soluble CD27 Soluble CD27 (sCD27) is a tumor necrosis factor family member secreted by WM cells and was proposed to be a faithful marker of disease burden in WM [69]. It was demonstrated to strongly correlate to serum IgM levels and clinical responses in patients with WM and is unaffected by the rituximab-induced flare or by plasmapheresis. However, it cannot be used in routine lab practice and, therefore, did not develop beyond research purpose.

17.10 Conclusion

Major progress have been made in understanding the pathophysiology of WM, and biological tests are now of great interest in the characterization of this disease. Biological markers may lead to personalized treatments in patients with WM. New biomarkers are under investigation, offering a quantitative alternative to traditional electrophoretic techniques, and could, therefore, be of great use in the management of patients with WM.

Disclosures of Potential Conflicts of Interest None.

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Part V

Response

Response Assessment in Waldenström's Macroglobulinaemia

18

Eva Kimby, Roger G. Owen, and Enrica Morra

18.1 Introduction

Waldenström macroglobulinaemia (WM) is a distinct B-cell lymphoproliferative disorder characterized by the presence of a monoclonal immunoglobulin M (IgM) in serum and an infiltration of a lymphoplasmacytic lymphoma in bone marrow [1, 2]. Recent studies have demonstrated that the majority of patients have a MYD88 L265P mutation and that this is central to the pathogenesis of the disorder but also is of diagnostic utility [3].

Besides the bone marrow, the tumour cells can infiltrate lymphoid organs, such as lymph nodes and spleen. The most common symptoms of the disease are fatigue, anaemia and thrombocytopenia. Symptoms can also occur as a consequence of the concentration and physical properties of the M-component, and these include hyperviscosity syndrome, cryoglobulinaemia, cold agglutinin disease, peripheral neuropathy and amyloidosis.

Formal criteria for assessment of treatment response were first proposed during the Second International Workshop on WM and were published in 2003 [4]. These were developed with the principle aim of promoting and facilitating the uniform reporting of clinical trials. Evolving biological knowledge about the disease and new targeted therapies leading to new treatment paradigms along with requirements

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_18

of regulatory agencies have resulted in re-evaluation of the response criteria following the Second, Third and Sixth International Workshops [4, 5, 6]. The consensus process by the eight (IWWM8) International Task Force on response assessment chaired by Roger Owen will further update the 2013 criteria. This effort will result in refined categorical response criteria with a better definition of bone marrow and lymph node response.

It is acknowledged in developing response criteria that they should be evidence based and that categories of response predict outcome, at least in terms of progression-free survival (PFS), but also allow for meaningful comparison with historical data.

Criteria for response in nodal non-Hodgkin lymphoma and classical Hodgkin lymphoma have also been updated recently, the so-called Lugano criteria [7], but these are currently not recommended in WM as evidence of their predictive value is lacking. In this disease, symptoms due to the elevated monoclonal IgM and bone marrow infiltration of tumour cells are dominating and it is unusual for patients to be treated on the basis of nodal and/or splenic disease in isolation [8].

The assessment of response in patients with IgM-related disorders such as anti-MAG neuropathy, cryoglobulinaemia and cold agglutinin disease requires specific criteria to assess clinical benefit.

Uniform reporting of response may aid physicians in the routine clinical management of individual patients. Symptomatic benefit in patients remains the main goal of therapy and this can occur also in the absence of high-quality categorical responses. However, reproducible response criteria are needed for prediction of outcomes in clinical trials and for allowing comparison with historical data.

18.2 Categorical Response Definitions

Categories remain based on assessment of sequential changes in IgM as these have been demonstrated to predict outcome in terms of PFS with rituximab-based therapy. The value of the response definition is less well established in patients with relapsed disease. Continued prospective evaluation of these criteria is encouraged in clinical studies.

The response categories are outlined in Table 18.1. Besides conventional complete response (CR) and partial response (PR), two specific response categories have been used in WM: very good PR (VGPR) and minor response (MR) [9, 10]. Also the category major response rate (MRR), defined as the proportion of patients obtaining a PR or better, is frequently used.

The most appropriate time point for categorical response assessment has been discussed. The varying kinetics of IgM responses across different therapies (see below) and the advent of continuous targeted therapies such as ibrutinib make the documentation of best response necessary [11]. The reporting of time to best response is also a clinically meaningful criterion particularly for patients where rapid disease control is relevant such as hyperviscosity syndrome.

It is essential that treatment responses continue to be critically evaluated in clinical studies and in this context, pre-planned bone marrow and extramedullary

Table 18.1 Categorial response definitions

Response category	Definition
Complete response (CR)	• Absence of serum monoclonal IgM protein by immunofixation • Complete resolution of extramedullary disease, i.e. lymphadenopathy and splenomegaly if present at baseline • Absence of lymphoplasmacytic lymphoma cells in bone marrow aspirate and trephine biopsy • No new signs or symptoms of active disease
Very good partial response (VGPR)	• Monoclonal IgM protein is detectable by immunofixation and IgM within normal range and/or • ≥90 % reduction in serum IgM level from baseline • No new signs or symptoms of active disease
Partial response (PR)	• Monoclonal IgM protein is detectable • ≥50 % but <90 % reduction in serum IgM level from baseline • No new signs or symptoms of active disease
Minor response (MR)	• Monoclonal IgM protein is detectable • ≥25 % but <50 % reduction in serum IgM level from baseline • No new signs or symptoms of active disease
Stable disease (SD)	• Monoclonal IgM protein is detectable • <25 % reduction and <25 % increase in serum IgM level from baseline • No new signs or symptoms of active disease
Progressive disease (PD)	• ≥25 % increase in serum IgM level from lowest nadir and/or[a] • New signs or symptoms of active disease. • Progression in anaemia and/or thrombocytopenia attributable to the disease

[a]An absolute increase of at least 5 g/l is required to define progression when the IgM level is the only applicable criterion

disease assessments are encouraged regardless of IgM responses. The time points chosen for such assessments may vary and should be determined by choice and duration of the therapies under evaluation.

All categories of response should be confirmed by a second assessment at minimum of 4 weeks after initial assessment and the date of the first assessment should be used for response duration calculations.

18.2.1 Complete Remission

Absence of serum monoclonal IgM protein by immunofixation and a normalized serum IgM level are required for CR. Full recovery of polyclonal IgG, IgM and IgA are not, however, required as they frequently remain reduced following a range of therapies [12]. Bone marrow evaluation is mandatory for confirmation of CR, requiring absence of morphological infiltration by lymphoplasmacytic lymphoma in both bone marrow aspirate and trephine biopsy. Complete resolution of lymphadenopathy and splenomegaly, if present at baseline, confirmed by imaging and disappearance of WM-associated symptoms is also required for a CR.

There is only limited data on minimal residual disease (MRD) assessment in WM [13], but further studies are strongly encouraged given the prognostic value of MRD assessment in patients with CLL and myeloma [14, 15]. The most appropriate methodology has not yet been established, but multiparameter flow cytometry along with molecular methods utilizing either the MYD88 L265P mutation or the unique immunoglobulin gene sequence are all potentially applicable and have their merits. Prospective evaluation of these techniques is encouraged in clinical studies. Such studies should also evaluate methods that are applicable to the peripheral blood as non-invasive techniques are clearly desirable.

18.2.2 Very Good Partial Response

The VGPR category was introduced in the 2013 criteria [6], as it had been noted that a group of patients with this type of response had comparable prognosis to that of patients in CR [10]. Proposed criteria now suggest, given the large variations in IgM concentrations that occur between patients, that VGPR is defined by the presence of monoclonal IgM on immunofixation and serum IgM within the normal range and/or $\geq$90 % reduction in serum IgM. For VGPR, there is no requirement for complete resolution of extramedullary disease or bone marrow confirmation.

18.2.3 Partial Remission

Monoclonal IgM protein is detectable but with >50 % but <90 % reduction in serum IgM level from baseline and >50 % decrease in adenopathy/organomegaly, if present at baseline, on physical examination or on CT scan. No new symptoms or signs of active disease.

18.2.4 Minor Response

MR was also introduced in the 2013 criteria and is defined by a >25 % but <50 % reduction in serum IgM level from baseline. This category was introduced as a consequence of data that demonstrated an outcome benefit over non-responders [9, 10].

18.2.5 Stable Disease and Non-response

SD is defined as those patients who do not meet the criteria for either MR or progressive disease (PD). This category will largely consist of patients who have not experienced any symptomatic benefit and who require alternative treatment and are, thus, considered as “non-responders”. It may be appropriate to define non-response as lack of at least a MR following a minimum of 3 months therapy.

Repeat marrow assessment may be of value in this context particularly with treatments associated with slow or delayed IgM responses and selective bone marrow B-cell depletion (see below).

18.2.6 Progressive Disease

Progressive disease is defined by an increase of at least 25 % in IgM compared to the lowest recorded level (nadir) and needs to be confirmed by a repeat assessment. An absolute increase of at least 5 g/l is required to define progression, when the IgM level is the only applicable criterion.

The development of new signs and symptoms should be suspicious for progression, even in the absence of IgM increase. However, PD requires the demonstration that new symptoms are specifically due to WM and not to other causes such as treatment toxicity and other co-morbidities. In case of unclear anaemia and/or thrombocytopenia, a bone marrow evaluation is required to reaffirm or exclude progressive/refractory disease.

A new enlarged lymph node and/or progressive lymphadenopathy may require biopsy to confirm progressive disease. Histological transformation, Bing–Neel syndrome and the development of amyloidosis are all considered as progressive disease.

18.3 Survival Outcome Definitions and Other Efficacy Endpoints

The proposed efficacy measures for clinical trials are defined in Table 18.2. At this time, progression-free survival (PFS) remains the most appropriate primary survival outcome measure for use in clinical trials.

Table 18.2 Survival outcome definitions and other efficacy endpoints

Endpoint	Definition
Overall survival	Time from the initiation of treatment to death from any cause
Cause-specific survival	Time from the initiation of treatment to death censoring for deaths from unrelated causes
Progression-free survival (PFS))	Time from the initiation of treatment to disease progression or death from any cause
Time to progression (TTP)	Time from the initiation of treatment to disease progression with deaths due to unrelated causes censored
Disease-free survival (DFS)	Time from the first documentation of CR to disease progression with deaths due to unrelated causes censored
Duration of response (DOR)	Time from the first documentation of response to disease progression with deaths due to unrelated causes censored
Time to next treatment (TTNT)	Time from the initiation of treatment to initiation of next line of therapy

Data regarding time to achieve best response and time to next line of treatment (TTNT) are also important efficacy measures for patients with WM.

Overall survival (OS) is clearly a relevant criterion to report, but it is becoming increasingly difficult to interpret as patients may receive novel effective therapies at disease progression. Moreover, mortality unrelated to WM is a significant confounding factor especially for older patients [16, 17] and cause-specific survival is, therefore, of interest.

Disease-free survival (DFS) is often reported for patients achieving CR, while duration of response (DOR) is applicable for all responding patients.

18.4 Specific Comments for Response Evaluation in WM

18.4.1 Monoclonal Immunoglobulin M

Sequential quantitative assessment of IgM is the principal response measure in WM. Quantitative IgM responses are determined either by M protein quantitation or total serum IgM quantitation. Total IgM values as assessed by nephelometry are systematically higher than M protein values determined by densitometry [18, 19]. It is, therefore, crucial that sequential response assessments for individual patients are performed in the same laboratory with the same methodology (i.e. densitometry or nephelometry).

The percentage reduction in IgM has been demonstrated to predict progression-free survival (PFS) in patients treated with rituximab-based regimens [9, 10]. It remains unclear whether this is also predictive of overall survival outcome, as patients at progression may now receive highly active therapeutic agents leading to increase in overall survival [17].

The kinetics of IgM response can vary considerably with treatment. IgM reduction may be seen late with alkylating agents, purine analogues and monoclonal anti-CD20 antibodies (rituximab and ofatumumab) [20–22] and is mostly more rapid with bortezomib- and ibrutinib-based therapies [11, 23–25]. Another factor to consider is the "IgM flare" phenomenon, which has been described largely not only in the context of therapy with single agent rituximab [26] but also transient rising in IgM during temporary drug holds (as for surgery) with ibrutinib.

Discrepancies can be found between IgM and lymph node/bone marrow responses. For instance, purine analogue-based therapies may be associated with significant bone marrow response, but suboptimal IgM response [21, 27], while bortezomib and everolimus can be associated with excellent IgM response, but discordant marrow and tissue responses [23, 28].

A number of studies have shown the serum free light chain assay and the hevylite assay to be informative in WM [29–32]. There is, however, insufficient data to support their inclusion in the consensus criteria and further prospective study is encouraged. The serum free light chain assay is, however, useful in the monitoring of patients with WM-related amyloidosis and those rare patients with light chain associated cast nephropathy [33].

18.4.2 Bone Marrow

The assessment of bone marrow post-treatment is of value for response evaluation in WM, but is only mandatory for confirmation of a CR. Heterogeneity in marrow responses has been shown after some therapies, particularly those containing monoclonal antibodies, alkylating agents and purine analogues [21, 27]. These therapies appear to deplete the B-cell component, but spare the plasma cell component which may explain at least in part the delayed IgM responses seen with such therapies [20–22]. The CD20-positive clonal B-cells are often depleted after rituximab therapy, while CD138-positive clonal plasma cells may persist, which may influence disease phenotype at relapse/progression and subsequent choice of therapy.

Morphological quantitation of disease on trephine biopsies is subjective and is unlikely to be reproducible across institutions and is not used for categorization of response in WM. Flow cytometry can be used to quantitate residual B-cells and might be applicable to WM patients on the basis of the WM-specific $CD22^{weak}$ CD25+ immunophenotype [34]. In this setting, Garcia-Sanz and colleagues have demonstrated that a residual B-cell burden of $>5\,\%$ is associated with an inferior outcome [13].

Thus, accurate and reproducible quantitative methods are desirable in WM and planned sequential marrow assessments are encouraged in all clinical trials. Also, molecular methods utilizing either the MYD88 L265P mutation or the unique immunoglobulin gene sequence are potentially applicable.

18.4.3 Lymph Nodes/Splenomegaly

Regress of measurable/palpable lymphadenopathy/splenomegaly is considered indicative of a response to therapy in WM, but the impact on survival outcomes has not been established. In the updated Lugano criteria [7], a pathologic lymph node is defined by a longest dimension of >15 mm and pathological splenomegaly by a longitudinal measurement of >13 cm and response to therapy is evaluated with PET/CT. In WM, lymphadenopathy is documented in only around 15 % of newly diagnosed patients, but is more common if a CT is performed, as well as in patients with advanced disease and those without the MYD88 L265P mutation [8, 11, 35–37]. Evidence is, however, lacking for the need of CT and PET imaging for most categorical responses, and currently, CT is recommended only for the confirmation of CR in patients with evaluable nodal and/or splenic disease.

In WM patients with a suspected histologic transformation to a more aggressive disease, PET/CT might be useful to guide biopsy, like in patients with other indolent lymphoproliferative disorders [38, 39].

18.4.4 Haemopoietic Recovery

Incomplete or suboptimal haemopoietic recovery remains a difficult issue in WM response assessment since some treatments may themselves impact haemopoietic recovery. Anaemia remains one of the principal indications for therapy in clinical trials and the impact of therapy on haemoglobin should be recorded in parallel to conventional categorical response.

18.5 Conclusions

Evidence-based data for response evaluation in WM patients are still in progress. The updated consensus panel response definitions include evaluation of the monoclonal immunoglobulin M in serum, the tumour burden in bone marrow and in extramedullary sites and symptoms of disease. The value of quantitative assessment of the tumour in bone marrow, imaging techniques for extramedullary/nodal disease and assessment of haematological response is still under discussion. New biomarkers such as MYD88, CXCR4 and other mutations and exploratory endpoints associated with clinical benefit/improvement will need further studies.

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Part VI

Prognostic Factors

Risk Stratification in Waldenström Macroglobulinemia

19

Pierre Morel and Bénédicte Hivert

19.1 Introduction

Waldenström macroglobulinemia (WM) is a rare lymphoproliferative disorder characterized by the production of serum monoclonal immunoglobulin (Ig) M and lymphoplasmacytic (LPL) bone marrow infiltration [1, 2]. Whole genome sequencing identified a new specific molecular abnormality, the $MYD88^{L265P}$ mutation, in up to 90 % of patients as discussed elsewhere. These molecular studies also identified other abnormalities in a significant proportion of patients, such as mutations in the TNFAIP gene and the CXCR4 gene [3]. Median age at diagnosis is around 65 years in most series [4–10].

Assessing the prognosis in WM is not an easy issue. First, the patterns of evolution are very heterogeneous; for example, at least 25 % of patients are asymptomatic at diagnosis and 50 % of asymptomatic patients who are observed will not require therapy within 3 years [5, 11]; one in 10 within 10 years [5, 11]. Furthermore, the availability of many effective therapies during the recent years likely improved the outcome and survival; thus, the events required for the power of statistical tests or estimates are less and less frequent and consequently, more and more prolonged follow-up is mandatory. In addition, a multiplicity of events besides the progression of the disease such as treatment-related late adverse events

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_19

or unrelated comorbidities may occur more and more frequently during this prolonged follow-up.

19.2 Overall Survival Estimates

Median overall survival (OS) ranged from 60 to 77 months in patients who received alkylating agent or purine analogs alone as first-line therapy, with 10 year OS estimates at 41 % [4, 6, 12–14]. In a large population-based study of 1555 patients with LPL and WM diagnosed in Sweden from 1980 to 2005, 1- and 5-year relative survival rates improved significantly during the study period in all age groups. Patients diagnosed after 2000 had significantly lower excess mortality compared with patients diagnosed in the preceding periods [15]. Similarly, median overall survival of 5784 WM patients diagnosed between 1991 and 2010 from the Surveillance, Epidemiology, and End Results (SEER) database was 6 and 8 years for the 1991–2000 and the 2001–2010 periods, respectively. Thus, the former estimate was in agreement with previous estimates. Symptomatic and asymptomatic patients were not recognizable in these studies and the lead-time bias (i.e., smaller follow-up and exposure time to events in recent patients) may be an unavoidable limit of these comparisons [16].

In contrast with previous studies, no survival improvement was observed when comparing the outcomes of patients with symptomatic WM who started treatment before January 1, 2000 (mainly with alkylating agent-based regimens) and patients who started treatment later (mainly with rituximab-based regimens) [17]. The prolonged and indolent course of WM in some patients, the limited effectiveness of initial therapy in other patients with high-risk disease, may explain this disappointing result because of an insufficient follow-up duration in former patients or the persistence of a subset of latter patients refractory to rituximab-based regimen.

In addition, the advanced age of a large subset of patients is associated with the presence of competing causes of death unrelated to the disease. Unrelated deaths and progression of the WM with symptoms related to marrow failure or transformation to high grade lymphoma are the main causes of death [5, 6]. Second malignancy and infection were the causes of 31 and 19 % of deaths recorded in the Spanish series [5]. Transformation to high grade lymphoma occurred in approximately 2 % of patients [5, 18, 19] and solid tumors in 10–14 % of patients during the follow-up of patients with WM [5, 6, 18, 20] or even before the diagnosis [6]. Cumulative incidence of solid cancer and second malignancy has been estimated 17 and 8 % at 15 years, respectively, with an overall risk of second cancer in WM 1.69 times higher than expected ($P = 0.002$). WM patients were at increased risk for diffuse large B-cell lymphoma [standardized incidence ratio (SIR) 9.24, $P < 0.0001$], myelodysplastic syndrome/acute myeloid leukemia (SIR 8.4, $P < 0.0001$), and brain cancer (SIR 8.05, $P = 0.0004$) [18] without clearly identified risk factor [18, 21]. For this reason, disease-specific survival has been considered as an appropriate endpoint because it controls many unrelated deaths [5, 9, 22]. However, related and unrelated deaths may be very difficult to

distinguish for many (16 %) elderly patients because of the various disease-related complications that may occur during the course of the disease [6]. In the recently published analysis of SEER data, only death from leukemia, lymphoma, or myeloma was considered WM related and 29 % of causes of death were undefined. With these assumptions, the competing-risk analysis identified a significant relative improvement in WM-related as well as non-WM-related deaths during the last decade. In a Greek study, WM related causes of death were those due to progressive disease, transformation (myelodysplastic syndrome or diffuse large B-cell lymphoma), infections, or treatment-related complications. There was no difference in the crude mortality between patients who received primary therapy with rituximab and those who did not. However, patients who did not receive primary therapy with rituximab had significantly higher 5-year WM-related death rate than patients who received rituximab-based therapy but significantly lower 5-year WM-unrelated death rate, probably because more elderly and frail patients had received primary therapy with rituximab. The comparison of relative mortality of this subgroup of symptomatic patients with that observed in a general population of individuals with similar age and gender distribution may also accurately express the effects of the disease [23, 24].

Altogether, these data suggest that survival estimates should be considered very cautiously for estimating survival of currently managed patients. As indicated above, the downward trend in the occurrence of WM-related events and the improvement of outcome of the general population are associated with a trend upward of follow-up duration required for getting the required number of events for accurate estimates. Therefore, the treatment of patients enrolled in a prognostic study is frequently different from that proposed at the time of its publication. This is an unavoidable limitation in the clinical usefulness of available survival estimates.

19.3 Prognostic Value of the Presence of at Least One of the Criteria for Initiating Therapy

More than 10 years ago, a consensus panel agreed that initiation of therapy was appropriate for patients with constitutional symptoms, progressive or symptomatic lymphadenopathy or splenomegaly, anemia with a hemoglobin value of 10 g/dL or lower, thrombocytopenia with a platelet count lower than 100×10^9/L due to marrow infiltration, hyperviscosity syndrome, symptomatic sensorimotor peripheral neuropathy, systemic amyloidosis, renal insufficiency, or symptomatic cryoglobulinemia [25]. The presence of at least one of these initiation treatment criteria is a strong prognostic factor that should be systematically taken into account in prognostic studies: Asymptomatic WM had a mortality rate equivalent to that of the general population, whereas the standardized mortality ratio of patients with symptomatic WM was 5.4 [24]. However, some of the treatment initiation criteria (lymphadenopathy or splenomegaly, hyperviscosity syndrome, constitutional symptoms, IgM-related disorder) retained no prognostic role in most studies designed in symptomatic patients only [10].

19.4 Risk Assessment in Asymptomatic Patients

Asymptomatic WM is defined as a serum IgM monoclonal protein concentration of 3 g/dL or higher and/or bone marrow lymphoplasmacytic infiltration of 10 % or greater and no evidence of criteria for initiating therapy. The risk of progression of asymptomatic to symptomatic Waldenström macroglobulinemia has been estimated 6 % per year, and progression within 5 years had been observed in 55 % of patients with asymptomatic Waldenström macroglobulinemia [26]. Twenty-one percent of the previously untreated asymptomatic patients enrolled in the prospective SWOG study [8] required therapy later, with a median follow-up of 100 months. The median time to evolution has been estimated 46–141.5 months for indolent WM [26–28]. Main variables adversely related to evolution were high monoclonal IgM [26–28], hemoglobin concentrations [27, 28], the degree of bone marrow infiltration [26], and the degree of reduction of uninvolved Ig [26, 27]. In addition, a significant correlation between initial free light chain serum concentration and time to treatment initiation was observed in asymptomatic patients ($p = 0.047$) [29].

