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On Waldenström's macroglobulinemia and IgM related disorders

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Chapter 1

General introduction and
outline of the thesis

WALDENSTRÖM'S MACROGLOBULINEMIA

Waldenström's Macroglobulinemia (WM) was first described by the Swedish physician Jan Gösta Waldenström (1906-1996) in 1944.¹ He reported two patients with anemia, thrombocytopenia, a bleeding tendency and an increased erythrocyte sedimentation rate. He found an increase of lymphoid cells in the bone marrow and observed that there were no bone lesions, thus setting it apart from multiple myeloma. In their serum he found a high viscosity and the presence of a large globulin with a high molecular weight. Essentially, he described what we would still consider to be two very typical WM patients.

In the latest World Health Organization (WHO) classification (the 2017 revised 4th edition)², WM is defined as "lymphoplasmacytic lymphoma (LPL) with bone marrow involvement and an Immunoglobulin M (IgM) monoclonal gammopathy of any concentration". LPL is defined as "a neoplasm of small B lymphocytes, plasmacytoid lymphocytes and plasma cells, usually involving bone marrow and sometimes lymph nodes and spleen, which does not fulfill the criteria for any of the other small B-cell lymphoid neoplasms that can also have plasmacytic differentiation".

The majority of LPL are WM, but LPL also includes rare cases lacking the IgM paraprotein (<5%). These mostly have an IgA or IgG paraproteinemia, non-secreting LPL is very rare. This thesis focuses on WM and IgM related disorders.

Diagnostic classification

Bone marrow examination is essential for diagnosing WM since IgM paraproteinemia can also be associated with a number of other conditions, including IgM MGUS, IgM multiple myeloma (very rare) and other B-cell lymphoproliferative diseases such as marginal zone lymphoma and B-chronic lymphatic leukemia (CLL). Based on the bone marrow examination and the presence or absence of disease related symptoms, 4 disease categories can be defined: IgM MGUS, IgM related disorders, smoldering WM and WM (**Table 1, Figure 1**).³

Two definitions of IgM MGUS currently coexist. In the WHO classification 2017², IgM MGUS has become a separate entity (setting it apart from the much more common non-IgM MGUS) using the International Myeloma Working Group criteria.⁴ In the WHO classification, IgM MGUS is now defined as "a serum IgM paraprotein concentration < 30 g/L, bone marrow lymphoplasmacytic infiltration of <10% and no evidence of anemia, constitutional symptoms, hyperviscosity, lymphadenopathy, hepatosplenomegaly or other end-organ damage that can be attributed to the underlying lymphoproliferative disorder". The Consensus Panel of the Second International Workshop on WM (IWWM-2) on the other hand defines IgM MGUS as "IgM paraproteinemia of *any level* without equivocal

bone marrow infiltration and the absence of related symptoms”.³ The latter definition was used in the 2012 Dutch national guideline for WM, as summarized in **Table 1**.⁵

Table 1: Diagnostic Classification as used in the Dutch WM guidelines, based on IWWM-2^{3,5}

	IgM MGUS	Asymptomatic WM	Symptomatic WM	IgM related disorder
IgM M-protein (serum)	Yes	Yes	Yes	Yes
Lymphoplasmacytic infiltration (Bone marrow)	No	Yes	Yes	No
WM related signs or symptoms	No	No	Yes	Yes
Approach	Follow up (infrequently)	Wait and see	Start treatment	Depending on specific manifestation, start treatment if applicable
Risk of progression to WM	1.5% per year	50-60% after 5 years	Not applicable	Unknown

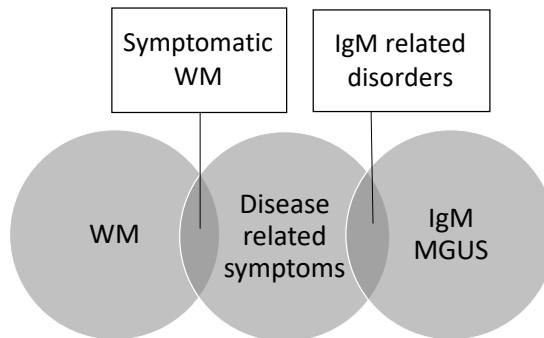


Figure 1: Disease Categories.

“IgM related disorders” is the term that was coined by the IWWM-2 for those patients that have IgM paraproteinemia and disease related symptoms, but without overt lymphoma infiltration in the bone marrow (“symptomatic MGUS”).³ Typical examples are IgM-related neuropathy, cryoglobulinemia and cold agglutinin disease. Smoldering WM is defined as WM without disease related symptoms. Symptomatic WM is defined as WM with disease related clinical features.

IgM MGUS has a small risk of progression to WM, non-Hodgkin Lymphoma (NHL), B-CLL or AL amyloidosis of 2% per year in the first 10 years after diagnosis and 1% per year thereafter.⁶ Smoldering WM has a much higher risk of progression: 68% at 10 years: 12% per year in the first five years and then 2% per year thereafter.⁷

Epidemiology

WM is a rare subtype of B-cell lymphoma that accounts for about 2% of all NHL cases. The incidence is approximately 6 per million persons per year in the US.⁸ In the Netherlands, the crude incidence is approximately 14 per million persons per year, and 6,5 based on the 'world standardized rate' (WSR). Around 250 new LPL/WM patients are diagnosed yearly and the 10-year prevalence in 2017 was 1700 in the Netherlands.⁹ The average age at diagnosis is 65 - 70 years and the incidence is higher in males than in females and higher in Caucasians than in non-Caucasians. IgM MGUS accounts for approximately 15% of all MGUS cases and is much more common than WM with a (strongly age dependent) prevalence of around 0.54 per 100 individuals who are 50 years of age or older.¹⁰ The incidence of IgM related disorders is unknown.

Prognosis

The prognosis of WM has improved over the last decades, and more recently reported median overall survival (OS) durations are around 8-9 years.^{11,12} The survival rates are strongly age dependent as illustrated by a recent Greek study where median OS was 5.3 years for patients > 75 years and 9.7 years for patients ≤ 75 years old.¹² Because WM occurs mainly in the elderly and can have an indolent disease course, part of the patients may die of a cause other than the lymphoma. Whether the aggressiveness of the disease is age dependent is unknown. The International Prognostic Scoring System for WM is based on five adverse prognostic factors: age > 65 years, Hemoglobin < 11,5 g/dl, platelet count < 100x10⁹/L, B2M > 3mg/L and serum IgM paraprotein > 7 g/dL. Low-risk patients with no or 1 risk factor and age < 65 years, intermediate-risk patients with 2 factors or only advanced age, and high-risk patients with more than 2 factors have 5-year survival rates of 87%, 68% and 36% respectively as published in 2009.¹³

Pathophysiology

The normal counterpart of the WM tumor cell is still a subject of debate. The WHO classification 2017 states that the postulated normal counterpart is "a post-follicular B cell that differentiates into plasmacells". The cell of origin is generally thought to be a memory B cell that has undergone somatic hypermutation but before isotype switching, based on the V_H regions analysis and the immunophenotype.¹⁴ However, a recent transcriptome study showed a lack of similarity between WM cells and normal memory B cells, while there was strong expression of genes associated with activated B cells that are transforming into plasmacells.¹⁵ Possibly, the normal counterpart of the WM tumor cell might be a rare memory B cell subset. Currently, the cell of origin question still remains unanswered.

All cases of WM are thought to be preceded by the premalignant state of IgM MGUS. Indeed, the immunophenotypic and molecular profiles of the clonal B-cells are highly

overlapping between IgM MGUS and WM, although the number of genetic abnormalities increases from IgM MGUS to smoldering WM to symptomatic WM.¹⁴

Interestingly, WM patients tend to have clustering of WM and other B-cell malignancies in their families. The genetic background of this familial predisposition has not been fully clarified¹⁶.

Recent insights regarding the molecular abnormalities in the WM tumor cell are discussed below.