19.5 Prognostic Factors in Symptomatic WM

Response rates between 26 and 92 % and from 55 to 96 % have been reported after single-agent first-line therapy [4, 8, 13, 30–33] and combination chemotherapy or chemo-immunotherapy, respectively [34–42]. Median response durations and/or progression-free survival (PFS) from 7 to 29 months have been observed in most reports on single-agent regimen [4, 8, 13, 31, 43–46]. These endpoints were around 36 months after purine analogs alone [47] and up to 42 months after chemo-immunotherapy [34, 39–42, 48].

Most prognostic studies focused on overall survival. Despite the description of treatment initiation criteria, many studies have pooled symptomatic and asymptomatic patients. The cutoff values and the statistical significance would probably have been different if studies had focused on the two main categories of WM patients, namely asymptomatic or symptomatic patients only.

The main adverse prognostic factors were older age [4–7, 9, 10, 23, 49, 50], anemia [4–8, 10, 23, 50], low albumin serum concentrations [6, 8, 23], high β2-microglobulin (β2M) values [5, 8, 9, 51], leukopenia [4, 6, 9], and thrombocytopenia [4, 6, 7, 9, 49, 50]. The different cutoff values associated with these covariates are summarized in Table 19.1.

An adverse prognostic value has been reported also in association with the following characteristics: a poor performance status (World Health Organization criteria) more than 1 [9, 49], hepatomegaly [5, 6], organomegaly, splenomegaly [9], 2 cytopenias or pancytopenia [6, 49], cryoglobulinemia [7, 23], male gender [4, 6], hyperviscosity [5, 9], urine monoclonal component [5], C-Reactive protein more than 1 mg/L [8], the presence of constitutional symptoms or weight loss [4, 5, 7], and a diffuse pattern of bone marrow histology [49].

Table 19.1 Hazard ratio associated with main clinical prognostic factors in WM patients at the time of first-line therapy

Characteristics	Cutoff	Prognostic information (hazard ratio)[a]	
		Treated patients only	All patients
Advanced age	>60 years	2.85 [4]	2.28 [4]
	>65 years	1.95 [50]; 2.5 [10]; 2.5 [9]	1.7 [6]; 2.6 [5]
	>70 years	3.15 [7]; 2 [8]	
Low hemoglobin concentration	<9 g/dL	1.95 [7]	
	<10 g/dL	2.28 [4]; 1.28 [50]; 1.4 [9]	2.32 [4]
	<10.5 g/dL		2.49 [5]
	<11.5 g/dL	1.7 [10]	
	<12 g/dL	1.9 [8]	2.18 [6]
Low platelet count	$<100 \times 10^9/mm^3$	1.94 [50]; 1.76 [10]	
	$<150 \times 10^9/mm^3$	2.27 [4]; 1.6 [9]	2.4 [4]; 1.67 [6]
Low absolute neutrophil count	$<1.5 \times 10^9/mm^3$	1.93 [10]	
High β2-microglobulin	>3 mg/L	2,5 [8]; 1.93 [10]	
	>4 mg/L	1.45 [51]	
	Continuous	1.4 [9]	
Low serum albumin concentration	<40 g/L	2 [9]	
	<35 g/L	1.45 [50]; 1.34 [10]; 1.7 [8]	
Monoclonal IgM concentration	<40 g/L	2.4 [8]	
	>45 g/L		2.48 [5]
	≥25 g/L		1.15 [6]
	≥70 g/L	1.83 [10]	

Abbreviations: *IgM* immunoglobulin M, *HR* hazard ratio

[a]All series included more than 200 patients, except [4, 7, 50] 122–167 patients

The international prognostic scoring system for WM (IPSSWM) has been designed only to predict survival after first-line therapy in symptomatic patients [10] in a series of 587 patients mainly treated with alkylating agent (63 %) or purine analog (33 %). Besides most of the adverse features listed above (age more than 65 years, hemoglobin less than or equal to 11.5 g/dL, platelet count less than or equal to 100×10^9/L, β2M more than 3 mg/L), IPSSWM also takes into account monoclonal IgM serum concentration more than 70 g/L for identifying three risk groups with significantly different 5-year survival rates [10]. Besides age, all other prognostic factors included in the IPSSWM indicate the presence of a high tumor

burden. IPSSWM and most other prognostic systems identified only limited subgroups of young high-risk patients (6–24 % of young patients) [6, 8, 10, 52]. Thus, the rarity of young high-risk patients may be a distribution feature mainly related to the disease itself rather than a drawback of scoring systems. IPSSWM has been validated in patients prospectively treated with alkylating agents and nucleoside analogs [21] and in patients who received rituximab-based therapy [22]. Several studies supported the usefulness of lactate dehydrogenase, mainly in the subgroup of IPSSWM high-risk patients [12, 49, 53].

Besides the clinical features, characteristics of the microenvironment may also play a role in the behavior of tumoral clone. The angiogenin concentration correlated with albumin levels, while vascular endothelial growth factor-A correlated with β2M. Angiopoietin-1/angiopoietin-2 ratio showed a negative correlation with β2M and positive correlations with albumin, hemoglobin, and lymphadenopathy [54]. High von Willebrand factor (VWF) concentration has been identified in 59 % of 72 consecutive patients. This characteristic was associated with an adverse survival and survival after first-line treatment. Moreover, VWF antigen (VWF:Ag) level and IPSSWM (or each parameter of this scoring system) retained independent prognostic values. Since IPSSWM was built as a combination of age and covariates mainly related to tumor burden, VWF:Ag may express the presence of a microenvironment favorable to growth and survival of tumor cells. High VWF concentration was associated with chronic endothelial activation, increased bone marrow microvessel density, and the presence of mast cells on vascular endothelial growth factor [55].

Genomic and molecular abnormalities of malignant lymphoplasmacytic cells may also account for the heterogeneity of the disease and provide useful prognostic information (Table 19.2). A 17p13 (TP53) deletion (8 % of the patients) has been associated with a shorter PFS and a shorter disease-free survival and trisomy 12 (less than 5 % of patients) with a shorter PFS (12.2 months versus 31.2) even when adjusting for treatment arm and IPSSWM risk groups [56].

Genetic polymorphisms may also influence the outcome of WM patients. Thus, IL6 (−174G/G) was associated with an adverse survival after first-line therapy only in patients aged 65 years or less, probably because this genotype was associated with young age [57]. The CXCL12 (−801G/G) genotype [58] has been associated also with adverse survival after first-line therapy. The clinical characteristics associated with $MYD88^{L265P}$ mutation and warts, hypogammaglobulinemia, infections and myelokathesis (WHIM) syndrome-like mutations in CXCR4 gene have been evaluated in two studies yet. Treon et al. [6] genotyped lymphoplasmacytic cells from 175 WM patients (symptomatic or asymptomatic). Schmidt et al. reported 90 patients including 51 LPL/WM patients (1 with IgG paraprotein). Both studies reported lower platelet count in $MYD88L^{L265P}P/CXCR4^{WHIM}$ patients. Bone marrow involvement was lower in $MYD88^{WT}/CXCR4^{WT}$ patients and higher in $MYD88^{L265P}/CXCR4^{WHIM}$ patients. Hyperviscosity syndrome was more frequent in the latter patients and absent in the former patients. $MYD88^{WT}/CXCR4^{WT}$ patients were older in the Treon series; the two patients with this unfrequent genotype of the series of Schmidt had cold agglutinin disease.

Table 19.2 Hazard ratio associated with main biological characteristics in WM patients

Unfavorable characteristics	Population characteristics			Prognostic information	
	Nb patients	Disease status	Treatment	Endpoint	Hazard ratio
VWF: Ag > 250 % [55]	10/55	Symptomatic, first line	Various	OS after first-line therapy	5.08
TP53 deletion [56]	11/140	Symptomatic, first line	Chlorambucil versus fludarabine	PFS	1.66
Trisomy 12 [56]	6/140	Symptomatic, first line	Chlorambucil versus fludarabine	PFS	2.58
IL6 (−174G/C) [57]	22/75 (12/29 in pts aged <65)	Symptomatic, first line	Various	OS after first-line therapy	4.29
CXCL12 (−801GG) [58]	48/82	Symptomatic, first line	Various	OS after first-line therapy	8.52
$MYD88^{WT}$ [59]	15/175	Symptomatic and asymptomatic	Various	OS	10.54 (age and B2M adjusted risk of death)

Abbreviations: *VWF Ag* von Willebrand factor antigen, *OS* overall survival, *PFS* progression-free survival, *B2M* β2 microglobulin

$MYD88^{L265P}$/$CXCR4^{WT}$ patients more frequently presented with lymphadenopathy in both studies; the difference was significant in the Treon's report. Patients with $MYD88^{WT}$/$CXCR4^{WT}$ had a shorter estimated overall survival (asymptomatic and symptomatic patients were pooled and there was a high proportion of early censored patients at the stopping date of the analysis).

Finally, microRNA expression may also play a prognostic role: decreased expression of six microRNAs significantly correlated with a low IPSSWM risk in a series of 20 patients [60].

19.6 Prognostic Role of Response to Treatment

Response criteria have been defined and updated in the last few years. Briefly, a complete response is defined by the resolution of all symptoms, normalization of serum IgM concentration with complete disappearance of IgM monoclonal protein by immunofixation, a bone marrow biopsy demonstrating no evidence of disease, and resolution of any adenopathy or splenomegaly. A minor response (MR), a partial response (PR), and a very good partial response (VGPR) were defined as achieving a 25–49 %, 50–90 %, and >90 % reduction in serum IgM levels, respectively [61]. However, delayed IgM monoclonal protein responses may cause important difficulties in response assessment [61]. In addition, discrepancies

between the kinetics of serum M protein reduction and the clearance of monoclonal B-cells from the bone marrow have been reported [62]. Nevertheless, the prognostic value of these discrepancies remains to be evaluated.

The prognostic role of response on OS had been reported earlier [4]. On one hand, achievement of at least a VGPR was associated with improved PFS ($P < 0.0001$) in 159 WM patients who received rituximab-based therapy [63] and in patients who received the fludarabine rituximab combination [35]. No difference in PFS was observed between CR and VGPR [63]. Similarly, a landmark analysis showed the absence of significant difference in subsequent PFS or survival between patients who achieved a negative immunofixation test within the first 6 months after autologous stem-cell transplantation (ASCT) and those patients who retained a positive immunofixation test [64]. On the other hand, patients who achieved a MR have been reported to do as well as those achieving an objective response after rituximab single agent therapy [65]. Furthermore, achieving at least a MR to salvage therapy with fludarabine containing regimen was also significantly associated with prolonged subsequent survival [66].

Using allele-specific PCR assay, $MYD88^{L265P}$ mutation was detectable and quantifiable in the blood of most (97 %) untreated WM and 62 % of previously treated WM. Similarly to serum IgM, peripheral blood $MYD88^{L265P}$ ΔCt is correlated to bone marrow disease burden. Peripheral blood $MYD88^{L265P}$ ΔCt correlated also with serum IgM and hemoglobin levels in IgM MGUS and WM patients (including previously treated patients) [67]. Therefore, the prognostic role of the information provided by this test besides serum IgM concentration and bone marrow assessment remains to be evaluated.

Finally, there is probably a need for improving response evaluation, taking the potential prognostic role of bone marrow evaluation and $MYD88^{L265P}$ load into account, in order to express the prognostic consequences of responses achieved with new treatments.

19.7 Risk Factors Associated with Type of Treatment and Predictive Factors of Response

Reports on new treatment approaches provided also valuable prognostic information for response achievement and/or duration of response. Most treatment studies reported prognostic factors similar to covariates included in the IPSSWM. However, specific predictive factors have been identified for cladribine or rituximab single-agent regimen. Most predictive factors for response and/or response duration are summarized in Table 19.3.

Drug resistance was observed in $CXCR4^{S338X}$ cells exposed to Bruton's tyrosine kinase, mammalian target of rapamycin, and phosphatidylinositol 3-kinase inhibitors, but not proteasome inhibitors [74]. Indeed, Cao et al. [4] showed that the most common $CXCR4^{WHIM}$-like somatic mutation in WM ($CXCR4^{S338X}$), present in up to 30 % of WM patients, confers decreased CXCL12-triggered

Table 19.3 Adverse prognostic factors for treatment effectiveness in Waldenström's macroglobulinemia

Treatment	Patients	Adverse characteristic for response, response duration, or survival		
		Response rate	Response duration, PFS	Subsequent survival
Fludarabine (71 patients) [68]	Previously treated	Short interval between first treatment and fludarabine		Hemoglobin < 9.5 g/dL, platelet $< 75 \times 10^9$/L
Fludarabine (182 patients) [8]	Untreated and previously treated	Age ≥ 70 years	β2-m ≥ 3 mg/L and mIgM < 40 g/L (PFS)	Age ≥ 70 years, previous therapy, disease duration >1 year, β2-m ≥ 3 mg/L, mIgM < 40 g/L, and CRP ≥ 1 mg/L
Fludarabine cyclophosphamide (49 patients) [37]	Untreated and previously treated		Age > 65 years and mIgM < 40 g/L	Age > 65 years and mIgM < 40 g/L
Cladribine (29 patients) [69]	Untreated and previously treated	**Low levels of human concentrative nucleoside transporter 1 (hCNT1)**		
Cyclophosphamide rituximab fludarabine (43 patients) [70]	Untreated and 1 prior treatment	High β2-m concentration		
Rituximab (58 patients) [30]	Untreated and previously treated	**Fcγ ReceptorIIIA(F/F) phenotype**		
Rituximab (159 patients) [63]	Rituximab-naïve patients	**Fcγ receptors (FcγR)3A-48 (L/L) and FcγR 3A-158 (F/F)**		
Rituximab (51 patients) [32]	Untreated and previously treated	mIgM ≥ 40 g/l and serum albumin < 35 g/L	mIgM ≥ 40 g/L (TTP)	Serum albumin <35 g/L
Bortezomib rituximab (37 patients) [71]	Previously treated	Age >65 years		
Ibrutinib (63 patients) [72] Previously treated		$MYD88^{WT}CXCR4^{WT}$ unfavorable; **$CXCR4^{WHIM}$ unfavorable among $MYD88^{L265P}$ patients**	High IPSSWM at progression, >3 previous regimen, and $MYD88^{WT}$ $CXCR4^{WT}$ genotype	
Perifosine (37 patients) [73]	Previously treated		β2-m > 3.5 mg/L	

Abbreviations: *β2-m* β2-microglobulin, *mIg M* monoclonal immunoglobulin M, *CRP* C-reactive protein, *PFS* progression-free survival, *TTP* time to progression. Only significant tests are reported in table. Predictive factor of a specific treatment are indicated in *bold* characters

CXCR4 internalization, enhanced AKT and ERK activation, and resistance to ibrutinib-triggered apoptosis in WM cells. Similarly, patients with advanced WM and with $MYD88^{L265P}$/$CXCR4^{WT}$ had significantly higher best serum IgM and hemoglobin response rates and higher major response rates after ibrutinib therapy than patients with $MYD88^{L265P}$/$CXCR4^{WHIM}$. However, patients with $MYD88^{WT}$ $CXCR4^{WT}$ retained lower major and overall response rates than remaining $MYD88^{L265P}$ mutated patients.

19.8 Risk Assessment After Relapse or Before Salvage Therapy

In patients with relapsing or refractory WM, overall response rate ranged from 89 to 90 % in recently reported trials of bortezomib and rituximab combined with dexamethasone [71], everolimus [75] and in the ibrutinib trial [72]. Median PFS were estimated 15.6, and 21 months in former trials respectively and 2-year was PFS 69 % in the latter report.

The SWOG study of the efficacy of fludarabine in WM included untreated and previously treated patients. The hazard ratio associated with previous therapy was estimated 1.61 [8]. Few reports focused on the prognosis after relapses or failures requiring therapy. Levy et al. [76] found a strong prognostic value associated with the Lille scoring system based on the total number of cytopenia (mainly anemia and thrombocytopenia), age, and serum albumin concentration. The prognostic role of IPSSWM has been assessed before salvage therapy including fludarabine in 51 patients [66]. Patients had been more heavily treated before enrollment than patients of the previous report by Levy et al. [76]. Patients at high risk before the initiation of salvage therapy had a shorter subsequent survival than remaining patients (Hazard ratio: 10.18, $p = 0.019$) without difference in survival of low- and intermediate-risk patients. The prognostic value of IPSSWM in patients with advanced WM has been recently confirmed in the ibrutinib study [72]. Kyriakou et al. analyzed 158 adult patients with WM reported to the European Group for Blood and Marrow Transplantation [64]: chemorefractory disease and a poor performance status at ASCT were associated with higher non-relapse mortality in univariate analysis. Having received at least three treatment lines before ASCT and having chemorefractory disease at ASCT were adverse prognostic factors for response rate after ASCT. Heavily pretreated patients, patients with refractory disease, and those with poor performance status had shorter PFS and OS in univariate analysis.

Future studies should assess the prognostic role of previous therapy because the hazard ratio estimate provided by the SWOG study has probably changed with the use of combination therapy as first-line therapy. In addition, it is conceivable that the type of previous therapy, initial response, and the response duration may also have a prognostic role in addition or in place of clinical covariates assessed at the time of initiation of salvage therapy.

19.9 Conclusion

The identification of asymptomatic and symptomatic disease and the assessment of IPSSWM remain the main tools for prognostic assessment in WM patients and for making treatment decision at the time of the initiation of the first-line therapy. However, therapy options have changed during the last few years. Therefore, available estimates of survival should be taken with caution when used for currently managed patients. IPSSWM has been designed to be improved, with the assessment of biological characteristics of the disease. However, appropriate demonstration of the prognostic role of new characteristics should take into account the information provided by conventional characteristics (symptomatic versus asymptomatic and IPSSWM). In addition, predictive factors and comorbidity assessment should also be considered for treatment decision.

Waiting an efficient surrogate endpoint of OS that could avoid the observation of a more and more prolonged follow-up, a sufficient number of events remains to be reported in prognostic studies. Hopefully, this is now a difficult paradigm because of the availability of very effective new agents.

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Part VII

Treatment Options and Recommendations

Indications for Treatment of Waldenström's Macroglobulinemia

20

Robert A. Kyle, Stephen M. Ansell, and Prashant Kapoor

Seventy-two years ago, Jan Gosta Waldenström described two patients with oronasal bleeding, thrombocytopenia, normochromic anemia, elevation of the erythrocyte sedimentation rate, lymphadenopathy, and low serum fibrinogen levels [1]. A large homogeneous gamma globulin component with a sedimentation coefficient of 19–20 and a molecular weight of approximately one million was present in both patients. The serum viscosity was elevated and the serum of one patient precipitated at low temperatures indicating cryoglobulinemia. The serum globulin was subsequently identified as an immunoglobulin and was later designated as IgM. The protein results from the proliferation of clonal cells with lymphocyte and plasmacytoid characteristics. The clone develops from a post-germinal center IgM memory B-cell that has undergone somatic hypermutation but not isotype class switching. The typical immunophenotype consists of surface IgM^+, $CD19^+$, $CD20^+$, $CD5^-$, $CD10^-$, and $CD23^-$. Mutation of the MYD88 gene (L265P) is present in approximately 90 % of patients with Waldenström's macroglobulinemia (WM) [2]. The presence of C1013G/CXCR4 [warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM)]-mutation is found in 28 % of WM patients [3]. Copy number alterations and structural variants have been reported [4]. Paiva et al. have recently reported that specific copy number abnormalities [+4, del (6q23.3–6q25.3), +12, and +18q11–18q23] increase from the precursor states, IgM MGUS and smoldering Waldenström's macroglobulinemia (SWM) [5]. There was considerable overlapping of phenotypic profiles in the three entities.

The diagnosis of Waldenström's macroglobulinemia (WM) depends upon the presence of an IgM monoclonal protein of any size in the serum and 10 % or more infiltration of the bone marrow aspirate or biopsy by monoclonal small

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_20

lymphocytes and/or plasma cells in an intertrabecular pattern [6]. This is manifested as a lymphoplasmacytic lymphoma.

The onset of WM is usually insidious and the patient complains of slowly developing weakness and fatigue. Constitutional symptoms including fever, night sweats, and weight loss may occur. Chronic oozing of blood from the nose and gums as well as pallor may be noted. Epistaxis and bleeding from the gastrointestinal tract may occur. Hepatomegaly is found in up to 25 % of patients at diagnosis, while splenomegaly and generalized lymphadenopathy are slightly less common.

Neurologic abnormalities are found in one-fifth of patients [7]. Peripheral neuropathy is characterized by a distal, symmetric, and slowly progressive sensorimotor process. The lower extremities are usually more involved than the upper extremities. Anti-myelin-associated glycoprotein (anti-MAG) is found in almost one-half of patients with the sensorimotor neuropathy, but the titer of anti-MAG correlates poorly with the clinical features. Infiltration of the meninges by plasmacytoid lymphocytes is uncommon. Retinal vein engorgement with vascular segmentation (sausaging), flame-shaped hemorrhages, and exudates are common with hyperviscosity, but papilledema is rare. Occasionally, diffuse pulmonary infiltrates or masses may be seen. Renal involvement is uncommon. A bleeding diathesis is common and results from interference of the IgM protein with clotting factors and platelet function as well as hyperviscosity.

20.1 Precursor Conditions

20.1.1 IgM Monoclonal Gammopathy of Undetermined Significance

MGUS of the IgM class was recognized in 213 Mayo Clinic patients residing in the 11 counties of Southeastern Minnesota from 1960 to 1994 [8]. The median age at diagnosis was 74 years (range 24–94 years) but only 1 % were younger than 40 years and almost two-thirds were older than 70 years. Fifty-eight percent were men. The size of the M protein ranged from unmeasurable (visible on electrophoresis but not quantifiable by densitometry) to 2.6 g/dL with a median of 1.2 g/dL. Fifteen percent of patients had a normal nephelometric IgM value (<300 mg/dL). IgM kappa light chain was found in 70 % and lambda in the remaining 30 %. Thirty-five percent had a reduction in one or more uninvolved immunoglobulins. Only three patients had more than 100 mg of urinary light chain/24 h. Anemia, thrombocytopenia, or renal insufficiency did not result from WM and was unrelated to the lymphoplasmacytic proliferative process.

These 213 patients were monitored for 1567 person-years during which time 71 % died. During follow-up, non-Hodgkin lymphoma developed in 17 persons, WM in 6, AL amyloidosis in 3, and chronic lymphocytic leukemia in 3 persons. The standardized incidence rate of progression was increased 15.9-fold compared to an expected rate of 1.8-fold based upon the Iowa SEER registry (Table 20.1). The cumulative probability of progression to one of these disorders was 10 % at 5 years, 18 % at 10 years, and 24 % at 15 years (Fig. 20.1). The overall average risk of

Table 20.1 Observed and expected progression and standardized incidence rates among 213 patients with IgM monoclonal gammopathy of undetermined significance

Disease progression	Observed	Expected[a]	SIR	95 % CI
Non-Hodgkin lymphoma	17	1.1	14.8	8.6–23.7
Amyloidosis	3	0.18	16.3	3.4–47.5
Macroglobulinemia	6	0.02	262	96.0–569.5
Chronic lymphocytic leukemia	3	0.53	5.7	1.2–16.5
Total group	29	1.83	15.9	11.0–22.8

MGUS was diagnosed between 1960 and 1994 in patients in southeastern Minnesota
SIR indicates standardized incidence rate; 95 % CI, 95 % confidence interval
[a]Iowa SEER Registry
This research was originally published in Blood. Kyle RA et al., Long-Term follow-up of IgM monoclonal gammopathy of undetermined significance. Blood. 2003;Vol 102:3759–3764. © the American Society of Hematology

progression was approximately 1.5 % per year. Patients were at risk for progression even after having had a stable MGUS for 20 years or more.

It is important to recognize that the rates of death due to cardiovascular or cerebrovascular diseases and nonlymphoid or plasmacytic malignancies were 31 % at 5 years, 52 % at 10 years, and 65 % at 15 years reflecting an older population. In a competitive model, the rates of progression to lymphoplasmacytic malignancies were 8 % at 5 years, 12 % at 10 years, and 15 % at 15 years (Fig. 20.2).

Risk factors for progression consisted of the concentration of the serum M protein at the time of recognition of MGUS ($p = 0.03$) and the serum albumin value ($p = 0.01$). These factors were the only independent predictors of progression on multivariate analysis. Reduction in one or more uninvolved immunoglobulins or the presence of a monoclonal light chain in the urine did not appear to be risk factors for progression in this series. The relative risk for progression was directly related to the concentration of the M protein in the serum at the time of recognition of MGUS. The risk of progression to lymphoma or a related malignancy 10 years after recognition of MGUS was 14 % for an initial M protein value of 0.5 g/dL or less but 41 % for those with an initial M protein of 2.5 g/dL (Fig. 20.3).

20.1.2 Smoldering Waldenström's Macroglobulinemia

Virtually all patients with symptomatic WM have passed through IgM MGUS and then SWM, but the latter phase is infrequently recognized. SWM is defined as the presence of a serum IgM monoclonal protein ≥ 3 g/dL and/or ≥ 10 % bone marrow lymphoplasmacytic infiltration without evidence of end-organ damage such as constitutional symptoms, symptomatic lymphadenopathy or hepatosplenomegaly, or hyperviscosity that can be attributed to a lymphoplasmacytic proliferative disorder [6, 9]. Clinically, all patients with SWM have had an IgM MGUS.

Nineteen percent of 452 patients with WM diagnosed at Mayo Clinic appeared to have had SWM. The majority of excluded patients had no bone marrow

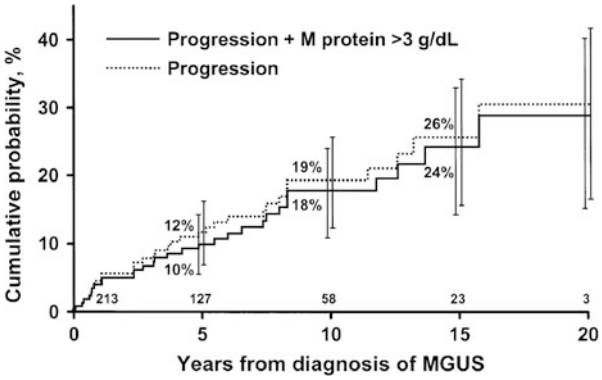

Fig. 20.1 Probability of progression in 213 patients. Patients were residents of southeastern Minnesota in whom monoclonal gammopathy of undetermined significance (MGUS) of IgM class was diagnosed from 1960 through 1994. Curve shows probability of progression of MGUS to lymphoma, Waldenström macroglobulinemia, primary amyloidosis, or chronic lymphocytic leukemia. Bars show 95 % confidence intervals. Numbers at bottom of the horizontal axis are numbers of patients at risk at each interval. This research was originally published in Blood. Kyle RA et al., Long-Term follow-up of IgM monoclonal gammopathy of undetermined significance. Blood. 2003; Vol 102:3759–3764. © the American Society of Hematology

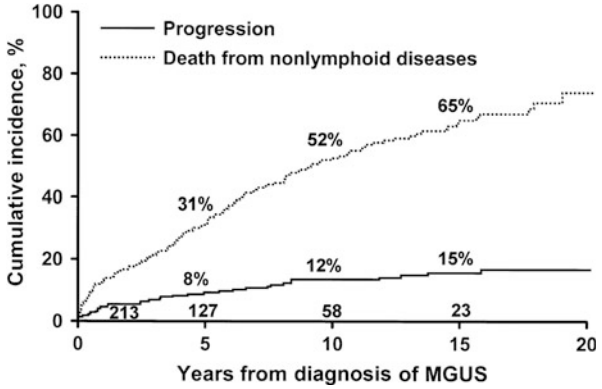

Fig. 20.2 Competitive model in 213 patients. Patients were residents of southeastern Minnesota in whom monoclonal gammopathy of undetermined significance (MGUS) of IgM class was diagnosed from 1960 through 1994. Upper curve shows probability of dying of nonlymphoid diseases. Lower curve shows probability of progression to lymphoma or a related disorder. This research was originally published in Blood. Kyle RA et al., Long-Term follow-up of IgM monoclonal gammopathy of undetermined significance. Blood. 2003; Vol 102:3759–3764. © the American Society of Hematology

examination results within 30 days of diagnosis and five others were excluded because of biclonal gammopathy. The median age of the 48 documented patients was 63 years with 2 % younger than age 40. Two-thirds were male. Asymptomatic palpable hepatosplenomegaly was present in 20 %, while asymptomatic

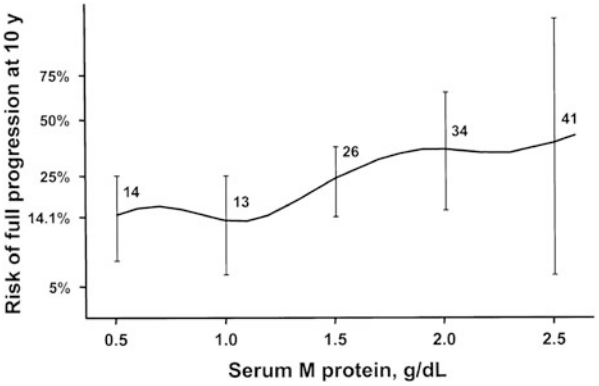

Fig. 20.3 Relative risk of disease progression in 213 patients. Patients were residents of southeastern Minnesota in whom monoclonal gammopathy of undetermined significance (MGUS) of IgM class was diagnosed from 1960 through 1994. Risk is by monoclonal protein value at diagnosis. Error bars show 95 % CI. This research was originally published in *Blood*. Kyle RA et al., Long-Term follow-up of IgM monoclonal gammopathy of undetermined significance. *Blood*. 2003;Vol 102:3759–3764. © the American Society of Hematology

lymphadenopathy was noted in 10 % [10]. An initial hemoglobin value < 10 g/dL was the result of unrelated causes and all had < 50 % lymphoplasmacytic infiltration of the bone marrow. The serum monoclonal (M) protein level at diagnosis ranged from 1.5 to 5.2 g/dL (median 3.3 g/dL). IgM kappa was present in 75 %. Levels of uninvolved (background, normal) immunoglobulins were reduced in 47 %. Only five patients had a urine M protein > 200 mg/24 h. Serum albumin ranged from 2.5 to 4.3 g/dL with a median of 3.6 g/dL. Ten percent had a serum albumin level < 3 g/dL. Lymphoplasmacytic infiltration of the bone marrow aspirate and biopsy ranged from 3 to 80 % (median 30 %).

During a median follow-up of 15.4 years, 71 % progressed to WM requiring chemotherapy, 1 to AL amyloidosis, and 1 to lymphoma (total progression 75 %). One would have expected 0.004 cases of WM on the basis of the Surveillance, Epidemiology, and End Results data. Thus, the relative risk of progression was increased 8034-fold for the whole cohort.

The cumulative probability of progression to symptomatic WM requiring therapy, AL amyloidosis, or lymphoma was 6 % at 1 year, 39 % at 3 years, 59 % at 5 years, and 68 % at 10 years (Fig. 20.4). Thus, the cumulative probability of progression was 12 % per year for the first 5 years and then 2 % per year for the next 5 years.

The percentage of bone marrow lymphoplasmacytic cells, size of the serum monoclonal spike, hemoglobin value, and the presence of IgA reduction were identified as significant factors for progression. The percentage of lymphoplasmacytic infiltration of the bone marrow was the most important risk factor for progression. The size of the serum M protein and hemoglobin value each

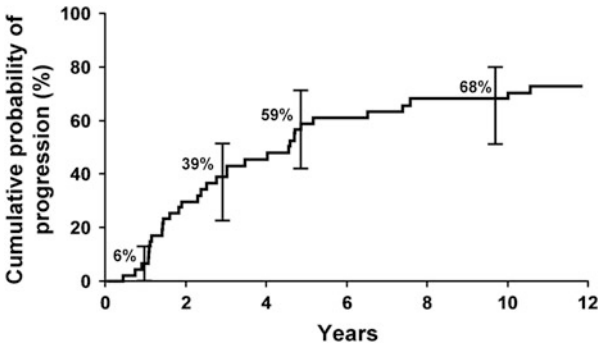

Fig. 20.4 Cumulative probability of progression to symptomatic WM requiring therapy, amyloidosis, or lymphoma in the cohort of 48 patients with SWM. This research was originally published in *Blood*. Kyle RA et al., Progression in smoldering Waldenström macroglobulinemia: long-term results. *Blood*. 2012;Vol 119:4462–4466. © the American Society of Hematology

contributed significantly to a model already containing the percentage of lymphoplasmacytic bone marrow cells (Table 20.1).

During the first 5 years, 92 % of 13 patients who had 50 % or more lymphoplasmacytic cells in the bone marrow at diagnosis progressed, while 46 % of those with a bone marrow lymphoplasmacytic infiltration < 50 % progressed ($p = 0.001$) (Fig. 20.5). Sixty-one percent of patients with an M protein ≥ 3 g/dL and 10 % or more bone marrow lymphoplasmacytic infiltration progressed at 5 years. The risk of progression at 5 years was 49 % for those with an M protein < 3 g/dL and a bone marrow lymphoplasmacytic level ≥ 10 %. The median level of lymphoplasmacytic infiltration in the bone marrow was 15 % in the 12 patients who did not progress during follow-up (Table 20.2).

Seventy-five percent of the 48 patients with SWM died during follow-up. The overall survival of the 48 SWM patients was 83 % at 5 years and 50 % at 9.6 years (Fig. 20.6). Seven patients died of cardiovascular disease or other unrelated disorders before developing WM or a related disorder. The median survival after progression to symptomatic WM was 5.1 years. Only five SWM patients are alive and still at risk for progression; they have been followed for 14–24.6 years [10].

20.1.3 Waldenström's Macroglobulinemia

The clinical features of WM relate to the organs involved. Clinically, it is important to recognize symptoms and signs caused by the clonal neoplastic cells and the rheologic consequences of the monoclonal IgM protein that is produced.

Two-hundred seventeen patients from 46 Spanish institutions diagnosed between 1989 and 1999 fulfilled the WM diagnostic criteria of (1) presence of a serum monoclonal IgM protein of ≥ 3.0 g/dL at diagnosis and (2) a bone marrow containing > 20 % lymphocytes or > 5 % lymphoplasmacytes [7]. Twenty-eight

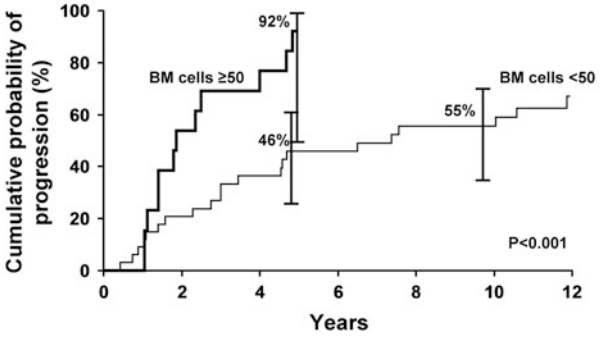

Fig. 20.5 Probability of progression to symptomatic WM requiring therapy on the basis of lymphoplasmacytic bone marrow infiltration. This research was originally published in *Blood*. Kyle RA et al., Progression in smoldering Waldenström macroglobulinemia: long-term results. *Blood*. 2012;Vol 119:4462–4466. © the American Society of Hematology

Table 20.2 Risk factors for progression to treatment among 48 patients with smoldering Waldenström's macroglobulinemia

Multivariate model		
	Hazard ratio	*p* Value
Bone marrow lymphoplasmacytic cells	1.31 (1.1–1.5)	<0.001
Hemoglobin value	0.7 (0.5–0.9)	0.002
Serum M Protein value	2.1 (1.3–3.5)	0.003
IgA reduction	2.4 (1.1–5.4)	0.036

This research was originally published in Blood. Kyle RA et al., Long-Term follow-up of IgM monoclonal gammopathy of undetermined significance. *Blood*. 2003;Vol 102:3759–3764. © the American Society of Hematology

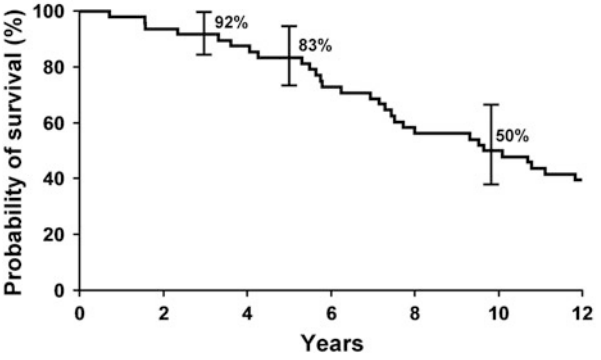

Fig. 20.6 Probability of survival of SWM in 48 patients. This research was originally published in *Blood*. Kyle RA et al., Progression in smoldering Waldenström macroglobulinemia: long-term results. *Blood*. 2012;Vol 119:4462–4466. © the American Society of Hematology

percent ($n = 61$) were asymptomatic at diagnosis, while 15 % ($n = 32$) did not receive chemotherapy and were classified as SWM.

The median age of the 217 patients was 69 years with a male/female predominance of 2:1. At diagnosis, the most common findings were anemia (38 %), hyperviscosity (31 %), constitutional B symptoms (23 %), bleeding (23 %), and neurologic symptoms (22 %) [7]. Evidence of hyperviscosity was found in the fundus of 34 %, while hepatomegaly (24 %), splenomegaly (19 %), and lymphadenopathy (25 %) were present on physical examination. The median IgM monoclonal protein was 4.4 g/dL, while monoclonal light chains were found in the urine of 31 %. The LDH was elevated in 11 %, while the β2-microglobulin value was increased in 54 %. Neutropenia was seen in 4.3 % and thrombocytopenia in 2.4 % at diagnosis. No lytic lesions were identified but severe osteoporosis was present in 7 %.

Twenty-eight percent ($n = 61$) were asymptomatic at presentation but 29 patients required therapy eventually and 15 % remained asymptomatic throughout and did not receive chemotherapy. The presence of hemoglobin < 12.5 g/dL, elevated beta-2 microglobulin (β2-M), or a monoclonal IgM > 4.0 g/dL predicted a shorter time free of therapy. Chlorambucil/prednisone was given in 43 %, continuous chlorambucil in 26 %, intermittent chlorambucil in 11 %, cyclophosphamide, vincristine, and prednisone (COP) in 13.5 %, and other therapy in the remaining 6.5 %. A complete response occurred in 2 %, partial response in 48 %, and minor response in 10 %. Progression-Free Survival (PFS) was 43 % at 5 years, while the projected overall survival was 55 % at 10 years. The most frequent cause of death was the development of second malignancies. The most frequent cause of death was solid tumors ($n = 10$), myelodysplasia (MDS) ($n = 3$), and aggressive lymphoma ($n = 3$). Infection was the cause of death in 19 %. Multivariate analysis revealed that the presence of symptomatic disease at diagnosis, age > 65 years, and hemoglobin < 11.5 g/dL were the most important causes of death.

Ghobrial et al. reported on 337 symptomatic patients with WM seen at the Mayo Clinic between 1960 and 2001 [11]. The inclusion criteria for diagnosis were: (1) symptomatic disease, (2) serum M spike ≥ 3.0 g/dL, (3) quantitative IgM ≥ 3000 mg/dL or IgM ≥ 1500 mg/dL or serum M spike > 1.5 g/dL with an associated ≥ 20 % bone marrow lymphoplasmacytic involvement. The median age at diagnosis was 64 years with 47 % > 65 years and 65 % were male. Enlargement of the liver or spleen was noted in 25 %. A prior MGUS was present in 19 %, while systemic amyloidosis occurred in 4 %. The median serum M spike was 3.8 g/dL and the quantitative IgM 5,140 mg/dL. IgM kappa was present in 75 %, IgM lambda 22 % and biclonal or indeterminate in 3 %. The median hemoglobin was 9.9 g/dL (range 4.0–15.5 g/dL). The hemoglobin was < 10 g/dL in 51 %. The leukocytes were $< 4.0 \times 10^9$/L in 20 %, while the platelets were $< 150 \times 10^9$/L in 26 %. The median serum viscosity was 3.2 (range 1.3–17.2) centipoise (cP). The median serum albumin was 3.5 g/dL, while 81 % were < 4.0 g/dL.

The median duration of follow-up of the 337 patients was 11 years with death occurring in 70 % of patients. The cause of death was due to WM or complications of therapy in 53 %. Myelodysplasia or leukemia occurred in 7 %.

The median survival of all patients was 6.4 years with a 5-year survival of 57 % and 10-year survival of 31 %. Multivariate analyses revealed that age > 65 years and organomegaly were the only two parameters significantly associated with a poor prognosis. The median survival of patients who had neither factor was 10.6 years but only 3.1 years for those who had both risk factors. The year of diagnosis had no effect on overall survival, but these patients were seen prior to the wide-scale introduction of rituximab or novel agents.

The β2-microglobulin value was available in only 98 patients but it added significantly to the model containing age > 65 years and organomegaly. A β2-microglobulin value ≥ 4 was associated with a threefold increase in the risk of death.

Approximately, 20 % of patients with WM present with neurologic abnormalities. Peripheral neuropathy is common and is usually a distal, symmetric, and slowly progressive sensorimotor process. The lower extremities are more involved than the upper extremities. If one utilizes electrophysiologic tests rather than clinical examination, the frequency of peripheral neuropathy is higher.

While peripheral neuropathy is a more common neurological manifestation, cranial nerve palsies, mononeuropathy, and mononeuritis multiplex may result from infiltration of the nerves by tumor cells, hyperviscosity, or a bleeding diathesis. The myelin sheath may contain IgM deposits, but it is not possible to determine whether the deposition of IgM is causative or whether it represents passive deposition of IgM in an already damaged nerve. Typically, biopsy of the sural nerve shows myelin degeneration but no significant infiltration by lymphocytes or plasma cells.

The central nervous system may also be involved with plasmacytoid lymphocytic infiltration of the meninges [12]. Multifocal leukoencephalopathy is rare. Sudden hearing loss may be a presenting symptom [13].

Hyperviscosity syndrome may produce headache, blurred vision, dizziness, vertigo, ataxia, diplopia, seizures, altered consciousness, or coma. Cerebral hemorrhage may occur. Hyperviscosity is frequently associated with retinal vein engorgement with vascular segmentation (sausaging) and flame-shaped hemorrhages, as well as exudates. Papilledema is rare.

Renal insufficiency is uncommon in WM despite the presence of deposits of monoclonal IgM on the basement membrane. The presence of lymphocytes or plasmacytoid cells identical to those seen in the bone marrow is frequent. Nephrotic syndrome is rare, but when present, is often due to systemic AL amyloidosis.

Occasionally, diffuse pulmonary infiltrates, masses, or pleural effusion may be seen. A review of 20 patients with WM by Winterbauer et al. revealed that five patients had pulmonary involvement consisting of multiple nodular infiltrates and/or pleural effusion. Cough and dyspnea were the major symptoms [14].

Rarely, deposition of IgM protein produces an amorphous hyaline-like material in the small intestine. Infiltration of lymphocytes and plasma cells may be seen, but diarrhea and steatorrhea are uncommon in WM. Retroperitoneal and/or mesenteric lymph nodes may be prominent. Erythematous urticarial skin lesions and the presence of an IgM monoclonal protein (Schnitzler syndrome) has been described and may occur in WM. Lymphoplasmacytic cells may also infiltrate the dermis and

produce lesions. Rarely, the IgM monoclonal protein may be deposited in the skin. Monoclonal IgM (type 1 cryoglobulin) may precipitate at room temperature and produce Raynaud's phenomena, urticaria, purpura, acrocyanosis, or tissue necrosis upon exposure to the cold [15].

A bleeding diathesis is common in WM and may result from interference of the IgM protein with clotting factors and platelet function. Purpura and gross bleeding especially from the gastrointestinal tract may be a major clinical problem. Thrombocytopenia from bone marrow infiltration, chemotherapy, hypersplenism, or idiopathic thrombocytopenic purpura (ITP) may play a role in bleeding.

It must be emphasized that IgM MGUS and SWM are asymptomatic and precede symptomatic WM. Often these two precursor states are not recognized and the patient is thought to present with symptomatic WM. The presence of IgM MGUS or SWM is not an indication for treatment. It oftentimes takes years to become symptomatic and many will die of an unrelated cardiac or cerebrovascular event or a malignancy unrelated to the lymphoplasmacytic infiltration.

20.1.4 Laboratory Features

Anemia is present in the majority of patients with WM and is due to inadequate red blood cell synthesis, decreased red blood cell survival, blood loss, or a combination thereof. An acquired hemolytic anemia with a positive Coombs test may occur. The plasma volume is increased and responsible for spuriously low hemoglobin and hematocrit levels. The sedimentation rate is usually very high and Rouleaux formation is striking. Lymphocytosis, neutropenia, and thrombocytopenia may all be found.

The presence of the monoclonal IgM protein may result in low values for serum cholesterol, elevated values for bilirubin, and abnormal inorganic phosphate levels. The serum creatinine is usually normal, while hyperuricemia is not uncommon. The β2-microglobulin value is increased in about half of patients and is an important prognostic factor.

Serum protein electrophoresis always produces a sharp, narrow spike, or dense band usually migrating in the gamma area. Immunofixation must be performed in order to confirm the presence of a monoclonal protein and to determine its type. Approximately, 75 % of monoclonal proteins are IgM kappa. The quantitation of IgM on nephelometry may be 2–3 g/dL more than the densitometric measurement of the serum spike [16]. Thus, serum protein electrophoresis should be used to measure the IgM levels rather than nephelometry [17]. It is essential that one measures the protein abnormality by the same technique during treatment and follow-up because of the variability in the size of the serum M spike and the quantitation of IgM by nephelometry.

The uninvolved IgG value is reduced in approximately 60 % of WM patients, while IgA is decreased in approximately 20 % [18]. The IgM monoclonal protein

may precipitate in the cold (type 1cryoglobulin) but they are usually asymptomatic unless an increased thermal insolubility occurs. Although monoclonal light chains are found in almost three-fourths of patients with WM, their level is usually low and poses no specific problems.