Clinical presentation

One of the striking aspects of WM compared to other lymphoid malignancies is its highly diverse clinical presentation. While anemia is the most frequent symptom and indication for treatment, a wide range of manifestations affecting nearly all organ systems can be associated with WM. Several pathophysiological routes can lead to clinical symptoms, examples are given below and are illustrated in **Figure 2**.

- Direct organ infiltration by the WM tumor cells (lymphadenopathy, Bing Neel Syndrome, cytopenias)
- Immunological effects caused by the LPL cell (anemia, B-symptoms)
- Direct damage by the IgM paraprotein or the accompanying circulating light chains (cast nephropathy, hyperviscosity syndrome)
- Deposition diseases related to the IgM or light chains (monoclonal immunoglobulin deposition disease, light chain deposition disease, AL amyloidosis)
- Auto-antibody activity of the IgM paraprotein (cold agglutinin disease, IgM related neuropathy)
- Clinical syndromes related to specific physicochemical properties of the IgM paraprotein (cryoglobulinemia, hyperviscosity syndrome)
- Transformation to aggressive large cell lymphoma

Anemia

Anemia is the most frequent symptom and indication for starting treatment in WM patients. The pathogenesis is multifactorial, and in most cases not caused by tumor infiltration only. The level of anemia is not strongly related to the tumor load in the bone marrow¹⁷. In some cases, WM patients suffer from auto-immune hemolytic anemia mostly due to cold agglutinins (IgM-antibodies, typically reactive against the I antigen on the red blood cell surface, causing complement-mediated hemolysis).

In addition, anemia in WM has been related to elevated levels of hepcidin, a key regulator of iron metabolism¹⁸. High hepcidin levels lead to suppression of iron uptake and release into blood plasma, resulting in reduced plasma iron levels and decreased hemoglobin production. Hepcidin levels increase during infection and inflammation, probably to prevent growth of pathogens by blocking their access to extracellular iron.