Although the hyperviscosity syndrome was initially noted in 1929 [19], it was rarely recognized clinically until the review by Fahey et al., more than 35 years later [20]. The serum viscosity value should be determined if the serum M spike is more than 4 g/dL or any patient with oronasal bleeding, blurred vision, or neurologic symptoms suggestive of hyperviscosity. Various instruments may be used to determine the serum viscosity but the Ostwald-100 viscometer is commonly used. Other techniques include the Wells-Brookfield or Sonoclot viscometers. There is often a poor correlation between the serum viscosity value and clinical symptoms. Generally, most symptomatic patients have a relative serum viscosity > 4 cP. Rarely, a patient may have symptoms of hyperviscosity when it is < 4 cP and some have no symptoms even when the viscosity is 6–10 cP. In general, the level of relative viscosity producing symptoms in an individual patient is usually the same. In short, if a patient has symptoms with a relative viscosity of 5 cP, they will become symptomatic when the relative viscosity returns to the same level. One must be aware that the viscosity–protein concentration curve of IgM is not linear. At low serum IgM levels, an increase of 1–2 g/dL produces little or no change in the serum viscosity, but if the IgM is 4–5 g/dL an increment of 1–2 g/dL greatly increases the relative viscosity. In addition to the size of the serum M protein, the molecular characteristics and aggregation of the proteins, microvasculature changes, hematocrit level, and cardiac status all play a role in relative viscosity [21].

The bone marrow aspirate is frequently hypocellular, but the biopsy specimen is usually hypercellular and reveals extensive infiltration with clonal lymphoid or plasmacytoid cells. Intranuclear vacuoles containing IgM protein (Dutcher bodies) are common. Mast cells are frequently increased and may help differentiate WM from other lymphomas or myeloma. The pattern of bone marrow involvement is frequently diffuse (45 %), but nodular–interstitial infiltration (22 %), mixed paratrabecular–nodular (20 %), or paratrabecular infiltration was reported in 13 % [22]. The cellular components are B-cells with a low proliferative rate. The typical immunophenotype consists of surface IgM^+, $CD5^-$, $CD10^-$, $CD11C^-$, $CD19^+$, $CD20^+$, $CD22^+$, $CD23^-$, $CD25^+$, $CD27^+$, $FMC7^+$, $CD103^-$, and $CD138^-$. The plasmacytic component is $CD138^+$, $CD38^+$ and $CD45^-$, or DIM. They express only one type of light chain (kappa or lambda).

20.1.5 Serum Free Light Chain Assay

The serum free light chain (FLC) assay measures the serum kappa and lambda light chain levels and is then expressed as the kappa to lambda ratio. Normal FLC ratios are seen in patients without plasma cell or B lymphocyte proliferative disorders

[23]. There is little data on FLC assays in WM but it appears that FLC assays correlate with other markers of tumor burden [24].

20.1.6 Differential Diagnosis

WM must be differentiated from other monoclonal conditions such as MGUS, multiple myeloma, chronic lymphocytic leukemia, and mantle cell lymphoma.

IgM MGUS is characterized by an IgM monoclonal protein < 3.0 g/dL, bone marrow containing < 10 % lymphoplasmacytic cells, the absence of symptomatic anemia, lymphadenopathy, hepatosplenomegaly or hyperviscosity, and the absence of constitutional symptoms. The features of IgM MGUS have already been discussed. Symptomatic WM must also be differentiated from SWM and this has been discussed in detail.

Multiple myeloma with an IgM monoclonal protein is very rare and comprises only 0.5 % of multiple myeloma. The absence of CD56 is helpful in differentiation. Bone lesions are common in IgM MM but are rarely seen in WM. Hyperviscosity, lymphadenopathy, and splenomegaly are infrequently seen in IgM multiple myeloma. Translocation (11;14) may be present in IgM myeloma but is not seen in WM [25]. Recurrent somatic mutation involving the MYD88 gene (L265P) is present in approximately 90 % of patients with WM and can serve as a useful diagnostic marker. In cases that are histopathologically difficult to interpret, MYD88 L265P mutation status should be checked using allele-specific polymerase chain reaction (AS-PCR) assay.

Chronic lymphocytic leukemia is easily differentiated from WM by its immunophenotype. The abnormal B cells in CLL are $CD5^+$, $CD23^+$, and $FMC7^-$. Mantle cell lymphoma has nuclear staining for Cyclin-D1 in more than 70 % of patients. Most patients with mantle cell lymphoma have a t(11;14) translocation. The lymphocytes are $CD5^+$ and $CD23^-$. Patients with WM must also be differentiated from patients with IgG or IgA MM who have many lymphocytoid cells in the bone marrow and no lytic bone lesions [26].

Hematopathologists find it challenging to differentiate WM from other indolent lymphoproliferative disorders such as marginal zone lymphoma (MZL) and splenic marginal zone lymphoma (SMZL). Kyrtsonis et al. reported that 60 % of WM express CD38 in contrast to 18 % of SMZL patients [27]. MALT lymphoma must be considered in the differential diagnosis and is distinct from LPL [28]. Similar to WM, a neoplastic lymphoplasmacytic infiltrate with cell surface/cytoplasmic IgM is observed in MZL. Markers such as CD25 are less frequently observed in SMZL compared to WM. In contrast, CD11c, CD22, and CD103 may be overexpressed in SMZL. Additionally, chromosomal aberrations can occasionally assist in distinguishing WM from SMZL, with 6q deletion notable in 30–50 % of WM cases and 7q loss (19 %), 3q (19 %), and 5q (10 % cases) gains encountered occasionally in SMZL. Furthermore, MYD88 L265P mutation occurs in only a minority (10 %) of cases of SMZL.

20.1.7 Prognostic Factors

The median survival of patients with WM has been 5 years but at least a fifth of these patients survived for more than a decade and 10–20 % die of unrelated causes [7, 29].

In a series of 167 patients with WM from a single institution, Facon et al. reported a median survival of 60 months [30]. The most important adverse prognostic factors for survival were a neutrophil value $< 1.7 \times 10^9$/L, age > 60 years, and hemoglobin < 10 g/dL. In another group of patients with WM requiring therapy, multivariate analysis revealed that age > 70 years, hemoglobin < 9 g/dL, and the presence or absence of weight loss or cryoglobulinemia were the most significant prognostic features [31]. In a series of 232 French patients with WM, age ≥ 65 years, albumin < 4 g/dL, and the presence of one or more cytopenias were the major risk factors affecting survival [32]. The 5-year OS was 87 % in patients with ≤ 1 risk factor, 62 % in those with two adverse risk factors, and 25 % in those with ≥ 3 adverse risk factors [32]. Utilizing serum β2-microglobulin, hemoglobin, and serum IgM concentration, low-risk patients were defined as having β2-microglobulin < 3 mg/L and Hb ≥ 12 g/dL; medium risk had β2-microglobulin < 3 mg/L, Hb < 12 g/dL or β2-microglobulin ≥ 3 mg/L, and serum IgM ≥ 4 g/dL; and high risk consisting of β2- microglobulin ≥ 3 mg/L and IgM ≥ 4 g/dL [33]. The 5-year OS rate with low risk was 87 %, medium risk was 63 and 53 %, and high risk was 21 %. At 10 years, an elevated serum lactate dehydrogenase (LDH) level was identified as an additional poor prognostic factor.

The International Prognostic Staging System for WM (IPSS WM) was developed by Morel et al. [34]. It is based on 587 patients from seven international institutions. The significant adverse features were age > 65 years, hemoglobin ≤ 11.5 g/dL, platelets $\leq 100{,}000 \times 10^9$/L, β-2 microglobulin > 3 mg/L, and serum IgM > 7 g/dL. Low-risk patients were < 65 years of age and had 0 or 1 risk factor. High-risk patients constituted 35 % of the group and had two risk factors, while intermediate risk patients accounting for 38 % had two risk factors or those greater than age 65. Five-year OS rates for patients in the low-, intermediate-, and high-risk groups were 87 %, 68 %, and 36 %, respectively ($p < 0.001$). Other investigators have included gender, the presence of B symptoms, IgM value, performance status, presence of hyperviscosity, bone marrow infiltration pattern, and cytogenetic changes as additional prognostic factors.

20.1.8 When Should WM Actually Be Treated?

In many instances, it is easy to determine that immediate therapy is required. The patient who presents with severe constitutional symptoms such as high fever, drenching night sweats, or marked weight loss requires treatment. Constitutional symptoms may also be due to transformation to a myelodysplasia/acute leukemia in patients with WM [35]. The presence of anemia producing dyspnea, chest discomfort on exertion, and marked fatigue also should be started on therapy.

The presence of hyperviscosity is an indication for immediate plasmapheresis and the institution of systemic therapy to reduce the size of the M protein. Symptomatic lymphadenopathy such as a "bull" neck requires treatment. Occasionally, hepatosplenomegaly will cause symptoms of fullness, discomfort or pain, and require chemotherapy. Retroperitoneal lymphadenopathy is usually asymptomatic and does not require therapy. The size of the M spike is not an indication for immediate therapy.

Although the M spike may be high and infiltration of the bone marrow with clonal lymphocytes and plasma cells may be striking, patients with minimal or no symptoms should be observed. Approximately, 10 % of patients with SWM may not require treatment for nearly a decade from diagnosis [7]. Moreover, active surveillance and delaying therapeutic intervention until development of symptoms have not been shown to adversely affect survival outcome in SWM [36]. The advice of Jan Waldentröm, "let well alone" must be kept in mind [37]. Although WM is a treatable disorder, it remains incurable despite the availability of many active agents, and clinicians should resist the temptation to initiate chemoimmunotherapy solely to improve the abnormal laboratory values in the patient. Waldenström also emphasized the need to listen to the symptoms of the patient and to perform a complete physical examination and to avoid treatment of mild symptoms. The patient does not require therapy simply because of the presence of a large monoclonal serum protein or marked infiltration of the bone marrow with lymphocytes and plasma cells consistent with the diagnosis of lymphoplasmacytic lymphoma. Waldenström said that if the patient is able to state on his tombstone, the words of the great Swedish poet, Stiernhielm, "Vixit, dum vixit, laetus" (He lived happily as long as he lived), the physician has succeeded in improving the quality of life of the patient. In short, the physician must not treat the patient too early or delay therapy in the presence of appropriate indications in a patient.

20.1.9 Recommendations for Follow-Up of "Watch and Wait" Patients

In the presence of SWM, follow-up is recommended in 3–4 months and, if stable, this can be lengthened to 6-month intervals. If stability continues, annual follow-up is reasonable. However, the patient must be advised to return to the physician in the event of any new symptoms or untoward problems.

There is no standard therapy for symptomatic WM. Several prospective trials have demonstrated the efficacy of various drugs or combinations, but it is difficult to compare these agents on the basis of response rates. Few prospective randomized studies have been performed.

In summary, a complete history and careful physical examination, including funduscopic examination (preferably by an ophthalmologist on a dilated pupil) to evaluate retinal vein engorgement with hemorrhages and exudates as well as the possibility of papilledema, must be performed. The use of densitometry is advised to determine the IgM level for serial evaluation. Nephelometry is also useful and

both determinations are advised. The results of densitometry and nephelometry should not be directly compared with each other. However, therapy may be started for patients who have a rapidly rising IgM level or have progressive signs or symptoms of WM. The presence of hemoglobin $\leq$10 g/dL and/or a platelet level of $<100 \times 10^9$/L that are attributable to the disease are additional indications for initiating treatment. Bulky adenopathy or symptomatic organomegaly also warrant therapy. The same is true for patients with recurrent high fever, drenching night sweats, significant weight loss, or severe fatigue. Symptoms of hyperviscosity, severe symptomatic neuropathy, coexisting amyloidosis, and symptomatic cryoglobulinemia are also indications for therapy. Immediate plasmapheresis is required for symptomatic hyperviscosity.

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Immunotherapy in Waldenstrom's Macroglobulinemia

21

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21.1 Introduction

Treatment strategies and modalities have changed at the beginning of the twenty-first century with the use of anti-CD20 monoclonal antibody rituximab. Alone or in combination, rituximab was widely used in WM patients. Since 2000, new monoclonal antibodies have been investigated, targeting CD20 or other antigens such as CD52 or CD22. The combination of anti-CD20 monoclonal antibody with chemotherapy is still a standard of care in this setting. Rituximab-based regimens remain a recommended primary therapy for most patients with WM [1–3].

21.2 Monoclonal Antibodies Used as a Single Agent

21.2.1 Rituximab

Rituximab is a chimeric monoclonal antibody, which targets the CD20 antigen, expressed on B-cells, including WM cells. Since the beginning of 2000s, rituximab is widely used in B lymphoproliferative diseases. Two schedules of administration were studied in monotherapy for WM: the standard one, in which one weekly infusion is administrated for 4 weeks, and the extensive one, in which responsive patients received four more infusions between the 12th and 16th week. In the standard schedule, overall response rates (ORRs) varied between 27 and 60 %, with 27–35 % of major response (≥partial response), with a median time to

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_21

response of 3 months and a duration of response (DOR) of 8 months, both in previously treated and untreated patients [4, 5]. Even for patient with minor response, an improvement of hemoglobin, platelet counts, and a reduction of lymphadenopathy and splenomegaly was observed. Extensive therapy allowed an ORR between 35 and 48 %, with a DOR longer than 29 months [6, 7]. High IgM level (>40 g/L) or low albumin (<35 g/L) is a predictive factor for a lower response and a shorter time to progression. Of note, response rate to rituximab is dependent on polymorphism of the Fc gamma RIIIa receptor: patients with the V158F variant gene (a valine amino acid instead of a phenylalanine at position 158) are more likely to respond to rituximab. Indeed, in a retrospective study, patients with the FCGR3A_158FF variant had a response rate of 9 % versus 40 % for patients with FCGR3A_158VV [43]. Rituximab is well tolerated; however, about 50 % of patients will experience a transient increase of the IgM level, named flare-up effect. No predicting factors, like baseline IgM level, plasma viscosity level, bone marrow infiltration, or previous therapy, had been identified. The flare-up effect is seen mostly during the 1st months of treatment and can persist until the 4th month. This phenomenon is associated with a higher risk of treatment failure, but physicians should be cautious to interpret this too quickly as lack of response or even progression, because decrease of IgM level can occur slowly. No effect on progression-free survival (PFS) and overall survival (OS) was observed [8]. Patients for whom baseline serum IgM level or serum viscosity is higher than 50 g/L or 3.5 cp, respectively, IgM flare-up can induce hyperviscosity-related event. Such patients at risk should undergo prophylactic plasmapheresis or avoid rituximab during the first course of chemotherapy until IgM levels decline to safer level. Late-onset neutropenia (LON) was also described with single-agent rituximab [9]. The underlying mechanism of LON is not understood, and a cellular immune mechanism or antibodies-mediated complement cytotoxicity are supposed [10]. An association between specific polymorphism in the immunoglobulin G Fc receptor FCγRIIIa V158F and LON was also described [11]. Predisposing factors of LON in hematologic malignancy are previous autologous stem cell transplantation, advance disease, purine analogues' exposure, and previous intensive chemotherapy, eventually associated with radiotherapy [9].

Because of the lower chance to respond if the IgM level is high, and the risk of an IgM flare when used alone, rituximab single-agent therapy is now essentially used for WM patients with immunological disorders secondary to the WM, such as anti-MAG neuropathy.

21.2.2 Ofatumumab

Ofatumumab is a humanized CD20-directed monoclonal antibody that targets a CD20 region different than that of rituximab. One phase II trial studied ofatumumab in monotherapy in 37 treatment-naïve or previously treated patients. Fifty-nine percentage of the patients achieved at least a minimal responses after both cycles (38 % a PR), with somewhat higher responses at higher doses (47 %

versus 68 %), in therapy-naïve (6/9, 67 %) and rituximab-naïve (9/12, 75 %) patients than in rituximab-exposed patients (13/25, 52 %). Two patients experienced an IgM flare with necessity of plasmapheresis [12].

21.2.3 Alemtuzumab

Alemtuzumab is a CD52-directed monoclonal antibody. CD52 is widely expressed on WM cells, but also on mast cells. Treon studied alemtuzumab in 28 patients with lymphoplasmocytic lymphoma (including 27 patients with WM), among which 23 patients were in relapse or refractory. Alemtuzumab was infused at 30 mg three times a week for 12 weeks. ORR was 75 %, with 36 % of major response, and a time to progression of 14.5 months. Toxicities were hematological (including 5, 24, and 10 % of grade 3–4 neutropenia, thrombocytopenia, and anemia, respectively) and infectious with 17.8 % of CMV reactivation. Furthermore, 14 % of patients developed late-onset autoimmune thrombocytopenia, at a median time of 12.9 months after treatment completion [14]. Based on these considerable toxicities, alemtuzumab applications have not become an accepted treatment approach in WM.

21.2.4 Anti-BLyS Monoclonal Antibody

The B-lymphocyte stimulator (BLyS) protein is a cytokine belonging to the tumor necrosis factor (TNF) family, implicated in B-cell survival and maturation and overexpressed in WM. One phase II study used the anti-BLyS monoclonal antibody, belimumab, in 12 patients with WM. No objective response was reported and 10 patients had stable disease. Belimumab has not been studied in combination therapy yet [16].

21.2.5 Ublituximab

Ublituximab is a novel chimeric anti-CD20 monoclonal antibody that has a high affinity for NK FcγRIIIa receptors. Preclinical studies showed that ublituximab is more efficient than rituximab in inducing NK cell degranulation and antibody-dependant cellular cytotoxicity [17]. A phase I/II trial with single-agent ublituximab in patients with rituximab relapsed/refractory NHL, including WM patients, is currently ongoing.

21.3 Combinations with Rituximab

Because rituximab is an active and a non-myelosuppressive agent, its combination with various chemotherapeutic agents has been extensively explored in WM. The combination of anti-CD20 monoclonal antibody and chemotherapy is considered as a standard of care in patients in first line. The choice of chemotherapy depends on the comorbidities, how fast control of the disease has to be achieved, and the phenotype of the disease.

21.3.1 Rituximab + Alkylators

CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) + rituximab compared to CHOP alone in first line was studied in a randomized multicenter phase III trial by the German Low Grade Lymphoma Study Group (GLSG). Sixty-nine patients, among them 48 patients with WM, were included. Thirty-four patients (23 WM) received RCHOP and 30 patients (25 WM) received CHOP. In WM patients, ORR (≥PR) was 91 % versus 60 % ($p = 0.0188$), and the median time to treatment failure was 63 and 22 months ($p = 0.0033$) in the RCHOP and CHOP arm, respectively. Adverse effects were mainly myelosuppression and granulocytopenia, with 72 and 57 % of grade 3–4 granulocytopenia and 6 and 13 % of grade 3–4 infections in the RCHOP and CHOP arm, respectively. Toxicities did not differ between the two arms [18]. DC (dexamethasone, cyclophosphamide) + rituximab combination was tested in a phase II trial in 72 untreated WM. A high response rate (83 %) was observed, with 7 and 67 % complete and partial responses, respectively. The 2-year PFS was 67 % in all cohort and 80 % in responders. Median time to response was, however, long with 4.1 months, suggesting that this combination is not appropriate if a rapid control of disease is necessary. Toxicities are mild, with only 9 % of grade 3–4 neutropenia [19]. This study was updated in 2012 with a follow-up longer than 6 years for all patients. Time to treatment failure and time to next treatment were 35 and 51 months, respectively. Majority of relapsing patients were still sensitive to other rituximab-based therapies. The 5-year estimated OS was 100 %, 67 %, and 48 % for patients with low-, intermediate-, and high-risk disease, respectively, based on IPSSWM. Regarding toxicities, one patient, having previously received fludarabine, developed a MDS and two patients (including one after multiple treatments which included alkylating agents and fludarabine) had a RT. This update indicates that DRC is an active and safe treatment choice in first line for WM with a manageable toxicity, even in frail patients [20].

In a retrospective study, Ioakimidis compared the outcome of WM patients in first line who received RCHOP ($n = 23$), RCVP (rituximab, cyclophosphamide, vincristine, prednisone, $n = 16$), or RCP (rituximab, cyclophosphamide, prednisone, $n = 19$). Response rates were similar between the three arms (96 %, 88 %, 95 %, respectively), but toxicities, mainly neutropenic fevers and treatment-related

peripheral neuropathies, were higher in patients receiving RCHOP and RCVP compared to RCP [21].

21.3.2 Rituximab + Purine Analogues

Rituximab + fludarabine (RF) or rituximab + 2CDA was studied both in first line and in secondary treatment in WM. Treon published a phase II trial regarding RF association in 43 patients, including 27 in first line. ORR was 95.3 %, with a 4-year PFS of 67 %, and a median time to progression of 77 months. Patients in first line had similar ORR than those in relapse/refractory disease; however, patients in first line or with major response (≥VGPR) had longer time to progression (77.6 months and >88.3 months, respectively) than patients in relapse/refractory disease (38.4 months) and minor response (36.9 months) [44].

R + cladribine (2CDA) association was studied by Laszlo. An ORR of 93 % with a median time to progression not reached after a median follow-up of 43 months was found [24]. Grade 3–4 toxicities in Treon's studies were similar in untreated and previously treated patients. Twenty-seven, 7, and 6 patients had grade 3–4 neutropenia, thrombocytopenia, and pneumonia (responsible for two deaths), respectively, 1 patient had hemolytic anemia and one had a flare-up effect. Two patients developed a HSV reactivation, but they did not have acyclovir prophylactic treatment, and 13 patients had dose reductions ($n = 5$) or treatment discontinuation ($n = 9$) secondary to toxicities. Of note, eight secondary cancers were reported, including six hematological cancers (three Richter transformations, two AML, and one myelodysplastic syndrome). Seven of these patients had been heavily treated before RF therapy. Laszlo found 37 % of grade 3–4 neutropenia but no grade 3 infections or flare-up effects.

Association of rituximab + alkylators + purine analogues was studied both with fludarabine and 2CDA. In 2006, Tam studied rituximab + cyclophosphamide + fludarabine (RFC) association in 77 patients with CLL or indolent lymphoma, including 10 patients with WM. Patients received rituximab at day 1 and fludarabine + cyclophosphamide from day 1 to 3 intravenously. Response rate for WM patients was 60 %, with five partial responses and one complete response [25]. One prospective study was published in 2012, regarding 43 WM, in which 28 were in first line. Treatment schedule was similar to Tam's study. ORR was 79 with 74.4 % of major responses. Interestingly, four patients had delayed responses, within the 6 months after treatment discontinuation, raising major response rate to 76.7 %. No statistical link between PFS and event-free survival (EFS) was found according to quality of response [26]. Finally, RFC was studied retrospectively in 82 patients including 25 patients untreated. Rituximab was administered intravenously at day 1 and fludarabine and cyclophosphamide from day 2 to 4 orally. ORR was 85.4 with 78 % of major responses. Again, with a median follow-up of 47 months, 25 patients had a late improvement of response, increasing rates of very good partial responses and complete responses from 18 to 38 % within a median of 13.9 months after treatment discontinuation, suggesting

that late assessment of response is interesting with this combination therapy [27]. The PFS was 79 and 67 % at 3 and 4 years, respectively. The duration of response (DOR) and PFS time were statistically longer in untreated patients (median not reached) compared to relapsing patients (median DOR 74 months, median PFS: 79 months) but DOR was not influenced by quality of response in both studies; toxicities were mainly hematological with 88.3 and 36 % of grade 3–4 neutropenia in Tedeschi's and Souchet's studies, respectively. Also, long-lasting cytopenia were reported in 44 and 29 % of patients, with a median time for resolution between 7 and 9 months, respectively. Six patients developed a grade 3–4 infection, during or within the 6 months after treatment discontinuation in Tedeschi's study, responsible for the death of two patients. Finally, secondary cancers were reported: three secondary myelodysplastic syndromes (MDS) in Tedeschi's study and two MDS, three Richter transformations, and six solid tumors in Souchet's study, raising the question of the feasibility of purine analogues and alkylators association in these settings.