Waldenstrom Macroglobulinemia: Disease Manifestations

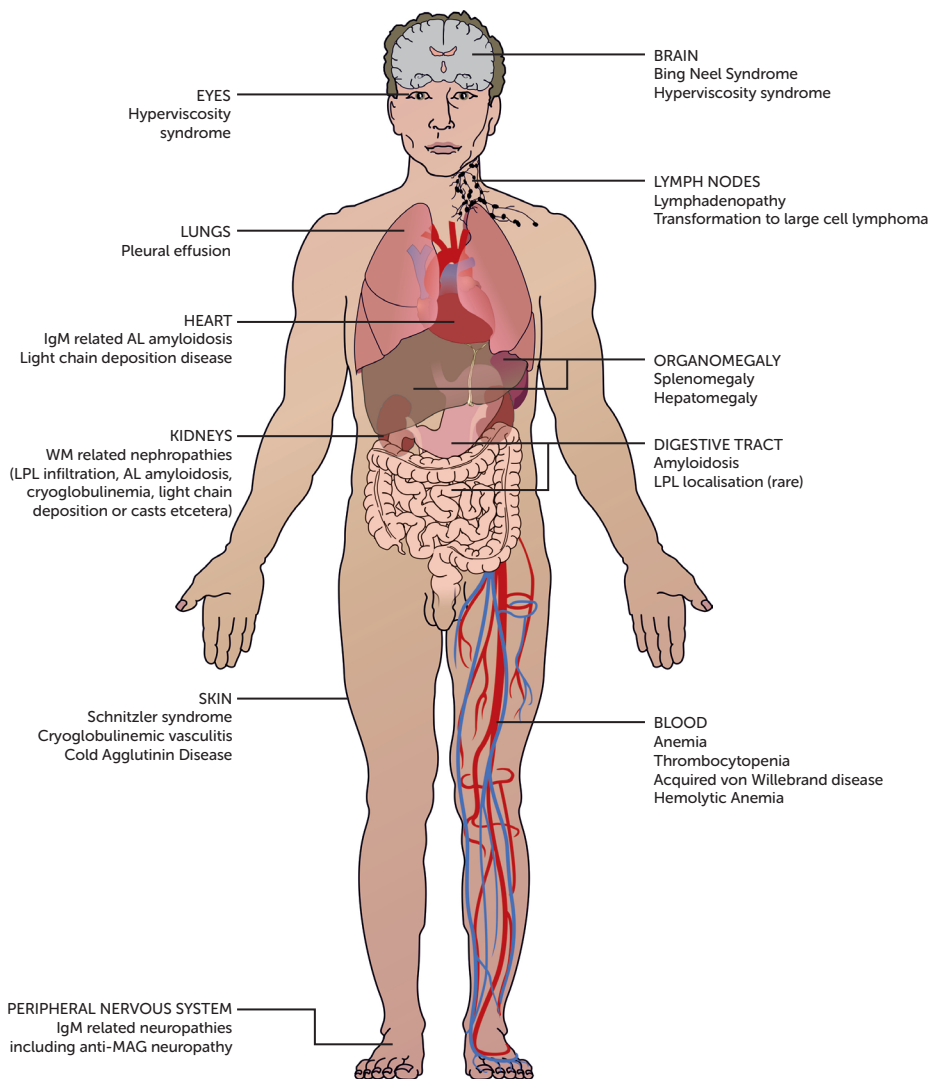


Figure 2: WM disease manifestations

Elevated hepcidin in inflammatory states including malignant lymphoma is thought to be responsible for anemia of chronic disease or chronic inflammation. This is also the case in WM where LPL cells have been found to produce hepcidin and hepcidin levels were shown to be inversely correlated to hemoglobin levels.¹⁹

Response assessment

Interestingly, although serum IgM is the hallmark of WM and the basis for the response criteria as defined by the IWWM-6 panel (**Table 2**)²⁰, the IgM level does not have a reliable relationship with the tumor load in the bone marrow¹⁷. Repeated bone marrow examinations can be informative when there is doubt about the response status or to confirm complete remission. Computerized tomography (CT) scanning is recommended prior to initiation of therapy and repeat scanning for those patients with measurable disease. Positron emission tomography (PET) can probably reveal a higher rate of extramedullary disease compared to CT but is not routinely recommended since prognostic correlations or clinical consequences have not been established²¹.

Table 2: Response criteria based on IWWM-6²⁰

Response category	Definition
Complete response (CR)	Absence of serum monoclonal IgM protein by immunofixation Normal serum IgM level Complete resolution of extramedullary disease, i.e., lymphadenopathy and splenomegaly if present at baseline Morphologically normal bone marrow aspirate and trephine biopsy
Very good partial response (VGPR)	Monoclonal IgM protein is detectable ≥ 90% reduction in serum IgM level from baseline Complete resolution of extramedullary disease, i.e. lymphadenopathy/ splenomegaly if present at 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 Reduction in extramedullary disease, i.e., lymphadenopathy/splenomegaly if present at 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 progression in extramedullary disease, i.e., lymphadenopathy / splenomegaly No new signs or symptoms of active disease
Progressive disease (PD)	≥25% increase in serum IgM level from lowest nadir (requires confirmation) and/ or progression in clinical features attributable the disease

IgM flare is the temporary surge in IgM levels seen in a portion of WM patients in reaction to rituximab therapy (or other anti-CD20 monoclonal antibodies). IgM flares are seen in up to 50% of patients treated with rituximab and are not associated with treatment failure. The IgM flare can last for several months and should not be confused with progression of disease.

Treatment

Although there is an increasing number of effective therapeutic options, WM remains an incurable disease. As in other indolent lymphomas, a “wait and see” policy is advocated in asymptomatic patients (smoldering WM) and IgM MGUS. Treatment of WM is indicated in symptomatic disease based on consensus criteria (**Table 3**).²² Treatment of IgM related disease can be necessary but treatment indications are less well defined and the choice of therapy can be challenging due to the absence of overt lymphoma and the lack of clinical trial data.

Table 3: Treatment indications as confirmed in IWWM-7²²

Clinical indications for initiation of therapy

- Recurrent fever, night sweats, weight loss, fatigue
 - Hyperviscosity
 - Lymphadenopathy which is either symptomatic or bulky (≥5 cm in maximum diameter)
 - Symptomatic hepatomegaly and/or splenomegaly
 - Symptomatic organomegaly and/or organ or tissue infiltration
 - Peripheral neuropathy due to WM
-

Laboratory indications for initiation of therapy

- Symptomatic cryoglobulinemia
 - Cold agglutinin anemia
 - Immune hemolytic anemia and/or thrombocytopenia
 - Nephropathy related to WM
 - Amyloidosis related to WM
 - Hemoglobin ≤10 g/dL
 - Platelet count <100 × 10⁹/L
-

A growing number of therapeutic agents is available for the treatment of WM. Until recently, none of them were specifically approved for WM and they were mostly “borrowed” from experience in other indolent B-cell lymphomas and multiple myeloma. However, WM patients have been found to experience a range of side effects that were not seen with the same drug in other patient populations. For example, the monoclonal CD20 antibody rituximab can cause a paradoxical temporary increase in the IgM level, a WM specific phenomenon known as “IgM flare”, which can lead to clinical symptoms due

to hyperviscosity syndrome. In addition, the immunomodulatory agent lenalidomide has been shown to lead to severe anemia in WM, to an extent that is not seen in MM, and higher than expected rates of neuropathy were seen after treatment with bortezomib, vincristine and thalidomide. More recently, unexpected severe DAT-negative hemolytic anemia and reticulocytopenia were found after combined treatment with a novel PD-1 antibody and a BTK inhibitor.^{23,24} These examples illustrate that the success of agents in other lymphomas or in multiple myeloma cannot always be extrapolated and therefore specific studies are needed in the WM patient population.

There is no consensus on the exact optimal treatment of WM in first line or in the relapse setting. The choice of treatment should be based on individual patient and disease characteristics. First line treatment should probably consist of combined immunochemotherapy in most patients, composed of combinations of rituximab and cyclophosphamide/dexamethasone, bendamustine, or bortezomib/dexamethasone. These combinations can provide durable responses with a long treatment-free interval²⁵. Rituximab monotherapy is an option for very frail patients and perhaps for immunological phenomena related to the IgM itself such as anti-MAG neuropathy. The oral BTK inhibitor ibrutinib is also an option for frail patients that are unfit for chemotherapy.

Plasmapheresis should be started immediately in patients with hyperviscosity syndrome. Patients with high IgM levels or hyperviscosity syndrome should not start with rituximab until the IgM is controlled with chemotherapy because of the risk of IgM flare.

In the relapse setting the same recommendations can be made, depending on the duration of the response to previous treatment as well as patient and disease characteristic. In addition, ibrutinib can be considered. Stem cell transplantation is not routinely advised but can be considered in highly selected cases (the typical example being rapidly relapsing disease in a transplant eligible patient). Recently, new proteasome inhibitors (carfilzomib and ixazomib) were found to be active in WM with a more favorable toxicity profile regarding neuropathy compared to bortezomib. This is relevant since WM-related neuropathy is already present in around 20% patients, and incidence of bortezomib related neuropathy is higher in WM than in multiple myeloma patients. Clinical trials using (combinations of) novel targeted agents such as novel BTK and proteasome inhibitors, new monoclonal antibodies against B-cell or plasmacell-specific antigens, CXCR4 antagonists, P13K inhibition, BCL2 inhibition, and checkpoint inhibitors are currently recruiting worldwide.

For many specific disease variants (for example Bing Neel syndrome, IgM amyloidosis, WM-related nephropathies) and most of the IgM related disorders prospective clinical trial data are lacking and the optimal approach to treatment is unknown. Treatment choices are based mostly on retrospective data and expert opinion combined with extrapolating data from WM or multiple myeloma.^{26,27}