However, R-FC is the most effective in relapsed patients with the longer PFS and duration of response, at least in a historical comparison with other salvage regimen [28]. Association of rituximab, cyclophosphamide, and 2-CdA (RC-2CDA) was less studied. Weber published results with 17 patients, all previously untreated, receiving a median of two courses of RC-2CdA. ORR was also high, 93 %, with a DOR of 60 months [29].

21.3.3 Rituximab + Immunomodulators

Thalidomide + rituximab association was studied in a phase II trial published by Treon. Twenty-five patients, including 20 in first line, received thalidomide 200 mg daily for 2 weeks, followed by 400 mg daily for 1 year. Rituximab was administered weekly from week 2 to 5, followed by 4 additional weekly infusions from week 13. ORR was 72 % and major response rate 64 %, with a median time to progression of 38 months. More than 40 % of patients developed peripheral neuropathies grade 2 or more. All patients required dose reduction and 11 patients had premature treatment discontinuation [30]. Rituximab and lenalidomide association was also studied by Treon in 16 patients (12 in first line). ORR was 50 %, and only 1 neuropathy was noted. However, 88 % of patients had reduction in their hematocrit, in spite of lenalidomide dose reduction. Lenalidomide with rituximab was associated with significant hematologic toxicity [23].

Lenalidomide should only be considered in the context of a clinical trial.

21.3.4 Rituximab + Bendamustine

Rituximab + bendamustine (RB) was compared to RCHOP in a phase III open-label trial A total of 546 patients were enrolled in this study for indolent NHL patients, including 41 patients with WM. Patients on the RB arm received bendamustine at

90 mg/m^2 on days 1, 2 and rituximab at 375 mg/m^2 on day 1 with the frequency of 4 weeks for each cycle. A similar ORR (95 %) was found, but a higher PFS was reported in the RB arm (median 69.5 months versus 28.1 months in the RCHOP arm), with a better tolerance of chemotherapy (lower rates of grade 3–4 neutropenia, infectious complications and peripheral neuropathies, and no alopecia) [31]. A randomized study evaluating rituximab as maintenance treatment after RB therapy in first line is currently ongoing. In the salvage setting, the outcome of 30 WM patients with relapsed/refractory disease who received bendamustine alone or with a CD20-directed antibody was reported by Treon et al. An overall response rate of 83.3 % and a median progression-free survival of 13.2 months were reported in this study. Overall, therapy was well tolerated though prolonged myelosuppression occurred in patients who received prior nucleoside analogue therapy [13]. Tedeschi reported an Italian retrospective study in 72 patients with relapsed/refractory disease. Overall and major response rates were 80.2 and 74.6 %, respectively. Major toxicity was grade 3/4 neutropenia occurring in 13 % of courses. There was no significant association between baseline features or patients' characteristics and response achievement. Median progression-free survival was not reached after a median follow-up of 19 months (range 3–54). None of the patients developed aggressive lymphoma or secondary myelodysplastic syndrome/acute myeloid leukemia. BR combination showed to be as effective as more intensive salvage regimens in pretreated WM patients. Treatment showed to be well tolerated even in elderly patients with limited episodes of myelosuppression and infections when compared to purine analogues including regimens [32].

21.3.5 Rituximab + Bortezomib

Treon studied rituximab, bortezomib, and dexamethasone association in 23 untreated patients, with administration of intravenous bortezomib at 1.3 mg/m^2 and dexamethasone 40 mg twice a week at day 1, 4, 8, 11 and rituximab 375 mg/m^2 at day 11, for four cycles as induction treatment and four more cycles at 3 months as maintenance treatment. ORR and major response rate were 96 % and 83 %, respectively, with a median time to response of 1.4 months and a late improvement of response after treatment discontinuation with a median time to best response of 15 months. Sixty percent of patients had treatment discontinuation after four cycles because of peripheral neuropathy. Follow-up was short (22.8 months), preventing long-term evaluation of efficiency and toxicity [22]. Treatment of bortezomib was then reduced to one injection a week at 1.6 mg/m^2 in an attempt to reduce the occurrence of peripheral neuropathy. According to this schedule, rituximab + bortezomib was studied in first line by Ghobrial in 26 patients, with bortezomib 1.6 mg/m^2 administrated intravenously at day 1, 8, and 15 during six cycles, in a 28-day cycle, and rituximab 375 mg/m^2 at each cycle during four cycles. Eighty-eight percent of patients obtained a response including 65 % of major response (58 % achieved $\geq$ PR, 8 % CRs/nCRs). The 1-year event free survival was 79 %. Response was obtained on IgM serum level and tumoral mass. Neurologic

complications were limited, and no grade 3–4 peripheral neuropathy was reported. Grade 3–4 neutropenia were noted in 12 % of patients [42]. Likewise, in relapse/refractory patients, ORR was 81 % with 51 % of major responses and a median PFS of 15.6 months. Sixteen percent of patients developed a grade 3 neutropenia and a grade 3 neuropathy occurred in only 5 % of patients. "IgM flare" developed in 40% of patients and 54 % of patients developed peripheral neuropathy but in none of grade ≥ 3 ([33]). Dimopoulos et al., in order to avoid "IgM flare", used an induction cycle of bortezomib (i.v. 1.3 mg/m^2 days 1, 4, 8, and 11), followed by four cycles of weekly bortezomib (i.v. 1.6 mg/m^2 for 4 weeks) with rituximab and dexamethasone on cycles 2 and 5. Peripheral neuropathy was observed in 46 % (grade ≥ 3 in 7 %) but only 5 (8 %) patients discontinued bortezomib due to neuropathy. Dimoupoulos reported the efficacy and toxicity of bortezomib, rituximab, and dexamethasone (BDR) in 59 naive-treatment patients. After a minimum follow-up of 32 months, median progression-free survival was 42 months, 3-year duration of response for patients with PR was 70 %, and 3-year survival was 81 %. Peripheral neuropathy occurred in 46 % (grade ‡3 in 7 %); only 8 % discontinued bortezomib due to neuropathy [34]. Neurotoxicity is the major concern with bortezomib because underlying IgM-related neuropathy or neuropathies due to age-related comorbidities (such as diabetes) is common. Weekly dosing and subcutaneous administration may reduce rates and severity of neuropathy. Bortezomib is not stem cell toxic and long-term follow-up in myeloma patients does not suggest a risk for secondary malignancies. Prophylaxis against herpes zoster is strongly recommended. Primary therapy with bortezomib is recommended for patients with high levels of IgM, with symptoms of or, at risk of developing hyperviscosity syndrome, symptomatic cryoglobulinemia or cold agglutinemia, amyloidosis, and renal impairment [3]. Conversely to BCR inhibitors, bortezomib is also effective in vitro on tumor cells with CXCR4 mutation [35].

More recently, the efficacy of carfilzomib in combination with rituximab and dexamethasone (CaRD) was reported in 31 patients [36]. Carfilzomib, rituximab, and dexamethasone (CaRD) produced overall and CR/VGPR responses in 87 and 36 % of frontline WM patients, respectively. With a median follow-up of 15.4 months, 20 patients remain progression free. CaRD activity was not impacted by MYD88 and CXCR4 mutations and represents a neuropathy-sparing option for treating WM patients.

21.4 Maintenance

One retrospective study used rituximab as maintenance therapy in 86 responsive patients who received previously combination therapy with rituximab. Maintenance treatment with rituximab seemed to extend PFS and OS, without major secondary effects [15]. However, this result need to be confirmed in prospective studies, and the use of rituximab in maintenance therapy is still discussed. A randomized prospective study is ongoing in Germany, analyzing the impact of

2-years rituximab in maintenance after an induction with rituximab + bendamustine in untreated patients.

21.5 Rituximab + BCR Inhibitors

Few patients have been treated with a combination of R + BCR inhibitors; 10 patients were reported by Gopal using the combination of rituximab + idelalisib in refractory/relapsing low-grade lymphoma. The overall response rate was 80 % and the PFS was 22 months [37].

Treon have recently reported the results of a prospective study of ibrutinib in 63 symptomatic patients with Waldenström's macroglobulinemia who had received at least one previous treatment [38]. The median time to at least a minor response was 4 weeks. The overall response rate was 90.5 %, and the major response rate was 73.0 %. The estimated 2-year progression-free and overall survival rates among all patients were 69.1 % and 95.2 %, respectively. Treatment-related toxic effects of grade 2 or higher included neutropenia (in 22 % of the patients) and thrombocytopenia (in 14 %), which were more common in heavily pretreated patients; postprocedural bleeding (in 3 %); epistaxis associated with the use of fish-oil supplements (in 3 %); and a trial fibrillation associated with a history of arrhythmia (5 %).

The efficacy of this combination depended on CXCR4 mutation, with a lower response rate in mutated patients asking the question whether treatment should be stratified according to the CXCR4 mutational status. Randomized studies are ongoing comparing the efficacy of R+ placebo versus R+ ibrutinib in relapsing and treatment-naïve patients.

21.6 Prognostic

The International Prognostic Scoring System, including 96 % of patients treated with alkylating agents and nucleoside analogs [39], is applicable in patients treated with rituximab-based regimen with a significant difference in overall survival with a 38 months median for high-risk patients [40].

21.7 Conclusion

Collectively, immunotherapy is the backbone of treatment for the majority of patients today. Rituximab in combination with chemotherapy is among the most effective treatment in WM and is uniformly recommended in national and international guidelines for first line and salvage treatment in this disease. Single-agent rituximab offers the possibility to elderly patients with comorbidities to control their disease, although to a lesser extent than rituximab/chemotherapy and also with a longer duration to first response. Because of its few side effects, rituximab is an

ideal backbone to add novel compounds such as the BCR inhibitors. Recently, impressive results has been seen in other indolent B-cell lymphomas, using PD1 inhibitors in combination with rituximab, following the concept to exploit the maximum of ADCC of rituximab by inhibiting co-inhibitory molecules on effector cells [41]. All this shows that immunotherapy is but also will stay one of the key therapeutic principles in patients with WM in the future.

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Signal Inhibitors in Waldenstrom's Macroglobulinemia

22

Steven P. Treon, Guang Yang, Zachary R. Hunter, and Jorge J. Castillo

22.1 Targeting Bruton's Tyrosine Kinase Signaling in WM

Whole genome sequencing has revealed activating somatic mutations in MYD88 (L265P) and the C-terminal domain of CXCR4 in Waldenström's macroglobulinemia (WM) [1, 2]. In WM cells, $MYD88^{L265P}$ triggers NFkB directed pro-survival signaling via two divergent pathways involving Bruton's tyrosine kinase (BTK) and IRAK1/IRAK4 (Fig. 22.1). Ibrutinib is an orally administered, small molecule inhibitor of BTK, which triggers apoptosis of $MYD88^{L265P}$-expressing WM cells [3, 4]. A prospective trial involving 63 previously treated WM patients administered ibrutinib (420 mg) until progression or intolerance [5]. Following treatment with ibrutinib, median serum IgM levels for the 63 WM patients who received ibrutinib declined from 3520 to 880 mg/dL at best response. In pre-therapy, 46/63 (73.0 %) patients had a serum IgM $\geq$3000 mg/dL and following treatment, at best response, 6/63 (9.5 %) patients had a serum IgM $\geq$3000 mg/dL. Median bone marrow involvement also decreased from 60 to 25 %, while hemoglobin increased from a median of 10.5 to 13.8 g/dL at best response. Responses included very good partial response ($n = 10$), partial response ($n = 36$), and minimal response ($n = 11$) for overall and major responses of 90.5 % and 73.0 %, respectively. The median time to at least minor and partial responses was 4 and 8 weeks, respectively. Overall responses were similar regardless of baseline age (<65 versus $\geq$65 years), ECOG status (0 versus $\geq$1), pre-therapy WM International Prognostic Scoring System score, serum β_2-microglobulin levels (<3.0 versus >3.0 mg/L), hemoglobin (<11 versus >11 g/dL), serum IgM (<4000 versus $\geq$4000 mg/dL),

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_22

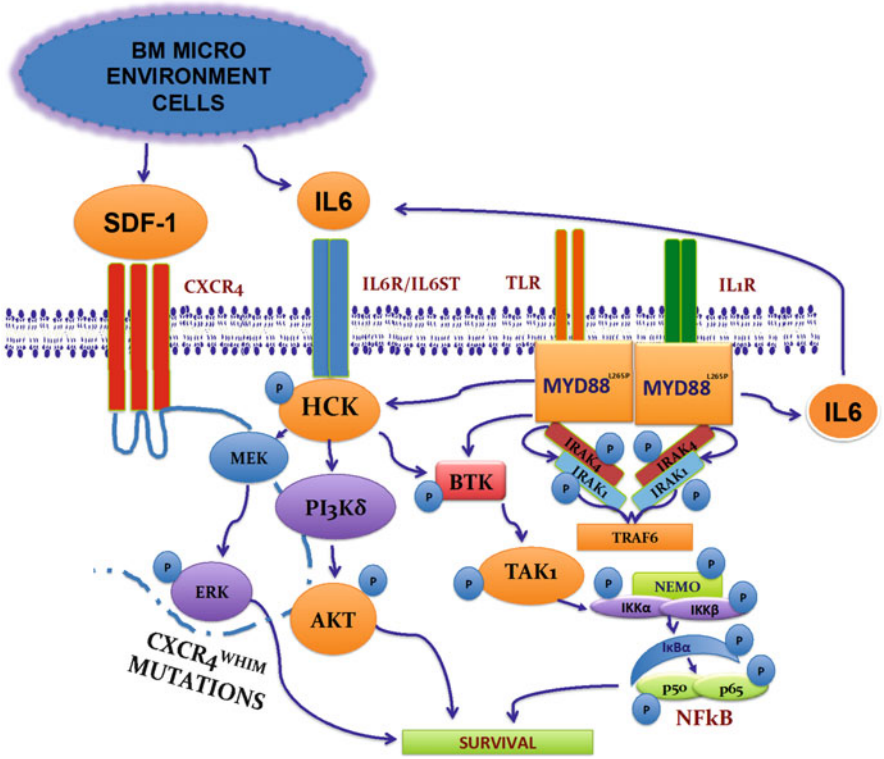

Fig. 22.1 Model of survival signaling directed by activating mutations in MYD88 and CXCR4 in Waldenstrom's macroglobulinemia

bone marrow disease involvement (<50 % versus ≥ 50 %), prior relapsed or refractory status, and prior lines of therapy (1–3 versus >3).

The activity of ibrutinib against extramedullary disease was also assessed in this study. CT-defined adenopathy (≥1.5 cm) was present in 37 of the 63 patients at baseline. Serial imaging for 35 of these patients showed decreased or resolved (67.6 %), stable (24.3 %), or increased ($n = 2.9$ %) adenopathy. Two patients came off study before repeat imaging was required. Among 7 patients with CT-defined splenomegaly (≥15 cm), spleen size was decreased (57.1 %), stable (28.6 %), or not evaluable (14.3 %) following elective splenectomy. The impact of ibrutinib against IgM-related morbidity was also assessed. Nine patients (14.3 %), 3 with positive anti-MAG antibodies, received ibrutinib for progressive IgM-related peripheral sensory neuropathy. All responded, and subjective improvements in peripheral sensory neuropathy occurred in five and remained stable in four during the treatment course. Symptomatic hyperviscosity related to progressive disease that required plasmapheresis prompted start of ibrutinib in 4 patients. All responded and none required additional plasmapheresis by the end of Cycle 2. One patient required plasmapheresis for acquired Factor VIII deficiency. He responded and did not require further plasmapheresis. The

spontaneous bleeding events that prompted therapy also resolved and this patient continued on ibrutinib.

With a median on treatment duration of 19.1 (range: 0.5–29.7) months, the estimated progression-free and overall survival at 24 months was 69.1 % and 95.2 %, respectively. For patients with progressive disease, the median time to progression was 9.6 (range: 3.5–19.4) months if transformation cases were censored and 9.5 (range: 3.5–19.4) months if transformation events were included. Subset analysis showed that >3 prior lines of therapy and high pre-therapy IPSS score were associated with inferior progression-free survival.

Overall, treatment was well tolerated among previously treated WM patients who received ibrutinib. Grade ≥3 neutropenia and thrombocytopenia occurred in 14.32 % and 12.72 % of patients and occurred more commonly in patients with ≥3 prior therapies. Ibrutinib-related neutropenia and thrombocytopenia were reversible, though necessitated dose reduction and/or treatment discontinuation in 3 and 4 patients, respectively. Grade ≥2 bleeding events occurred in 4 patients (2 epistaxis, 2 post-procedure bleeding). Fish oil supplements contributed to both grade 2 epistaxis events and resolved with their discontinuation. Infections at least possibly associated with ibrutinib were few, and in most cases, IgA and IgG hypogammaglobulinemia preexisted. Ibrutinib did not significantly alter serum IgA and IgG levels as has also been observed in chronic lymphoid leukemia and mantle cell lymphoma patients [6, 7]. Collectively, the safety and IgA/IgG-sparing effects distinguish ibrutinib from other salvage options in WM [8, 9]. Transient increases in serum IgM levels, however, commonly occur during hold periods for toxicity or procedures and recover with reinstitution of therapy.

Atrial fibrillation related to ibrutinib occurred in 3 patients in this study, all of whom had a prior history of paroxysmal atrial fibrillation. Atrial fibrillation resolved after ibrutinib was held, without cardiological intervention, and protocol therapy resumed uneventfully in all three patients. Atrial fibrillation associated with ibrutinib therapy was also been observed in patients with previously treated CLL in approximately 5 % of patients. Off-target effects may be responsible for both atrial fibrillation and bleeding diathesis associated with ibrutinib.

In a more recent study, the impact of MYD88 and CXCR4 mutation status was also assessed for ibrutinib response [10]. Both L265P and non-L265P MYD88 activating mutations, as well as CXCR4 WHIM mutations, were assessed. Activating CXCR4 somatic mutations in WM are similar to germline mutations found in WHIM (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis) syndrome patients [11]. At least 30 different $CXCR4^{WHIM}$ somatic mutations are present in WM [2, 12]. Tumor cells engineered to express $CXCR4^{WHIM}$ mutations show enhanced activation of pro-survival factors, AKT and ERK (Fig. 22.1), and decreased in vitro ibrutinib-related apoptosis [13–15]. Overall and major response rates were highest in patients with $MYD88^{L265P}CXCR4^{WT}$ (100 % and 92 %), followed by $MYD88^{L265P}CXCR4^{WHIM}$ (86 % and 62 %) and $MYD88^{WT}CXCR4^{WT}$ (60 % and 0 %) [10]. Overall and major response rates improved with prolonged therapy (>6 cycles) in patients with $MYD88^{L265P}CXCR4^{WT}$ and $MYD88^{L265P}CXCR4^{WHIM}$, with more pronounced improvements occurring for the latter [5]. Best serum IgM and hemoglobin responses were also impacted by tumor

genotype, with improvements most evident in patients with MYD88^{L265P}CXCR4^{WT} and least in those with MYD88^{WT}CXCR4^{WT} [5]. Lastly, MYD88^{WT}CXCR4^{WT} mutation status was associated with inferior progression-free survival [5]. Therefore, the use of ibrutinib should be reserved for those patients with MYD88 mutated disease, and genotyping of tumor cells for MYD88 status should be considered in all WM patients being considered for ibrutinib therapy [8].

The single agent activity of ibrutinib in WM has also been reported in two other studies [16, 17]. The outcome of four previously treated WM patients who received ibrutinib as part of a Phase I study was reported in one of these studies wherein patients received 12.5 mg/kg/day to 560 mg a day of ibrutinib. Three of 4 WM patients included in this study achieved a major response and continued to respond >4 years following start of ibrutinib therapy [16]. Treatment was well tolerated for these individuals. One patient required dose reduction from 560 to 420 mg for atrial fibrillation. Dimopoulos et al. [17] reported the outcome of 31 rituximab refractory WM patients with a median prior therapies of four (range 1–8) that received treatment as part of an open-label, multicenter study. With a median follow-up of 7.7 months, the overall response rate was 84 % with major responses achieved in 65 % of patients. Treatment was well tolerated with common grade ≥3 adverse events that included neutropenia (13 %), anemia, diarrhea, hypertension, and thrombocytopenia (6 % each). No episodes of atrial fibrillation were observed within the follow-up period in this study. One patient with wild-type MYD88 disease had early progressive disease. A frontline study examining the activity of ibrutinib has also been initiated and will also be examining subclonal evolution through longitudinal deep genomic sequencing. In addition to ibrutinib, other oral BTK-directed inhibitors are being investigated for their activity in WM patients including ACP-196, ONO/GS-4059, and BGB-3111.

While ibrutinib targets BTK, suppression of other kinases such as HCK may also contribute to responses in WM patients. HCK and IL6 transcription are both upregulated by MYD88 L265P [18]. IL6 triggers activation of HCK through IL6ST, and activated HCK triggers BTK, AKT, and ERK-directed pro-survival signaling. Ibrutinib binds to and suppresses HCK activation and blocks ATP kinase activity. In MYD88-mutated WM cells, transduction with either wild-type HCK or HCK bearing a mutated gatekeeper (T333M) leads to higher levels of ibrutinib resistance versus transduction with either wild-type BTK or BTK bearing a mutated ibrutinib binding site (C481S). The use of a more potent HCK inhibitor (A419259) versus ibrutinib also showed higher levels of WM cell killing compared to ibrutinib in MYD88-mutated WM and ABC DLBCL cells identifying HCK as a novel therapeutic target in WM [18].