Recent breakthroughs

MYD88

The past years have seen several major breakthroughs in the WM field. In 2012, the presence of a somatic point mutation in MYD88 was demonstrated in the bone marrow of > 90% of WM patients.²⁸ This mutation consists of a (T→C) at position 38182641 in chromosome 3p22.2, resulting in an amino-acid substitution from leucine to proline in the MYD88 TIR domain: the MYD88 L265P mutation (MYD88^{L265P}). MYD88^{L265P} is absent in healthy donors and multiple myeloma patients. It is present in several other B-cell malignancies albeit at a much lower rate. Patients with MYD88^{L265P} present with higher bone marrow infiltration and higher IgM levels compared to patients with wild type MYD88 (MYD88^{WT}). In IgM MGUS the mutation is found in up to 50-80% of patients^{29,30}. The presence of the mutation in IgM MGUS is associated with a higher risk of progression to WM or other lymphoproliferative disorders. WM patients with MYD88^{WT} have a higher rate of transformation to DLBCL and a shorter survival compared to patients with MYD88^{L265P} in one study.³¹ A higher rate of transformation, but no difference in survival was found in another study³², although in this study there was an unusually low rate of MYD88^{L265P} (only 79% of WM cases).

When treated with ibrutinib, patients with MYD88^{L265P} or other mutations in MYD88 reached deeper responses compared to patients with MYD88^{WT}.^{33,34}

MYD88 functions as an adaptor protein in Toll-like receptor (TLR) and interleukin-1 receptor (IL-1R) families signaling. After stimulation of the TLR or IL-1R, MYD88 homodimerizes to the activated receptor forming a complex including IRAK1/4 (the myddosome), which ultimately leads to nuclear factor κB (NF-κB) activation.³⁵ MYD88^{L265P} triggers spontaneous myddosome assembly without external stimuli, resulting in constitutive NFκB activation (**Figure 3**).¹⁶ In WM cells, inhibition of MYD88/IRAK signaling has been shown to decrease NF-κB signaling leading to enhanced apoptosis while MYD88^{L265P} overexpression leads to enhanced cell survival.¹⁶ Interestingly, inhibition of MYD88 in MYD88^{L265P} WM cells also causes a decrease in Bruton Tyrosine Kinase (BTK) activity, whereas overexpression of MYD88^{L265P} enhances BTK phosphorylation. IRAK and BTK seem to both activate NF-κB independently and the combined use of IRAK and BTK inhibitors lead to synergistic WM tumor cell killing in vitro.³⁶

CXCR4

The presence of somatic activating mutations in the c-terminal domain of the CXCR4 gene in approximately 30% of WM patients was reported in 2014.³⁷ Later, using a combination of Sanger sequencing and highly sensitive allele-specific polymerase chain reaction (AS-PCR) assays, CXCR4 mutations were found in up to 43% of patients and almost exclusively in patients with MYD88^{L265P}.³⁸ CXCR4 mutations seem unique to WM as they have not been found in other diseases, except in a small fraction of patients with marginal zone

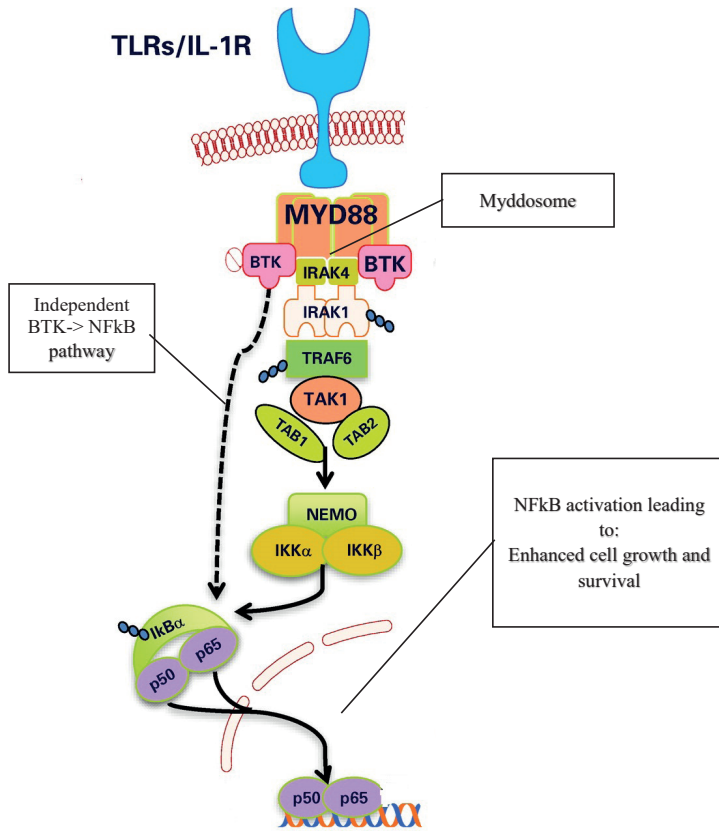


Figure 3: MYD88 directed signaling in WM, this figure is modified from Z.R. Hunter *et al.*, JCO 2017¹⁶ with permission of the author.

lymphoma. Interestingly, germline mutations similar to the somatic mutations found in WM are found in patients with WHIM syndrome (warts, hypogammaglobulinemia, infection and myelokathexis). WHIM syndrome is a rare immunodeficiency characterized by retention and increased apoptosis of neutrophils in the bone marrow, leading to neutropenia. In WM, many (>30) different nonsense and frameshift CXCR4 mutations have been described, all in the C-terminal domain. The CXCR4 mutations in WM are mostly subclonal and often polyclonal, suggesting that they arise after the initial MYD88^{L265P} mutation³⁷. In a recent transcriptome study, genes associated with the CXCR4 pathway (such as CXCL12 and VCAM1) were overexpressed regardless of the presence of a CXCR4 mutation, suggesting a role for CXCR4 signaling in many if not all WM patients.¹⁵

Although there are some differences in clinical presentation (less lymphadenopathy, higher bone marrow infiltration and IgM levels), CXCR4 mutations do not seem to impact

prognosis.¹⁶ When treated with ibrutinib, MYD88^{L265P} positive patients without CXCR4 mutations reach quicker and deeper responses.³³

Ibrutinib

The pivotal clinical study introducing ibrutinib in the field of WM was published in 2012 and led to its registration as the first drug specifically approved for WM by the Federal Drug Agency and European Medicines Agency.³³ Ibrutinib yielded an overall response rate of 91% of 63 heavily pretreated WM patients. This high response rate was recently confirmed in an open label phase 3 study of ibrutinib monotherapy³⁹, a prospective randomized trial of rituximab versus rituximab-ibrutinib⁴⁰, and a prospective first study of ibrutinib monotherapy in treatment-naïve patients⁴¹. However, in real life a substantial (25-30%) portion of patients discontinue the drug within the first two years, mostly due to disease progression or toxicities.^{33,42}

Ibrutinib decreases the constitutive activation of the NFκB pathway that is directed by the myddosome, most likely by affecting the interaction between BTK and MYD88. Also, ibrutinib inhibits HCK, a protein that upon activation by MYD88 leads to increased survival of WM cells via the AKT and ERK pathways.⁴³ Interestingly, treatment with ibrutinib does not generally lead to complete remissions, and a rapid rebound of the IgM level is seen when the drug is discontinued. However, it does lead to rapid decreases of the IgM levels and improvement of anemia in most patients. Bone marrow responses are somewhat slower and not always correlated with the IgM response. In fact, improvements in IgM and hemoglobin were seen even in patients with hardly any changes in bone marrow infiltration. A mechanism other than tumor cell killing seems to be responsible for the clinical benefit in this setting. This phenomenon has not been fully clarified but might be related to effects of BTK inhibition on non-tumoral cells including the microenvironment.

OUTLINE OF THE THESIS

This thesis comprises studies conducted to gain further insight into the various disease manifestations of WM and IgM related disorders, ultimately aiming to advance optimal diagnosis, treatment and patient outcomes, engaging in (inter)national collaboration when needed and incorporating the recent breakthroughs in the field that are described above when possible.

Direct invasion of WM tumor cells in the central nervous system (CNS, the cerebral spinal fluid, the meninges and/or the brain parenchyma) is called Bing Neel Syndrome (BNS).²⁷ It is a rare WM manifestation (only 13 cases of BNS were identified in a retrospective cohort of 1523 WM patients).⁴⁴ There is no consensus on the best treatment strategy. Fludarabine is an oral nucleoside analogue that is known to be highly active

in WM and capable of crossing the blood-brain barrier. It was found to be active in CNS localization of B-CLL.⁴⁵ We therefore considered it a potential effective treatment option for BNS. **Chapter 2** describes a study on fludarabine-based treatment in four consecutive patients with five episodes of BNS.