22.2 Targeting PI3Kδ/AKT Signaling in WM

PI3Kδ is triggered by activating mutations in MYD88 and CXCR4 [13, 14, 18, 19]. The use of inhibitors that target PI3Kδ/AKT pathway has been investigated in WM. Everolimus is an oral inhibitor of the AKT–mTOR pathway. Inhibition of this pathway leads to apoptosis of primary WM cells and WM cell lines [20]. Sixty patients with a median of three prior therapies were treated with everolimus in a joint Dana Farber/Mayo Clinic study [21]. The ORR was 73 %, with 50 % of

patients attaining a major response. The median PFS in this study was 21 months. Grade 3 or higher related toxicities were observed in 67 % of patients with cytopenias constituting the most common toxicity. Pulmonary toxicity occurred in 5 % of patients and dose reductions due to toxicity occurred in 52 % of patients. A clinical trial examining the activity of everolimus in 33 previously untreated patients with WM was recently reported by the WMCTG that included serial bone marrow biopsies in response assessment [22]. The ORR in this study was 72 %, including partial or better responses in 60 % of patients. However, discordance between serum IgM levels upon which consensus criteria for response are based and BM disease response was common and complicated response assessment. At assessments performed at 6 months, 10 of 22 (45.5 %) patients for whom both serum IgM and BM assessments were performed, discordance between serum IgM and BM disease involvement was observed. Among these patients, 2 had no change and 8 had increased bone marrow disease involvement despite decreases in serum IgM levels. Grade $\geq$2 hematologic and non-hematologic toxicities related to everolimus were predominately hematological, including anemia (39.4 %), thrombocytopenia (12 %), and neutropenia (18.2 %). Non-hematological toxicities included oral ulcerations (27.3 %) that improved with oral dexamethasone swish and spit solution and pneumonitis (15 %); the latter leading to treatment discontinuation in 5 patients.

The PI3Kδ inhibitor, idelalisib, has also been examined in 10 previously treated WM patients that were treated in a prospective, multicenter study with various indolent B-cell malignancies. A 70 % overall response rate was observed among the WM patients included in this series [23]. Idelalisib has received approval by the U.S. FDA for other malignant B-cell indications, and a prospective study to more clearly define the role of this agent in previously treated WM patients will be commencing.

22.3 Targeting TLR Signaling in WM

Knockdown of TLRs 7 and 9 has been shown to block NFkB signaling in $MYD88^{L265P}$–expressing cells and induce cytotoxicity [24]. IMO-8400 is an oligonucleotide designed to inhibit Toll-receptors (TLRs) 7, 8, and 9 and induces cytotoxicity in $MYD88^{L265P}$–expressing WM cells [25]. A clinical trial examining the safety and efficacy of IMO-8400 in WM has been initiated. IRAK1 and IRAK4 are kinases that mediate $MYD88^{L265P}$-directed NFkB signaling [3, 26]. Inhibition of these kinases leads to apoptosis of WM cells and the combination of inhibitors targeting BTK and IRAK1/4 induces synergistic tumor cell killing [3, 26]. Compounds that inhibit IRAK signaling have been developed and are under intense preclinical investigation for use in $MYD88^{L265P}$ relevant diseases at this time [27, 28].

22.4 Targeting BCL-2 Signaling in WM

The antiapoptotic factor, BCL-2, is overexpressed in WM disease, though the etiology for this finding remains to be clarified [29, 30]. The BCL-2 inhibitor, ABT-199, induces apoptosis and shows at least additive antiapoptotic activity against WM cells co-treated with either ibrutinib or idelalisib [31]. In a prospective clinical study that included previously treated patients with various indolent B-cell malignancies, 3 of 4 WM patients demonstrated a response including one CR [32]. A prospective study to evaluate ABT-199 in WM patients with previously treated disease is contemplated, and future studies examining ABT-199 in combination with other active agents in WM including ibrutinib or idelalisib will also be of interest.

22.5 Targeting CXCR4 Signaling in WM

The C-terminal domain of CXCR4 is mutated in 30–35 % of patients and enhances SDF-1a triggered AKT and ERK signaling in WM cells engineered to express nonsense or frameshift mutations [2, 13–15]. Ibrutinib suppresses AKT and ERK signaling in CXCR4 wild-type expressing cells, but not mutated CXCR4 cells. AKT is hyperphosphorylated in malignant lymphoplasmacytic cells taken from the biopsies of WM patients with mutated CXCR4 and remains constitutively active despite ibrutinib therapy [13]. In preclinical studies, use of CXCR4 antagonists (plerixafor, ulocuplumab) blocked SDF-1a rescue of apoptosis mediated by ibrutinib, idelalisib, and other WM relevant therapeutics [13–15]. A long-term study of plerixafor in patients with germline WHIM syndrome showed that CXCR4 inhibition was both safe and effective, and a trial examining the combination of ibrutinib with the CXCR4 antagonist antibody ulocuplumab in CXCR4-mutated WM patients is being planned [33].

Table 22.1 Targeted therapies in Waldenstrom's macroglobulinemia

Agent	Target	Pathway	Status	Supporting references
Ibrutinib	BTK, HCK	MYD88	Approved for WM (FDA, EMA)	Treon et al. [5], Furman et al. [16], Dimopoulos et al. [17]
ACP-196	BTK	MYD88	Investigational	www.clinicaltrials.gov
ONO/GS 4059	BTK	MYD88	Investigational	www.clinicaltrials.gov
BGB-3111	BTK	MYD88	Investigational	Tam et al. [34]
Idelalisib	PI3Kδ	MYD88, CXCR4	Approved (CLL), investigational (WM)	Gopal et al. [23], www.clinicaltrials.gov
Everolimus	MTOR	MYD88, CXCR4	Approved (multiple indications), investigational (WM)	Ghobrial et al. [21], Treon et al. [22], www.clinicaltrials.gov
Ulocuplumab	CXCR4	CXCR4	Investigational	Study pending
ABT-199	BCL-2	BCL-2	Investigational	Davids et al. [32], www.clinicaltrials.gov

22.6 Summary

In summary, the genomic revelations made possible by whole genome sequencing have enabled the development of targeted therapies for the treatment of WM. Other agents targeting MYD88-, CXCR4-, and BCL-2-related pathways are currently in clinical trials and will likely bring forward additional targeted agents for the treatment of this disease. Lastly, tumor genomics will play an even greater role in the future for designing personalized treatment approaches for WM patients (Table 22.1).

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Immunomodulatory Agents and Proteasome Inhibitors in Waldenstrom's Macroglobulinemia

23

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23.1 Immunomodulatory Drugs

The success of immunomodulatory drugs (IMIDs) and proteasome inhibitors in multiple myeloma prompted in great part their investigation in Waldenstrom's Macroglobulinemia (WM). The IMIDs have been investigated as single agents and in combination therapy (Table 23.1). Dimopoulos et al. [1] observed an overall and major response rate of 25 % in among 20 previously untreated and treated WM patients who received single-agent thalidomide. Dose escalation from the thalidomide (starting dose of 200 mg daily) was hindered by adverse events, particularly the development of peripheral neuropathy that obligated dose reduction or discontinuation of therapy in 25 % of patients. Low-dose thalidomide (50 mg daily) was then explored in combination with dexamethasone (40 mg once a week) and clarithromycin (250 mg orally twice a day) by Coleman et al. [2] in 12 previously treated WM patients. The overall and major response rate in this small study was 83 %, and time to progression was 7 months. Despite the lower thalidomide dose, 40 % of patients in this study discontinued thalidomide due to neurotoxicity. Dimopoulos et al. [3] also explored the use of thalidomide (200 mg daily) along with dexamethasone (40 mg once a week) and clarithromycin (500 mg orally twice a day) in 12 previously treated WM patients. The overall and major response rates (i.e., at least partial response) in this study were 42 % and 25 %, respectively. Thalidomide dose was reduced to 100 mg a day in 5 (42 %) patients due to neurotoxicity in this study.

The thalidomide derivative, lenalidomide, has also been explored as a single agent in WM. The results of a multicenter study in France that examined

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_23

Table 23.1 Immunomodulatory drug-based treatment studies in Waldenstrom's macroglobulinemia

Study	Regimen	*N*=	Population	ORR (%)	Major RR (%)	Response duration
Dimopoulos et al. [1]	Thalidomide	20	Untreated, previously treated	25	20	N/A
Coleman et al. [2]	BLT-D	12	Previously treated	83	83	7 mo (TTP)
Dimopoulos et al. [3]	CTD	12	Previously treated	42	25	N/A
Leleu et al. [4]	Lenalidomide	17	Previously treated	36	N/A	16 mo (TTP)
Treon et al. [8]	ThalR	25	Untreated, previously treated	72	64	35 mo (TTP)
Treon et al. [9]	LenR	16	Untreated, previously treated	50	25	17 mo (TTP)
Treon et al. [10]	PDR	7	Previously treated	43	43	15 mo (DOR)

BLT-D clarithromycin (Biaxin™), low dose thalidomide, dexamethasone; *CTD* clarithromycin, thalidomide, dexamethasone; *ThalR* thalidomide, rituximab; *LenR* lenalidomide, rituximab; *PDR* pomalidomide, dexamethasone, rituximab; *N/A* not available; *mo* months; *TTP* time to progression; *DOR* duration of response

lenalidomide were recently reported [4]. Seventeen previously treated patients with a median of one prior therapy were enrolled. The maximum tolerated dose achieved in this Phase I/II study was 15 mg/day 21 days out of 28. Only 7/17 (41 %) patients completed 1 year of single agent lenalidomide on this dose. On an intent-to-treat basis, the overall response rate was 36 %. A flare in serum IgM levels was observed in five patients. With a median follow-up of 36 months, 14 patients progressed with a median time to progression of 16 months. The most common adverse event was fatigue of at least grade 2 that was reported in 50 % of study patients. Grade $\geq$3 anemia (14 %) and neutropenia (43 %) were the main hematological toxicities. No grade $\geq$3 thrombocytopenia was observed. Moreover, no second primary malignancy or thromboembolic events were reported.

The combination of IMIDs with rituximab has also been explored. Thalidomide and lenalidomide were shown to significantly augment rituximab-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) against lymphoplasmacytic cells [5]. An expansion of natural killer cells was also observed with thalidomide, which in previous studies was shown to be associated with improved rituximab response [6, 7]. In view of these data, the WMCTG conducted three Phase II clinical trials in symptomatic patients with WM combining either thalidomide, lenalidomide or pomalidomide with rituximab [8–10]. In the thalidomide plus rituximab study, 25 untreated and previously treated patients not exposed to either thalidomide or rituximab were enrolled. Intended therapy consisted of thalidomide administered at 200 mg daily for 2 weeks, followed by 400 mg daily thereafter for 1 year [8]. Patients received four weekly infusions of rituximab at 375 mg/m^2

beginning 1 week after initiation of thalidomide, followed by four additional weekly infusions of rituximab at 375 mg/m^2 beginning at week 13. A flare in serum IgM was experienced in 50 % of patients on thalidomide plus rituximab. The overall and major response rates were 72 % and 64 %, respectively. The median time to progression for all evaluable patients was 35 months in this series. Dose reduction of thalidomide occurred in all patients and led to discontinuation in 11 (44 %) patients. Among 11 patients experiencing grade $\geq$2 neuroparesthesias, 10 resolved to grade 1 or less at a median of 7 months. Given the high incidence of treatment-related neuropathy, the investigators recommended that lower doses of thalidomide (i.e., $\leq$200 mg/day) should be considered in this patient population.

In the WMCTG study that investigated lenalidomide and rituximab, 16 patients were initiated on lenalidomide at 25 mg daily on a syncopated schedule, wherein therapy was administered for 3 weeks, followed by a 1-week pause for an intended duration of 48 weeks [9]. Patients received 1 week of therapy with lenalidomide, after which rituximab 375 mg/m^2 was administered weekly on weeks 2–5 and then on weeks 13–16. A flare in serum IgM was experienced in 75 % of patients. The overall and major response rates in this study were 50 % and 25 %, respectively, and the median TTP for all evaluable patients was 17 months. In two patients with bulky disease, significant reductions in extramedullary disease were observed. An acute decline in hematocrit was also observed during the first 2 weeks of lenalidomide therapy in 13 (81 %) patients with a median absolute decrease in hematocrit of 4.8 %, resulting in anemia-related complications and hospitalizations for four patients. Despite dose reduction, most patients in this study continued to demonstrate aggravated anemia with lenalidomide. There was no evidence of hemolysis or general myelosuppression with lenalidomide in this study, and the mechanism for lenalidomide-related anemia in WM patients remains to be determined. Toxicities associated with lenalidomide led to its discontinuation in 14 (88 %) patients in this study, and the use of this agent in WM patients remains limited.

The combination of pomalidomide, dexamethasone, and rituximab (PDR) was also explored by the WMCTG in previously treated WM patients in a dose escalating Phase I study [10]. Intended therapy consisted of 52 weeks of daily pomalidomide 0.5–4 mg with rituximab 375 mg/m^2 and dexamethasone 40 mg given intravenously every week on weeks 1–4 and 12–15. Seven patients were enrolled (3 at 0.5 mg and 4 at 1.0 mg of pomalidomide) before study closure for rituximab-related IgM flaring that required recurrent plasmapheresis. Among the seven enrolled patients, 3 (43 %) attained a major response (1 VGPR; 2 PR), and three patients were nonresponders. One patient was not evaluable for response. Median time to response was 2.1 months. Three patients required emergent plasmapheresis for an IgM flare and led to discontinuance of protocol therapy. The median response duration was 15.1 months, and all three patients continued to respond at study reporting.

23.2 Proteasome Inhibitors

Unlike IMIDs, proteasome inhibitors (PIs) are widely used in the treatment of WM. Preclinical studies have elucidated multiple mechanisms of action for PIs in WM. Most of the preclinical work has focused on bortezomib, the first-in-class PI. Bortezomib blocks the ubiquitin–proteasome degradation pathway through reversible inhibition of the 26S proteasome, affecting signaling pathways that include NF-kB [11]. The induction of endoplasmic reticulum stress has also been implicated as a mechanism for bortezomib activity leading to disruption of the unfolded protein response that prompts WM cell apoptosis [12, 13]. PIs may also impact the supportive bone marrow microenvironment in WM as has also been implicated for its activity in multiple myeloma [12, 13]. Bortezomib has also demonstrated synergistic and/or additive preclinical activity in combination with other agents including steroids, rituximab, and signaling inhibitors in WM cells.

Given the above findings, PIs have undergone clinical investigation as a single agent and as part of combination therapy (Table 23.2). In a multicenter study by the WMCTG, previously treated WM patients received single agent bortezomib (1.3 mg/m^2 IV) on days 1, 4, 8, and 11 out of a 21 day cycle [14]. Twenty-seven patients received up to 8 cycles of bortezomib, and the overall and major response rates in this study were 85 % and 48 %, respectively. Responses were rapid in this trial, with median times to first and best responses of 1.4 months and 4.1 months, respectively. The median TTP for all patients was 7 months. A trend for a longer median TTP was observed in patients achieving a major response (9 months) in this study.

Similar results were also achieved with single agent bortezomib given in a similar manner as the WMCTG trial by the National Cancer Institute of Canada (NCIC) [15]. Twenty-seven untreated and previously treated patients were enrolled in the NCIC trial, wherein overall and major response rates were 78 % and 44 %, respectively. The median time to first response was 1.5 months. Nodal responses were assessed in this study and were slower, though occurred in most patients with a median time to response of 12 weeks. The median PFS for all patients was 16 months in this study. Dimopoulos et al. [16] also investigated the activity of single agent bortezomib in 10 previously treated patients. A major response was observed in 6 patients (60 %), while two (20 %) additional patients attained a minor response for an overall response rate of 80 %. The median time to a major response was 16 weeks, and the TTP was expected to exceed 11 months for responders. While changes in serum IgM are used to determine responses in WM, discordance between serum IgM and bone marrow burden reductions has been reported in studies examining single agent bortezomib [14, 17].

To improve depth of response as well as progression-free survival, combination therapies with bortezomib have been evaluated. The combination of bortezomib, dexamethasone, and rituximab (BDR) was investigated by the WMCTG as primary therapy in 23 WM patients [18]. Patients received, by intravenous injection, bortezomib at 1.3 mg/m^2 along with dexamethasone 40 mg on days 1, 4, 8, and 11 and rituximab was given on day 11 of each 21 day cycle. In this study, four

Table 23.2 Proteasome inhibitor-based treatment studies in Waldenstrom's macroglobulinemia

Study	Regimen	*N*=	Population	ORR (%)	Major RR (%)	Response duration
Treon et al. [14]	Bortezomib	27	Previously treated	85	48	7 mo (TTP)
Chen et al. [15]	Bortezomib	27	Untreated, previously treated	78	44	16 mo (TTP)
Dimopoulos et al. [16]	Bortezomib	10	Previously treated	60	20	>11 mos (DOR)
Ghobrial et al. [20]	VR	26	Untreated	88	65	79 % (1 yr EFS)
Ghobrial et al. [21]	VR	37	Previously treated	81	51	16 mo (TTP)
Agathocleous et al. [22]	VR	10	Previously treated	90	90	N/A
Treon et al. [18, 19]	BDR	23	Untreated	96	83	66 mo (TTP)
Dimopoulos et al. [23]	BDR	59	Untreated	85	68	43 mo (PFS)
Ghobrial et al. [24]	RVR	36	Previously treated	89	56	21 mo (PFS)
Treon et al. [25]	CaRD	31	Untreated, previously treated	87	68	N/R after median follow-up of 15 mo
Siegel et al. [26]	Oprozomib	17	Previously treated	59	29	N/A

BDR bortezomib, dexamethasone, rituximab; *VR* bortezomib, rituximab; *RVR* RAD001 (Everolimus™), bortezomib (Velcade™), rituximab; *CaRD* carfilzomib, rituximab, dexamethasone
N/A not available; *N/R* not reached; *TTP* time to progression; *DOR* duration of response

cycles were administered as induction therapy. Twelve weeks after induction therapy, one cycle of therapy was given every 12 weeks for a total of four cycles as maintenance therapy. The overall response rate was 96 %, with 83 % of patients achieving a major response. The median time to response was 1.1 months in this study. An IgM flare was observed in only 9 % of patients. In a follow-up report, the median time to progression was 66 months with this regimen [19]. Treatment-related neuropathy contributed to bortezomib discontinuation in 60 % of patients. With the longer follow-up, complete resolution or partial resolution of treatment-related neuropathy to grade 1 was observed in most patients [19]. Dexamethasone-related hyperglycemia, myopathy, and gastritis also prompted omission or dose reduction of steroids in many patients. Furthermore, the addition of steroids to bortezomib was deemed responsible for herpes zoster that occurred in four of the first seven patients entered in this trial, who did not receive herpes zoster prophylaxis, leading to institution of prophylaxis while on active therapy plus six

additional months following end of therapy. The use of famotidine during active therapy along with use of lower dexamethasone dosing with BDR was also recommended by the study investigators.

In an effort to ameliorate steroid-related toxicity, Ghobrial et al. examined bortezomib in combination with rituximab (VR) in both untreated [20] and previously treated [21] patients with WM. In these trials, bortezomib was administered intravenously weekly at 1.6 mg/m^2 on days 1, 8, 15, and 28 days for six cycles and rituximab given at 375 mg/m^2 weekly during cycles 1 and 4. The overall response rate for the 26 untreated patients who received VR was 88 %, with a major response of 65 % [20]. The median time to progression at time of reporting was not reached, with an estimated 1-year event free rate of 79 %. Common grade 3 and 4 therapy-related adverse events included reversible neutropenia in 12 %, anemia in 8 %, and thrombocytopenia in 8 % of patients. Treatment-related neuropathy was observed in 15 % of patients at grade 2 and in none of the patients at the grade 3 or 4 level. Among 37 previously treated patients with VR, the overall response rate was 81 %, including 51 % of patients who achieved a major response [21]. The median TTP in this study was 16.4 months. The most common grade 3 and 4 therapy-related adverse events included reversible neutropenia in 16 %, anemia in 11 %, and thrombocytopenia in 14 %. Grade $\geq$3 treatment-related peripheral neuropathy occurred in two patients (5 %). In the above studies with VR, the rate of rituximab-related IgM flaring was 20–30 %. Agathocleous et al. [22] also examined VR in previously treated WM patients, in a study that also included other lymphoma histologies, and randomized between weekly and twice weekly intravenous bortezomib along with rituximab. Nine of 10 (90 %) WM patients in this study had a major response, and four of these patients remained free of progression after 2 years. The risk of neuropathy was not impacted by treatment arm (weekly versus biweekly bortezomib administration) in this study, though only a small number of WM patients were included in each arm.

In a prospective, multicenter clinical trial conducted by the European Myeloma Network (EMN), a novel schedule of administration for BDR was evaluated in 59 untreated WM patients [23]. Patients received twice weekly intravenous bortezomib (1.3 mg/m^2 days 1, 4, 8, and 11 of a single 21-day cycle) for the first cycle, then weekly (at a dose of 1.6 mg/m^2 on days 1, 8, 15, and 22 of 35-day cycles) for cycles 2–5, together with dexamethasone (40 mg). Rituximab (375 mg/m^2) was dosed weekly in cycles 2 and 5. Unlike the WMCTG trial, no maintenance therapy was employed. An overall response rate of 85 % was attained, including a major response in 68 % of patients. The median PFS in this study was 43 months, and patients with VGPR or better had significantly longer PFS. Treatment-related peripheral neuropathy (PN) occurred at grade 2 in 17 % and grade 3 in 7 % of patients. Rituximab-related IgM flaring was observed in 11 % of patients without need for preemptive plasmapheresis.

The combination of the mTOR inhibitor everolimus with bortezomib and rituximab (RVR) has also been examined in a prospective trial in patients with previously treated WM [24]. Thirty-six patients received six cycles of RVR. Each 28 day cycle consisted of everolimus 10 mg by mouth daily; bortezomib 1.6 mg/m^2

was given intravenously weekly on days 1, 8, 15, and for cycles 1 and 4 only; and rituximab 375 mg/m^2 was given intravenously on days 1, 8, 15, and 22. Following six cycles of therapy, patients continued on everolimus alone as maintenance therapy until progression. Most patients on RVR had received prior rituximab and/or bortezomib. An overall response rate of 89 %, with a major response rate of 53 % was observed in this study. The median progression-free survival was 21 months. No grade $\geq$3 peripheral neuropathy was observed in this study, though cytopenias were commonly observed that included grade $\geq$3 anemia (30 %), neutropenia (13 %), and thrombocytopenia (13 %).

Given the reported neuropathy-sparing effects of carfilzomib in myeloma patients, the combination of carfilzomib, rituximab, and dexamethasone (CaRD) was examined in WM patients naïve to bortezomib and rituximab [25]. CaRD therapy consisted of intravenous carfilzomib 20 mg/m^2 (cycle 1), 36 mg/m^2 (cycles 2–6) with dexamethasone 20 mg on days 1, 2, 8, 9 and rituximab 375 mg/m^2 on days 2, 9, and every 21 days. Maintenance therapy followed 8 weeks later with intravenous carfilzomib 36 mg/m^2 and dexamethasone 20 mg on days 1, 2 and rituximab 375 mg/m^2 on day 2 every 8 weeks for eight cycles. The overall response rate in this study was 87 % and 68 % of patients achieved a major response. MYD88 and CXCR4 tumor mutational status was examined in this study and did not appear to impact response attainment. With a median follow-up of 15.4 months, 20 patients remained progression free at the time of reporting. Grade 2 peripheral neuropathy occurred in one patient with underlying disease-related peripheral neuropathy, and no grade 3 or higher treatment-related neuropathy events were observed. Other grade $\geq$2 toxicities included asymptomatic hyperlipasemia (41.9 %), reversible neutropenia (12.9 %), and cardiomyopathy in one patient (3.2 %) with multiple risk factors. Declines in serum levels of IgA and IgG were common and contributed to recurring sinobronchial infections and IVIG use in a few patients.

Oral PIs are also under investigation in WM patients, and their preliminary experience has been notable for their neuropathy-sparing effects. Oprozomib is an oral epoxyketone proteasome inhibitor that is an analog of carfilzomib. An ORR of 59 % with a major response rate of 29 % was observed in a Phase II study in 17 previously treated WM patients treated with single agent oprozomib [26]. Gastrointestinal intolerance has been a prominent toxicity of oprozomib necessitating antiemetic use. Clinical trials examining the oral proteasome inhibitor ixazomib have also been initiated in combination with dexamethasone and rituximab (IDR) in symptomatic untreated, as previously treated WM patients. Ixazomib was recently approved by the U.S. FDA for treatment of myeloma patients. Results from the IDR trials in WM patients are awaited.

In summary, IMIDs and PIs have shown significant activity in WM. IMIDs have activity in WM, however, their use in this disease is hampered by their toxicity profile. Neurotoxicity of thalidomide is of major concern, while the more potent IMIDs, lenalidomide and pomalidomide, seem to be rather poorly tolerated. In contrast, the high rates of efficacy, particularly in combination therapy, as well as the overall tolerability of PIs have resulted in their adoption as important mainstays of WM therapy. The development of strategies to mitigate neuropathy risk of PIs,

including use of weekly, subcutaneous administration for bortezomib and exploration of novel neuropathy sparing agents will invariably lead to further advances in the use of this class of agents in the treatment of WM.

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High-Dose Therapy and Haemopoietic Stem Cell Transplantation in Waldenström's Macroglobulinaemia

24

C. Kyriakou

24.1 Introduction

Waldenström's macroglobulinaemia (WM) is, according to the World Health Organisation classification system, a lymphoplasmacytic lymphoma that is characterised by bone marrow infiltration by lymphoplasmacytic cells and associated IgM monoclonal gammopathy [1–3].

WM is a rare disease with overall incidence of approximately three per million persons per year, accounting for approximately 1–2 % of all non-Hodgkin lymphomas. The highest incidence of WM occurs among older individuals, with a median age at diagnosis in the 60s [4, 5].

Despite its rarity, our understanding of the disease biology has significantly improved in recent years with the identification of recurrent mutations in the MYD88 and CXCR4 genes. Based on the diversity of clinical and biological features observed in WM patients, therapy should be highly personalised taking into account several individual patient and disease factors such as age, co-morbidities, constitutional symptoms, need for rapid disease response, candidacy for high-dose therapy and haemopoietic stem cell transplantation, IgM levels, cytopenias, presence of hyperviscosity, coagulopathy, cryoglobulinaemia, cold agglutinin disease and peripheral neuropathy.

WM patients with asymptomatic disease should be observed since they have similar survival to the general population [6–8]. The international consensus panel recommendations clearly identified the symptoms and/or signs that indicate the need to start therapy. The standard therapies for symptomatic WM patients at presentation or relapse include a variety of chemotherapeutic agents, monoclonal antibodies and new agents alone or in combinations. However, the rarity of the disease has prevented the development of well-designed prospective comparative

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_24

clinical trials that are necessary for any drugs or combinations to achieve formal indication. Accordingly, WM has no standard formally approved therapy. This reinforces the recommendations of experts, such as those provided by the consensus panel during the 7th International Workshop for WM [9]. The International Prognostic Scoring System for WM (IPSSWM) has been validated as a reliable tool for treatment decision and it is important to use for the risk stratification for the treatment of WM patients [10–13].

The data on therapies for WM come mainly from non-randomised studies. Based on the available data, as well as the experience in treating patients with low-grade lymphomas, the recommended treatment options for symptomatic disease include anti-CD20-based combinations with alkylating agents, steroids, proteasome inhibitors and purine analogues. The combination of anti-CD20 with dexamethasone cyclophosphamide (DRC), or bendamustine (BR), or fludarabine (RFluCy) or proteasome inhibitors (BRD) have achieved high response rates with relatively less toxicity and adequate duration of responses. The use of chemotherapeutic drug can enable better nodal disease debulking, whilst proteasome inhibitor can achieve rapid response. The use of anti-CD52 antibody has also produced good responses although with higher associated toxicity.

At disease relapse, treatment options depend on the duration and response to prior therapies, the patient's overall condition, age and candidacy for ASCT. Administering the same regimen used as initial treatment could be considered in patients who achieved response that lasted $\geq$12 months, otherwise, alternate single or combination treatments used as primary treatment options are effective salvage therapies. Further advances in new agents keep multiple options available for disease relapse.

24.2 Is There a Role for High-Dose Therapy and Autologous Stem Cell Transplantation in the Treatment of WM?

In the era of a plethora of highly effective and new promising drugs for an indolent disease that frequently affects elderly patients, the role of high-dose therapy and stem cell transplantation (SCT) has remained a controversial option.

From the biological point of view, WM is considered a transition from the mature B lymphoid cell after antigen stimulation in peripheral lymph nodes to the final antibody-secreting plasma cell. Therefore, WM is an intermediate disease between mutated CLL and MM and shares several characteristics with both entities, although the gene transcription profile of WM resembles more that of chronic lymphocytic leukaemia (CLL) than that of multiple myeloma (MM) [14]. Both WM characteristics have prompted the use of treatment strategies derived from B-CLL and MM, although the responses have not been predictable as might have been expected according to previous experience with these two entities. The value of SCT for CLL has been well documented [15], although the incidence of WM is considerably lower from both CLL and MM [14, 16]. There is no known typical adverse cytogenetics in WM as happens in the case of MM in which SCT is

regularly contemplated and 17p deletion is an uncommon abnormality [17, 18]. Furthermore, WM is a far less kinetically active cancer than MM [19, 20]. Thus, the low proliferative index rate and the favourable genetics of WM might suggest that high-dose myeloablative therapy could result in deeper and more durable response since the cells have not developed drug resistance and are unlikely to regrow in a short time. As a consequence, someone would expect better outcomes from SCT in WM.

24.3 High-Dose Therapy and Autologous Stem Cell Transplantation

For stem cell transplant candidates at first or subsequent relapses, the choice of first line therapy is important. The use of stem cell toxic therapeutic agents could compromise adequate peripheral blood stem cell mobilisation. Reports on the use of purine analogues in stem cell harvest failure have been reported in other low-grade lymphoma and CLL [21]. Updated treatment recommendations from the 7th International Workshop on WM and the NCNN guidelines specifically advise avoidance of purine nucleoside analogues for potential transplant candidates [22].

Furthermore, the successful haemopoietic stem cell mobilisation is directly related to the number of previous therapy lines and disease response following multiple relapses and consideration of stem cell collection is suggested. Due to borderline patient presentation or relapse age together with financial pressures for stem cell harvest, cryopreservation and storage, the harvest for "rainy days" has not been a standard practice in most nations.

Published literature on SCT for WM was limited to small case series (largest series on ASCT are summarised in Table 24.1). The German group was investigating in a prospective study the feasibility of high-dose therapy and SCT as part of first-line treatment of indolent lymphomas. Twelve WM patients were included, and after an optional cytoreduction with alkylators or fludarabine, they received salvage with Dexa Mini-BEAM for stem cell mobilisation and remission induction followed by myeloablative therapy with total body irradiation (TBI)/high dose cyclophosphamide and stem cell reinfusion. The first 5/12 patients' grafts were vigorously B-Cell depleted. All patients achieved very good partial response and 6 patients had disease progression at a median time of 69 months and with median time to retreatment of 82 months [23].

In another pilot study, five patients were treated with the stem cell transplant as part of initial therapy of WM using Cyclophosphamide/Granulocyte Colony-Stimulating Factor (G-CSF) for stem cell harvest and Melphalan 200 mg/m^2 as conditioning. The authors reported blood and biochemical responses at a median follow-up of 66 months (range, 56–75); after the ASCT, all patients were evaluable, alive without clinical or serological signs of disease progression and maintain a stable very good partial response [24].

Table 24.1 Autologous stem cell transplantation in WM

Author or Series	Year	*N*	Median follow-up (months)	PFS	OS
Initial therapy					
Dreger	2007	12	69	Median PFS: 69 months	100 % at 69 months
Caravita	2009	5	66	100 % at 66 months	–
Kyriakou	2010	69	54	52 % at 5 years	77 % at 5 years
Relapse					
Anagnostopoulos	2006	10	63	65 % at 3 years	70 % at 3 years
Gilleece	2008	9	44	DFS: 43 % at 4 years	73 % at 4 years
Kyriakou	2010	158	50	40 % at 5 years	69 % at 5 years

PFS progression-free survival, *OS* overall survival, *DFS* disease-free survival

Another group reported the outcome of ASCT on six WM patients, two having ASCT as first line therapy and four following disease relapse. Interestingly, this study identified the failure to harvest patients exposed previously on Fludarabine. Melphalan was used as conditioning regimen apart from one patient who had addition of TBI. The responses reported were high with patients been free of disease at follow up at 52, 15, 12 and 2 months, respectively [25].

In another study, eight WM patients underwent ASCT with melphalan-based regimen and seven achieved partial responses, one complete response and five of these eight patients were alive and relapse free at 6–77 months after ASCT [26].

In another publication, on 24 heavily pretreated WM patients who received ASCT with either melphalan or cyclophosphamide-based conditioning and nine of them had additional TBI, the results showed responses in 23/24 patients and 9/23 evaluable patients achieved a complete response [27].

The first report of the French experience on ASCT in 19 WM patients was published in 2003. The median time from diagnosis to transplant was 36 months. High-dose therapy yielded a 95 % response rate and within a median follow-up of 18 months, 12 patients were alive at 10–81 months and eight patients were relapse free at 10–34 months [28].

A retrospective review from the Center for International Blood and Marrow Transplant Research database reported the ASCT outcomes on nine WM patients with relapse or refractory disease and one patient treated as part of first line therapy. The reported progression-free and overall survivals at 3 years with median follow up of 63 months were 65 % and 70 %, respectively [29].

The British Society of Blood and Marrow Transplantation reported nine patients with lymphoplasmacytic lymphoma who were treated at subsequent relapses with

ASCT. The median follow-up was 44 months with a 4-year PFS of 43 % and an OS at 4 years of 73 % [30].

The updated French experience on ASCT in 32 heavily pretreated WM has also been reported by Dhedin et al. Conditioning was with BEAM regimen in 13, melphalan and TBI in 9, TBI/cyclophosphamide in seven patients and with a median follow-up of 45 months, the median EFS was 32 months and the 5-year survival was 58 %. The TRM was 12.5 %. Thus, ASCT can achieve long-term responses even in heavily pretreated patients.

The largest cohort, so far, was published on 158 WM patients registered at the European Bone Marrow Transplant Registry (EBMT) and transplanted between 1991 and 2005. The median age at ASCT was 53 years, and at presentation, 37 % of the patients had intermediate-risk and 54 % had a high-risk disease as defined by the ISSWM. Twenty-one percent of the WM patients were autografted in first VGPR (VGPR1), 9 % in second VGPR (VGPR2) and 5 % in the third or later VGPR (VGPR3). Thirty-one percent of the patients were autografted in first partial response (PR1) and 19 % in the second or later PR (PR2). G-CSF alone or in combination with chemotherapy in 77 % was used for stem cell mobilisation. Conditioning regimen was chemotherapy-based in the majority of the patients. At a median follow-up of 4.2 years, CR was reported in 22 %, VGPR in 50 %, PR in 15 and 11 % had disease relapse or progression. Sixty-eight percent of the patients were alive and 32 % died, with 26 % dying of disease progression. Incidence of non-relapse mortality (NRM) was 3.8 % at 1 year, 4.6 % at 3 years and 5.6 % at 5 years post-ASCT. This study reported cumulative incidence of secondary malignancies after ASCT of 8.4 % at 5 years. In addition, analysis had stressed the prior use of fludarabine that was found to be an independent factor for secondary malignancies and also was associated with harvest failure and need for further mobilisation attempts. The OS rate at 5 years was 68.5 and 49 % patients were alive and remained progression free, with estimated PFS rates of 39.7 % at 5 years. Forty-five percent have relapsed or progressed within a median time from transplantation of 1.3 years and the estimated relapse rate was 48 % at 5 years. Chemorefractory disease, $\geq$3 prior therapy lines and poor performance were unfavourable factors for NRM, relapse rate (RR), PFS and OS. A subgroup analysis was performed on 69 patients autografted after a first response (VGPR1/PR1) and the estimated PFS at 5 years was 51.5 % and OS at 5 years was 77 % [31].

Although the published data are from small series and all reports are from retrospective studies, none of them reported high ASCT-related toxicity and all suggested that ASCT is a feasible treatment option for WM and produces durable responses. The international WM and the EBMT consensus recommendations suggest that ASCT should not be offered as first line therapy but should be considered a salvage treatment option for transplant eligible patients at first or subsequent disease relapse. It is important for the ASCT candidates to avoid first-line therapies with stem cell toxic chemotherapeutic combinations (Table 24.2). ASCT could also be a bridging therapy with lasting responses in the era of major advances in novel agent development. ASCT is a feasible and effective treatment option for transplant eligible patients but should be offered at early relapses. It can

Table 24.2 Primary or salvage treatment regimen for Waldenstrom's macroglobulinaemia by possible stem cell toxicity

Waldenstrom's macroglobulinaemia *Treatment regimens for fist or subsequent line therapies that might be associated with stem cell toxicity*
Possible stem cell toxicity and/or unknown
Bendamustine ± rituximab
Cladribine ± rituximab
Chlorambucil
Fludarabine ± rituximab
Fludarabine/cyclophosphamide ± rituximab
Non-stem cell toxic regimens
Alemtuzumab
Bortezomib ± rituximab
Bortezomib/dexamethasone
Bortezomib/dexamethasone ± rituximab
Cyclophosphamide/doxorubicin/vincristine/prednisolone/rituximab
Everolimus
Ibrutinib
Ofatumumab
Rituximab
Rituximab/cyclophosphamide/dexamethasone

result in higher, deeper and lasting response rates with relatively low toxicity. ASCT is not beneficial for patients exposed to more than three line therapies or with chemorefractory disease.

24.4 Is There a Role for High-Dose Therapy and Allogeneic Stem Cell Transplantation in the Treatment of WM?

If the place of high-dose therapy and autologous stem cell transplantation in the treatment algorithm of the WM is gradually becoming clearer, the role of alloSCT remains still controversial. Data on alloSCT for patients with WM are limited. Certainly, the relatively high age of patients at diagnosis, the increasing therapeutic options for the WM patients and the difficulty to identify those patients destined to do poorly at diagnosis all may have contributed to the limited data. In addition, the unacceptably high NRM reported after myeloablative conditioning (MAC) alloSCT also played a role [28, 29]. NRM after alloSCT decreased in virtually all lymphoma subtypes over the last decade and this has largely been attributed to the introduction of reduced intensity conditioning (RIC). RIC protocols were designed to reduce NRM but to preserve the clinically important graft-versus-lymphoma effect. Initial data on alloSCT for WM are mainly from case reports on patients with refractory or multiply relapsed WM.

A retrospective review on MAC alloSCT in five WM treated with MAC using busulfan and cyclophosphamide combination reported that with a median follow up of 32 months, four of the five patients were alive and disease free, and in addition, a

further gradual drop of the serum IgM levels in all patients was observed, suggesting an active graft-versus-WM effect [32].

An earlier case report on a 62-year-old man with refractory WM underwent a RIC alloSCT [33] and small case series on three WM patients from the MD Anderson group [34] reported encouraging outcomes. In another report by Meniane et al., a 52-year-old patient received RIC alloSCT for primary refractory WM disease but without adequate clinical response. Following donor lymphocyte infusion for mixed chimerism and refractory disease, the patient converted to full donor chimerism and developed limited chronic GVHD but the disease response was escalated to complete remission [35].

A systematic review, in six heavily pretreated WM patients, having alloSCT reported prolong survival in three of the six patients. Two patients died from NRM and one from disease progression [36].

The French group reported results in 10 WM patients treated with MAC alloSCT and six patients were alive at 3, 23, 50, 59, 74 and 76 months; however, the NRM was high at 40 % [28].

In another review presented by Dhedin et al., at the International WM Workshop in 2006, 11 WM patients received RIC and 11 patients had MAC alloSCT. The reported median EFS had not been reached, the OS was 68 % at 5 years and NRM was 27 % for the RIC group. For the patients treated with MAC alloSCT, the median survival was 36 months, OS at 5 years was 54 % and NRM was 36 %.

Results were reported on 26 WM patients from the Center for International Blood and Marrow Transplant Research registry. MAC alloSCT was used in 58 % of this group and after a median follow-up of 65 months, 35 % of all patients were alive. NRM was 40 % and estimated PFS and OS at 3 years were 31 % and 46 %, respectively [29].

Meniane et al. reviewed reports on 46 WM patients receiving alloSCT. Twelve patients were transplanted using RIC and 34 using the MAC regimen. Survival was 46 % and NRM 40 % at 3 years [35].

The British group reported the experience of seven patients treated with RIC and 2 with MAC alloSCT. The median follow up was 32 months and NRM was 44 % at 1 year and the DFS and OS at 4 years were 44 % and 56 %, respectively [30].

Although the data from these initial reports were on small patient series, all reported outcomes on heavily pretreated WM patients and pointed to high transplant related toxicity but also suggested that the alloSCT is feasible in a population of WM patients who were refractory or had multiply relapsed disease and could result in prolong survival.

Garnier et al. reported on 25 WM patients, 13 treated with MAC and 11 with RIC alloSCT. Forty-four percent had primary refractory disease and a median of three prior therapy lines. The overall response rate was 92 %, with 50 % of the evaluable patients obtaining complete response. With a median follow-up of 64 months among survivors, the 5-year PFS and OS were 58 % and 67 %, respectively. This report showed that allogeneic stem cell transplants yielded a high rate of complete responses and were potentially curative in poor-risk group of WM patients [37].

Maloney et al. presented, at the fifth International Worskshop for WM in 2008, data on 13 patients with advanced stage WM who underwent allograft with low dose TBI (2 Gy) and fludarabine. The 4-year PFS and OS rates, in a heavily pretreated, chemorefractory group of WM patients, were 60 %, NRM was 31 % and response rate was 91 %; those patients who developed extensive cGVHD had longer survival, suggesting the existence of a graft-versus-WM effect.

The Lymphoma Working Party reported the experience on 86 WM from the European Bone Marrow Transplant Registry. Thirty-seven were treated with myeloablative and 49 with reduced intensity conditioning. Eight patients had failed a previous autologous stem cell transplant and 68.6 % had chemosensitive disease at the time of alloSCT. The median follow-up of survivors was 50 months and the overall response rate was 75.6 %. The non-relapse mortality at 3 years was 33 % for the MAC and 23 % for the RIC patients. The 3-year relapse rate was 11 % for myeloablative transplants and 25 % for reduced-intensity transplants. The 5-year PFS and OS were 56 % and 62 %, respectively, for myeloablative transplants and 49 % and 64 % for reduced-intensity transplants. Chronic GVHD was associated with higher non-relapse mortality and a lower relapse rate, leading to the improved PFS. This report showed that allogeneic stem cell transplant can induce durable remissions in heavily pretreated patients. The low relapse rate in patients developing chronic GVHD suggests the existence of a clinically relevant graft-versus-Waldenstrom macroglobulinemia effect [38] (largest series on alloSCT are summarised in Table 24.3).

Although there are major diagnostic and therapeutic advances in the management of WM patients, the disease remains still incurable with the current available therapies and following multiple relapses, the duration of responses, if still responding, shortens and ultimately the disease becomes refractory. There is also a recognised by the ISSWM group of patients with poor prognosis disease. The International consensus recommendations suggest that alloSCT should be ideally undertaken in the context of a clinical trial. Due to the rarity of the disease, older group of patients and the smaller subgroup of WM patients that might need the alloSCT approach, clinical trial with alloSCT for WM is challenging. The information

Table 24.3 Allogeneic transplantation in WM

Series						
Author	Year	*N*	Follow-up (median)	NRM	PFS	OS
Anagnostopoulos	2006	MAC: 21 RIC: 5	75 months	40 % at 3 years	31 % at 3 years	46 % at 3 years
Garnier	2010	MAC: 12 RIC: 13	63 months	25 % at 1 year	58 % at 5 years	67 % at 5 years
Kyriakou	2010	MAC: 37	63 months	33 % at 3 years	56 % at 5 years	62 % at 5 years
		RIC: 49	44 months	23 % at 3 years	49 % at 5 years	64 % at 5 years

MAC myeloablative conditioning regimen, *RIC* reduced intensity conditioning regimen, *NRM* non-relapse mortality, *PFS* progression-free survival, *OS* overall survival

from the published data so far, although from small series and retrospective studies, suggests that a group of young alloSCT eligible WM patients can be rescued after multiple line therapies and with chemorefractory disease. The reduced intensity conditioning has become the choice of alloSCT in a rather elderly group of patients in an attempt to reduce toxicity. The association of cGVHD with better PFS and lower relapse rates, together with the fact that donor lymphocyte infusion can escalate disease response, favours the existence of graft-versus-WM effect. AlloSCT is associated with relatively higher toxicity. Patients who survive the toxicity of alloSCT can be potentially cured from this approach. This treatment option should remain as possible line therapy at advanced stage unless patients belong to the primary refractory group to conventional therapies group and then someone should consider the overall short- and long-term benefit of these patients and proceed to the procedure at earlier stage. It should remain an option for WM patients who experience failure of other therapies including ASCT.

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Long-Term Toxicity of Therapy in Waldenström Macroglobulinemia

25

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25.1 Chemotherapy-Related Second Hematologic and Non-Hematologic Cancers

t-MDS/AML has been frequently reported as a long-term complication of WM [1–10]. The incidence of t-MDS/AML in WM ranges from 1.2 to 8.9 % (Table 25.1). The real incidence may be underestimated due to the need of a bone marrow examination to confirm the diagnosis.

Disease transformation to DLBCL represents a well-known complication of chronic lymphocytic leukemia and follicular lymphoma. This event is less commonly described in other indolent lymphoproliferative disorders and mostly appears in heavily pretreated patients. In WM patients, the occurrence of transformation has been more frequently reported after NA treatment. The incidence of transformation ranges from 1.4 to 10 % (Table 25.1) [2, 4–6, 8–10]. The cumulative incidence of all hematologic malignancies is 4.2 % at 10 years [15].

Few data are available concerning the incidence of SSTs in WM. Varettoni et al. [16] reported a risk of second cancer in pretreated WM patients 1.69 times higher than expected. The cumulative incidence of solid tumors among survivors of WM is 12 % at 10 years, while the cumulative incidence of any secondary malignancy is 9.5 % at 5 years and 16.1 % at 10 years [15].

Disease-related immunodeficiency and therapy-related DNA damage have been postulated as possible mechanisms predisposing to second cancers in non-Hodgkin lymphomas. Some authors underline the crucial role of the immune system in the oncogenesis in WM, reporting an increased risk of second hematologic and non-hematologic cancers also in untreated patients [16].