Although WM-related nephropathy is an established indication for initiation of treatment, its epidemiology, prognostic impact and clinical features are largely unknown.²² Only few cases have been published, mostly as single case reports or small retrospective case series without a comparison group of WM patients without renal complications. We aimed to facilitate better recognition, diagnosis and treatment regarding this WM disease manifestation. **Chapter 3** addresses the incidence, characteristics and prognostic implications of WM related nephropathy in a cohort of 1391 WM patients.

Monoclonal IgM-related neuropathy is a heterogeneous group of disorders causing significant morbidity. These neuropathies are rare and their clinical presentation and prognosis can be variable. Optimal diagnosis, treatment and response assessment are challenging. These challenges also hamper the initiation of clinical trials in this field. **Chapter 4** contains a consensus approach by the 8th International Workshop on WM (IWWM-8) panel regarding diagnosis and management of peripheral neuropathies associated with IgM paraproteinemia, including WM.

Anti-Myelin-Associated Glycoprotein (MAG) peripheral neuropathy (PN) is the most frequent type of paraprotein-related neuropathy. It is typically a demyelinating neuropathy with ataxia, tremor and sensory disturbances leading to significant disability in up to 50% of patients. Although anti-MAG PN is sometimes seen in WM patients, it is often an IgM related disorder, meaning overt bone marrow infiltration by LPL is lacking. Progressive disease-related disability is considered an indication to start treatment. However, although there is (low quality) evidence that rituximab monotherapy is effective at least in some patients, there is no consensus on the optimal treatment approach and a high clinical need for effective therapies.⁴⁶ Clinicians have been hesitant to use chemotherapy in these patients without overt malignant disease, and agents with neurotoxic side effects are not suited since these patients already have a peripheral neuropathy. However, several new targeted “chemo-free” agents have and will become available for WM, often with little or no (neuro)toxicity. To explore whether these agents might be of potential value in anti-MAG PN, it is particularly relevant to establish a pathophysiological link between the two conditions. **Chapter 5** addresses the presence of MYD88^{L265P} in the bone marrow of patients with anti-MAG PN, in order to further explore the pathophysiological link of anti-MAG PN with WM.

Cytokines, including chemokines, are protein mediators of immunological processes that are important in lymphoma pathogenesis including WM. Cytokines can be produced either by LPL cells or by cells that are part of the BM microenvironment including the host immune system. They may modulate the growth and migration of LPL and microen-

vironmental cells.⁴⁷ The MYD88 and CXCR4 mutations seen in WM are associated with proinflammatory processes including chemotaxis, however the cytokine profiles of the different molecular WM subsets are unknown. Therefore, we were interested in cytokine and chemokine levels in WM patients with and without MYD88 and CXCR4 mutations.

While ibrutinib leads to rapid suppression of IgM levels and resolution of anemia, these effects are highly discrepant with the much slower effect on the bone marrow infiltration. This suggests that part of the clinical responses is possibly mediated by an immunological process rather than by direct tumor cell killing. Therefore, we studied cytokine and chemokine levels in previously treated WM patients participating in a clinical trial of ibrutinib in order to further explore the immunological characteristics of the response to this drug.³³

Anemia in WM patients may be related to elevated levels of hepcidin, and hepcidin is mediated via inflammatory cytokines.¹⁹ We hypothesized that ibrutinib might lead to a rise of hemoglobin via suppression of inflammatory cytokines and hepcidin.

Chapter 6 studies the serum cytokine and chemokine profile in WM patients compared to healthy donors and in relation to MYD88 and CXCR4 mutation status, as well as cytokine, chemokine and hepcidin changes in response to ibrutinib therapy.

In **Chapter 7** this thesis concludes with a summary and general discussion of the results. **Chapter 8** contains a summary in Dutch.

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