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_25

Table 25.1 Incidence of t-MDS/AML and of transformation to DLBCL in WM patients according to the type of treatment

Study (Year)	*N*	Median F-U (months)	Treatment	t-MDS/AML	Transformation to DLBCL
Facon et al. [1]	167	–	AA	2/167 (1.2 %)	
Leblond et al. [2]	71	34	NA	1/71 (1.4 %)	5/71 (7 %)
Kyle et al. [3]	46	–	AA	4/46 (8.6 %)	
Garcia-Sanz et al. [4]	217	–	AA	3/217 (1.4 %)	3/217 (1.4 %)
Leblond et al. [5]	46	34	NA	4/45 (8.9 %)	3/45 (6.7 %)
Leblond et al. [5]	46	34	AA	2/45 (4.5 %)	2/45 (4.5 %)
Tamburini et al. [6]	49	43	NA + AA	2/49 (4 %)	5/49 (10 %)
Ghobrial et al. [7]	237	–	AA	16/237 (6.7 %)	
Rakkhit et al. [8]	111	55	NA	1/111 (0.9 %)	11/111 (10 %)
Leleu et al. [9]	193	60	NA	3/173 (1.6 %)	9/193 (4.7 %)
Leleu et al. [9]	136	60	AA	0/136 (0 %)	1/136 (0.4 %)
Treon et al. [11]	43	40.3	R + NA	3/43 (6.9 %)	3/43 (6.9 %)
Lazlo et al. [12]	29	43	R + NA	0/29 (0 %)	0/29 (0 %)
Tedeschi et al. [13]	43	38.8	R + NA	3/44 (6.9 %)	0/44 (0 %)
Leblond et al. [10]	169	36	NA	0/207 (0 %)	11/207 (5.3 %)
Leblond et al. [10]	170	36	AA	3/207 (1.4 %)	14/207 (6.8 %)
Tedeschi et al. [14]	40	51	R + NA	2/40 (5 %)	1/40 (2.5 %)

25.2 Cytotoxic Agents Associated with Second Hematologic and Non-Hematologic Cancers

The drugs associated with higher risk of these complications are the AA and the NA.

AA damage DNA either by methylation or by DNA interstrand crosslink formation. AA tend to be associated with t-MDS/AML, with a gradual dysplastic clinical onset and a latency period of 5–10 years, with unbalanced chromosomal aberrations mainly involving chromosomes 5 and 7. The prognosis of t-MDS/AML is poor, with a median survival of 5 months.

NA share structural similarities with nucleotides and can be incorporated into DNA or RNA, causing inhibition of cell proliferation and DNA repair. The DNA damage of marrow progenitor cells induced by fludarabine may also account for the impairment of peripheral blood progenitor cells collection. The combination of fludarabine with cyclophosphamide or other AA may increase the risk of t-MDS/AML due to their synergistic effect in producing DNA damage [9]. The median time to t-MDS/AML is 5 years. High occurrence of abnormalities of chromosomes 5 and 7 has also been described in t-MDS/AML secondary to NA therapy. The incidence of t-MDS/AML and of DLBCL transformation after AA and/or NA is shown in Table 25.1.

25.2.1 Alkylating Agents

Alkylating agents such as chlorambucil and cyclophosphamide have been extensively used as first or subsequent lines of therapy in WM. Chlorambucil has been used for almost five decades. Kyle et al. [3] compared, in a prospective randomized study, continuous and intermittent chlorambucil therapy in 46 untreated WM patients. t-MDS/AML was developed in 4/46 patients. All four patients had received intermittent chlorambucil at a total dosage of 2000–3300 mg.

Garcia-Sanz et al. [4] analyzed late adverse events in 217 patients treated with chlorambucil ± prednisone (80 %) or other AA-based regimens. The most frequent cause of death was the development of second malignancies, which was responsible for 16 deaths. MDS occurred in three patients, transformation to DLBCL in three patients, and solid tumors in 10 patients.

The largest experience on long-term AA toxicity has been reported by Ghobrial et al. [7]. The analysis included 237 patients with newly diagnosed symptomatic WM. After a median follow-up of 11 years, death due to t-MDS/AML occurred in 16 patients (6.7 %) indicating that this may be a serious and fatal complication of AA therapy.

25.2.2 Nucleoside Analogs

The role of NA, in particular of fludarabine alone or in combination, in leukemogenesis and transformation to DLBCL has been extensively investigated.

The risk of t-MDS/AML or transformation to DLBCL specifically attributable to fludarabine-based therapy is difficult to quantify because indolent lymphoproliferative diseases have a long natural history often involving exposure to multiple lines of cytotoxic treatment and to radiation. Prior cytotoxic therapy appears highly likely to contribute to the risk of t-MDS/AML following fludarabine treatment.

Concerning the use of fludarabine in monotherapy, Leblond et al. [2] reported the effects of fludarabine in 71 patients previously treated with alkylating agents. After a median follow-up of 34 months, one patient developed t-MDS/AML (1.4 %) and 5 (7 %) a transformation to DLBCL. The same group, in 2001, compared in a randomized study the efficacy and toxicity of fludarabine alone versus CAP regimen (cyclophosphamide, doxorubicin, prednisone) in 92 WM patients in first relapse after AA. After a median follow-up of 34 months, the incidence of t-MDS/AML was 8.9 % in the NA arm and 4.5 % in the CAP arm. The incidence of transformation to high-grade lymphoma was 6.7 % in the NA arm and 4.5 % in the CAP arm (Table 25.1).

A large retrospective study with the intent to determine the incidence of transformation to DLBCL and of t-MDS/AML was conducted at Dana-Farber Cancer Institute by Leleu et al. [9]. Among 439 patients with WM, 193 and 136 were previously treated with and without a NA, respectively, and 110 had similar long-term follow-up without treatment. The median follow-up for all patients was

5 years. Overall, 12 patients (6.2 %) either developed DLBCL transformation (nine patients; 4.7 %) or t-MDS/AML (three patients; 1.6 %) among NA-treated patients, compared with one patient (0.4 %) who developed transformation in the non-NA treated group ($P < 0.001$). No such events occurred among untreated patients. The predominant transformed histology was DLBCL for eight patients in the NA group and one patient in the non-NA. Among NA-treated patients, the 15-year probabilities of developing either transformation to DLBCL or t-MDS/AML were 21 % and 8 %, respectively. DLBCL transformation and t-MDS/AML occurred at a median of 5 years from onset of NA therapy. All NA-treated patients who developed t-MDS/AML died at a median of 5 months, while the median survival of NA-treated patients who developed transformation to DLBCL did not differ from other NA-treated patients as a result of salvage treatment either with R-CHOP or with R-CHOP followed by autologous stem cell transplantation.

25.2.3 Combination Therapy of NA with AA

Not only prior exposure to AA but also the combination of NA with AA may potentiate DNA damage and contribute to oncogenesis.

The effects of the combination of fludarabine and cyclophosphamide (FC) have been reported by Tamburini et al. [6] in 49 WM patients (35 previously treated and 14 untreated). After a median follow-up of 41 months, 5/49 patients (10 %) developed transformation to DLBCL and two (4 %) had t-MDS/AML.

25.2.4 Fludarabine Versus Chlorambucil in Terms of Late Effects

In 2012, Leblond et al. [10] reported the results of the first randomized trial in WM comparing chlorambucil and fludarabine in patients with WM/lymphoplasmacytic lymphoma. A total of 339 patients with WM were enrolled, 169 in the fludarabine arm and 170 in the chlorambucil arm. With a median follow-up of 36 months, no statistically significant differences were found between the two arms in terms of transformation to DLBCL (14 in the chlorambucil arm and 11 in the fludarabine arm) or development of t-MDS/AML (3 t-MDS/AML, 1 hypereosinophilic syndrome, 1 Burkitt, and 1 follicular lymphoma in the chlorambucil arm; 1 mastocytosis in the fludarabine arm). Surprisingly, a significantly higher number of solid tumors were evidenced in the chlorambucil arm (20 versus 5 cases in the fludarabine arm), with a 6-year cumulative incidence rate of second malignancies of 20.6 % versus 3.7 % in the fludarabine arm ($P = 0.001$).

25.2.5 Combination of AA and/or NA with Rituximab

In WM, the efficacy and safety of the combination of rituximab with fludarabine have been investigated by Treon et al. [11] in 43 pretreated and untreated patients

with progressive disease. With a median follow-up of 40.3 months, three patients (6.9 %) developed DLBCL, three patients t-MDS/AML, and two patients a SST. The median time from therapy initiation to transformation to DLBCL was 21 months and to t-MDS/AML was 39.4 months.

Rituximab has the ability to sensitize cells to both fludarabine and cyclophosphamide, enhancing their cytotoxic activity. The efficacy of the fludarabine, cyclophosphamide, and rituximab combination (FCR) has been explored by Leblond et al. in 55 WM patients (15 treatment naive and 40 relapsed/refractory). After a median follow-up of 28 months, t-MDS/AML was observed in two heavily pretreated patients (3.6 %), while three patients developed a solid tumor.

Tedeschi et al. [13] treated 43 WM patients with FCR in a multicenter prospective study (65 % at first line therapy and 35 % pretreated with one line of chemotherapy). After a median follow-up of 38.8 months, three patients heavily pretreated with AA developed t-MDS/AML with complex karyotype after 5, 24, and 60 months, respectively. None of the patients showed transformation to DLBCL. The same group treated 40 relapsed/refractory patients with the FCR combination [14]. After a median follow-up of 51 months, two patients (5 %) developed t-MDS/AML and 1 (2.5 %) transformed to DLBCL.

Although data on fludarabine are more frequently reported, few trials on long-term toxicity after Cladribine (2-CdA) therapy have been published. In a retrospective study, Rakkhit et al [8] reported the incidence of transformation and of second malignancies in 111 WM patients treated for progressive disease. Patients received 2-CdA alone or in combination with other agents including prednisone, cyclophosphamide, and rituximab. After a median follow-up of 55 months, 11 patients (10 %) showed transformation to aggressive lymphoma, with a median time to transformation of 37 months; 12 patients (11 %) developed a SST; and one patient (0.9 %) AML.

The combination of rituximab with subcutaneous 2CdA (0.1 mg/kg for five consecutive days, administered monthly for four cycles) has been reported by Lazlo et al. [12] in a prospective study including 29 patients newly diagnosed or previously treated without NAs. With a median follow-up of 43 months, no patient developed transformation to DLBCL or t-MDS/AML.

25.3 Combination Therapy with Bendamustine and Rituximab

Treon et al. [17] treated 30 relapsed/refractory WM patients with bendamustine and rituximab (BR). Late toxicities attributed to BR included prolonged myelosuppression (four patients including three patients previously treated with NA). One responding patient transformed to DLBCL following four cycles of BR and subsequently succumbed for lymphoma. Prior therapies for this patient included CVP-R, Velcade/Rituximab, and CHOP-R. One patient developed myelodysplasia without cytogenetic abnormalities after five cycles of BR; prior therapies included fludarabine/rituximab and CP-R.

Rummel et al. [18] compared, in a phase III randomized study, six cycles of BR with six cycles of R-CHOP as first-line therapy in 514 patients with follicular lymphoma (FL), mantle cell lymphoma, and indolent non-FL (41 of them had WM). Long-term effects were similar in both groups: second malignancies occurred in 20 patients of the BR arm compared to 23 in the R-CHOP arm, with one t-MDS/AML in each group. Bendamustine, more recently introduced in the therapeutic armamentarium of WM, requires further studies to determine the impact of this drug on long-term adverse events.

25.3.1 Conclusive Remarks on Cytotoxic Agents Associated with Second Malignancies

Genetic instability caused by chemotherapy increases the possibility of transformation to DLBCL or t-MDS/AML. Even if multiple data are available about fludarabine-related increased risk of transformation to DLBCL or t-MDS/AML, its oncogenic potential seems enhanced by prior or concomitant alkylating agent-based therapy.

The higher incidence of second solid tumors seems to be peculiar to WM, compared with other non-Hodgkin lymphomas, and is enhanced by the use of alkylating agents.

Lastly, despite the reported clinical efficacy of NA-containing combinations, these appear not to be indicated in patients who are potential candidates to consolidation with high-dose therapy with autologous stem cell transplantation. In fact, NA therapy may impair for a long time the possibility of mobilizing peripheral blood progenitor cells.

Regarding the potential long-term adverse events following bendamustine treatment in patients with WM, prolonged follow-up is needed to precisely assess the risk of transformation to DLBCL or development of t-MDS/AML and secondary solid tumors.

25.4 Rituximab-Related Late Adverse Events

Late adverse events related to rituximab are uncommon in WM. They are mainly represented by late-onset neutropenia (LON) and by viral infectious complications.

25.4.1 Late-Onset Neutropenia

Most investigators define LON as grade III–IV neutropenia occurring at least 4 weeks after the last dose of rituximab, in the absence of an alternative explanation. LON has been extensively described in patients with other non-Hodgkin lymphoma by Wolach et al. [19], while there are few studies specifically addressing

LON in patients with WM. The incidence of LON is generally reported to be in the range of 3–27 %. Several retrospective studies have been performed to evaluate the characteristics of patients developing LON after rituximab treatment [19–24]. LON is more frequently observed in patients receiving rituximab after ASCT. Other factors significantly associated with LON are the number of rituximab doses (cutoff of 4), previous exposure to chemotherapy, advanced disease stage, and more intensive cytotoxic regimens.

The mechanism of LON has not yet been defined. It has been attributed to an antibody-mediated process, to a lymphocyte subpopulation imbalance (based on the demonstration of T-LGL in peripheral blood and bone marrow), and to a polymorphism in immunoglobulin G Fc receptor FCgammaRIIIa 158 V/F. Although, in some cases, bone marrow examination may result normal, the most common finding is a myeloid maturation defect.

The clinical implications of LON may be relevant as it may affect subsequent treatment strategies. Infection complications, however, are infrequent. Data from major retrospective studies show an infection rate of 16.9 %. The risk of infections is strictly dependent on depth and duration of neutropenia. Other risk factors are the concurrent and prolonged hypogammaglobulinemia and the cellular immune dysfunction induced by rituximab therapy. The role of therapy with granulocyte-colony stimulating factor (G-CSF) is an unanswered issue as in most series data are not conclusive. In some cases, however, a prompt response to G-CSF has been reported.

LON has not been reported after rituximab maintenance or after alkylating-based immunochemotherapy. A high incidence of long-lasting neutropenia episodes has been reported after purine analog-based immunochemotherapy (fludarabine plus rituximab or fludarabine and cyclophosphamide plus rituximab). In these cases, myelosuppression should be considered as the consequence of drug toxicity, increased by the concomitant administration of rituximab and alkylating agents, rather than an imbalance of targeted B-cells.

In the series of 43 patients receiving fludarabine and rituximab reported by Treon et al. [11], 27 patients developed neutropenia; in three cases, grade 4 neutropenia lasted more than 6 months and a prompt recovery was observed after G-CSF. Similarly, in the fludarabine, cyclophosphamide, and rituximab study of Tedeschi et al. [13], 19 patients (44 %) experienced long-lasting episodes of neutropenia after the last course but this did not translate in an increased rate of infections. In four of these patients, bone marrow biopsy showed a hypocellular marrow (personal data) demonstrating that neutropenia should be considered as a consequence of NA-induced myelosuppression increased by the concomitant administration of rituximab and alkylating agents.

Up to now, only one retrospective study by Treon et al. [25] evaluated the efficacy of rituximab as maintenance therapy in WM after rituximab combination treatment. No significant difference in absolute neutrophil count was observed during the follow-up period in the 85 patients receiving maintenance therapy compared to the control group.

25.4.2 Viral Infectious Complications: Progressive Multifocal Leukoencephalopathy

Rituximab therapy has been associated with viral infectious complications such as hepatitis B reactivation, cytomegalovirus, herpes simplex virus, varicella zoster virus, West Nile virus, and JC polyoma virus (JCV). PML is a rare demyelinating disease of the central nervous system that results from reactivation of latent JCV. Up to 92 % of the adult population is JCV seropositive. The risk of PML in patients with hematologic malignancies is estimated to be 0.07 %, with the highest incidence (0.5 %) being reported in chronic lymphocytic leukemia.

In the series of 52 patients with lymphoproliferative disorders reported by Carson et al. [26], three patients had WM. Median time from first rituximab dose to PML diagnosis was 16 months (1–90) and was 5.5 months (0.3–66) from last rituximab to PML. The case fatality was 90, 100 % among PML cases diagnosed within 3 months from the last rituximab dose versus 84 % among PML presenting after 3 months from rituximab discontinuation.

PML should be always suspected in patients treated with rituximab developing confusion/disorientation, motor weakness/hemiparesis, poor motor coordination, speech changes, and vision changes. A definitive diagnosis of PML is primarily confirmed by magnetic resonance imaging and by the documentation of cerebrospinal fluid JCV DNA by polymerase chain reaction or by brain biopsy. Treatment is most often ineffective.

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Treatment Recommendations in Waldenström Macroglobulinemia

26

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26.1 Introduction

The Second International Workshop on WM (IWWM-2) provided criteria for the clinicopathological diagnosis of WM, as well as indications for initiation of therapy in WM patients. IWWM consensus panels have since provided treatment recommendations that were updated in 2008 (IWWM-4), in 2012 (IWWM-7) and in 2014 (IWWM-8) [1, 3, 4, 11, 13]. The combination of an anti-CD20 monoclonal antibody with chemotherapy remains a recommended therapy in most patients with WM. The choice of the optimal chemoimmunotherapy should depend on the patient's comorbidities, consideration of short- and long-term toxicities, necessity for rapid disease control, and candidacy for future autologous stem cell transplant. Despite considerable heterogeneity in biology and clinical course, many mature B-cell malignancies are highly sensitive to kinase inhibitors that disrupt BCR signaling. Thus, inhibition of BCR signaling is emerging as a new treatment paradigm for WM patients.

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V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5_26

26.2 Treatment Indications

Several patients with a diagnosis of WM may not need immediate therapy. Criteria for the initiation of therapy include IgM-related complications and/or cytopenias, constitutional symptoms, and bulky extramedullary disease [5]. For patients who do not fulfill the criteria and in whom only laboratory evidence may indicate a possible development of progressive disease (such as a minor decrease in hemoglobin level with asymptomatic anemia or mild increases in IgM) or mild increase of lymphadenopathy or splenomegaly without discomfort for the patient, close observation is recommended. Plasmapheresis should always and immediately be used for patients with symptomatic hyperviscosity. Plasmapheresis alone is not an effective treatment of the disease and must be followed by a rapidly acting cytoreductive treatment.

26.3 Treatment Strategies

Rituximab alone can be considered for WM patients with immunological disorders secondary to WM, such as anti-MAG neuropathy, or in frail patients less likely to tolerate chemoimmunotherapy. Rituximab should be avoided in patients with high IgM levels due to concerns of an IgM flare and prompting symptomatic hyperviscosity.

Because rituximab is an active and a non-myelosuppressive agent, its combination with various chemotherapeutic agents has been extensively explored in WM. The combination of anti-CD20 monoclonal antibody and chemotherapy is considered standard of care in patients in first line treatment. The choice of chemotherapy depends on the comorbidities, how fast control of the disease is required, and the phenotype of the disease.

Chemoimmunotherapy combinations with rituximab (R) and cyclophosphamide/dexamethasone (DRC) or bendamustine (Benda-R) or bortezomib/dexamethasone (BDR) provide durable responses and are still indicated in most patients [6–8].

DRC is an active and safe treatment choice in first line for WM with a manageable toxicity, even in frail patients and can be considered for the first line treatment setting. Fludarabine-based combinations can be considered in fit patients with relapsed/refractory WM who have failed other, less toxic treatments. In young patients who are ASCT eligible, stem cells should be collected before fludarabine administration. Benda-R is effective in treatment-naïve and as well as previously treated WM patients. Treatment is well tolerated even in elderly patients with limited episodes of myelosuppression and infections when compared to purine analogue-based regimens. In elderly patients, as well as those with renal impairment, dose of bendamustine needs to be lowered.

Primary therapy with bortezomib is recommended for patients with high levels of IgM, with symptomatic hyperviscosity, cryoglobulinemia or cold agglutinemia, amyloidosis, and renal impairment, or in young patients in whom avoidance of

alkylator or nucleoside analogue therapy is desired. Bortezomib should ideally be given once weekly and possibly by subcutaneous route but, in case of urgent reduction of the IgM level, bortezomib can be started at a dose of twice a week for one or two cycles and then be changed to once weekly dosing in order to reduce risk of neurotoxicity.

Carfilzomib-based therapy represents an emerging neuropathy-sparing option for proteasome inhibitor-based therapy for WM. Cardiac toxicity has been reported in 3–4 % of MM patients and could be an issue especially in elderly WM patients with preexisting cardiac conditions. Other open issues include the optimal dose of carfilzomib and the optimal schedule of administration.

Rituximab maintenance therapy is an option in WM patients; given the benefits observed in other indolent lymphomas, as well as the results from a retrospective outcome study, though confirmatory prospective data, as well as studies to address the optimal dose, schedule, and duration of maintenance rituximab, are needed.

Immunomodulatory agents (lenalidomide, pomalidomide) and m-TOR inhibitors (everolimus) should be considered in the context of a clinical trial until further data are available.

The approval of the BTK inhibitor ibrutinib in the USA and in Europe represents a novel and effective treatment option for both treatment-naïve and relapsing patients. Ibrutinib is a BTK inhibitor effective in high-risk CLL and in mantle cell lymphoma patients. There is a strong rationale to use this drug in WM patients given that BTK is activated by mutated MYD88 and enhances the survival of WM cells by activation of NFKB. The efficacy of this drug may be also depend on CXCR4 mutations, with a lower response rate and delay in response in mutated patients asking the question whether treatment should be stratified according to the CXCR4 mutational status. Ibrutinib is an option in symptomatic WM patients [2, 9, 12]. However, the optimal use of therapy with ibrutinib (i.e., first line, or relapse) and use as a single agent or in combination (i.e., with rituximab or other anti-CD20 antibodies, proteasome inhibitors such as bortezomib or carfilzomib, or other agents) continue to be a subject of investigation. Stem cell transplantation should be discussed in selected WM cases, though should take into account the numerous available alternative treatment options, and should preferably be considered in the context of clinical trials.

In conclusion, many options are available in first line: chemoimmunotherapy with anti-CD20 monoclonal antibodies or the combination of anti-CD20 MoAbs with proteasome inhibitors. The aim of the first line treatments is to reach a high response rate with a prolonged PFS. Clinical trials are needed with chemotherapy-free combinations with new compounds alone or in combination with anti-CD20 antibodies. Ibrutinib is approved in the front-line setting, and front-line trials with ibrutinib and other BCR inhibitors are needed for assessing the efficacy and tolerability of these agents in treatment-naïve patients.

In relapsed/refractory setting, investigation of BCR inhibitors along with existing and novel compounds in patients must be performed. Combination with proteasome inhibitors would be of interest for overcoming resistance and interfering with the two key pathways that are affected by MYD88. Obinutuzumab

with its shown efficacy in CLL and also now in follicular lymphoma will be of interest as a combination partner in WM. The use of CXCR4 antagonists such as plerixafor or ulucuplomab may also offer an opportunity to extend the activity of therapeutics impacted by the CXCR4 mutation in WM patients. Active enrollment in clinical trials is endorsed by the panel for most patients with WM.

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Index

V. Leblond et al. (eds.), *Waldenström's Macroglobulinemia*,
DOI 10.1007/978-3-319-22584-5