

Waldenström's macroglobulinemia: a clinical perspective in the era of novel therapeutics

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Waldenström's macroglobulinemia (WM) is a rare, low-grade malignancy with no established standard of care. Rituximab regimens are most commonly used, supported by their efficacy in hematologic malignancies, including WM. A growing number of investigational regimens for WM have been evaluated in phase II clinical trials, including single-agent and combination strategies that include newer-generation monoclonal antibodies (ofatumumab and alemtuzumab), proteasome inhibitors (bortezomib and carfilzomib), immunomodulatory agents (thalidomide and lenalidomide), phosphoinositide 3-kinase/protein kinase B (Akt)/mammalian target of rapamycin pathway inhibitors (everolimus and perifosene), a Bruton's tyrosine kinase inhibitor (ibrutinib), and a histone deacetylase inhibitor (panobinostat). Other novel agents are in early-stage development for WM. International treatment guidelines for WM suggest suitable regimens in the newly diagnosed and relapsed/refractory settings, in accordance with patient age, disease presentation, and efficacy and safety profiles of particular drugs. These factors must be considered when choosing appropriate therapy for individual patients with WM, to maximize response and prolong survival, while minimizing the risk of adverse events. This review article provides a clinical perspective of the modern management of patients with WM, in the context of available trial data for novel regimens and recently updated treatment guidelines.

Key words: efficacy, novel agents, safety, treatment guidelines, Waldenström's macroglobulinemia

Introduction

Waldenström's macroglobulinemia (WM) is a rare, incurable, low-grade lymphoplasmacytic lymphoma characterized by the presence of immunoglobulin-M (IgM)-secreting clonal cells in the bone marrow [1, 2]. Approximately 25% of patients with WM have family members with a history of lymphoproliferative disorders, and first-degree relatives have a 20-fold higher risk of developing WM than those in the general population [3, 4]. A somatic activating mutation in the *MYD88* (*MYD88^{L265P}*) gene is found in >90% of patients; mutations in the *CXCR4* gene are also common. Both *MYD88* and *CXCR4* mutations may be associated with clinical outcomes and response to targeted therapies [5].

The clinical manifestations of WM include cytopenias, hyper-viscosity, hemolytic anemia, peripheral neuropathy (PN), hepatomegaly, splenomegaly, and organomegaly, with accompanying symptoms of recurrent fevers, night sweats, fatigue, and weight loss [6, 7]. Symptomatic patients with WM should receive treatment; recommendations on the treatment of WM have recently

been updated [7, 8]. However, until very recently, there were no approved regimens or consensus standard of care. Here, we review the potential of novel agents to broaden the WM treatment landscape.

Clinical data supporting WM therapies

Most therapies utilized in clinical practice for patients with WM are already approved for other hematologic malignancies. Several other drugs are in early-stage development and have relatively limited published data. The mechanisms of action of different agents support the rationale for their investigation in clinical trials of patients with WM, based on what is known about the pathogenesis of the disease. Efficacy and safety data from phase II studies of patients with WM are summarized in Tables 1 and 2.

Monoclonal antibodies

Rituximab is a monoclonal antibody directed against the CD20 antigen present on the surface of B cells. CD20 is a member of the tumor necrosis factor signaling pathway, and the binding of rituximab to CD20 results in B-cell lysis [26]. Approved for non-Hodgkin's lymphoma and chronic lymphocytic leukemia (CLL), rituximab has become a dominating component of the regimens

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Table 1. Efficacy data from phase II studies of investigational therapeutic regimens for WM

Agent/regimen	WM study population (n)	ORR (%)	CR (%)	Median TTP (months)	Median PFS (months)
Rituximab [9]	Previously untreated (n = 17)	35	0	13	–
Rituximab [10]	Previously treated with rituximab-based induction (n = 86)	98	16.3	–	56.3
Ofatumumab [11]	Previously untreated (n = 9) Relapsed (n = 28)	59	0	–	–
Alemtuzumab [12]	Symptomatic (n = 28) ^a	75	4	14.5	–
Bortezomib/dexamethasone/rituximab [13]	Previously untreated (n = 23)	96	13 (+9 nCR)	>30	–
Bortezomib/dexamethasone/rituximab [14]	Previously untreated (n = 59)	85	3	–	42.0
Weekly bortezomib/rituximab [15]	Previously untreated (n = 26)	88	4 (+ 4 nCR)	Not reached	–
Weekly bortezomib/rituximab [16]	Relapsed/refractory (n = 37)	81	5	16.4	15.6
Carfilzomib/rituximab/ dexamethasone [17]	Previously untreated (n = 31)	87 ^b	3	–	–
Thalidomide/rituximab [18]	Previously untreated (n = 20) Relapsed/refractory (n = 5)	72	4	34.8	–
Lenalidomide/rituximab [19]	Previously untreated (n = 12) Relapsed (n = 4)	50	0	17.1	–
Everolimus [20]	Previously untreated (n = 33)	72	0	–	–
Everolimus [21]	Relapsed/refractory (n = 60)	73	0	25.0	21.0
Enzastaurin [22]	Relapsed/refractory (n = 42)	38	0	10.9	–
Perifosene [23]	Relapsed/refractory (n = 37)	35	0	12.6	12.6
Ibrutinib [24]	Relapsed/refractory (n = 63)	90.5	0	9.6	Not reached
Panobinostat [25]	Relapsed/refractory (n = 36)	47	0	–	6.6

^aTotal of 27 patients with WM (trial enrolled patients with lymphoplasmacytic lymphomas).

^bResponse not affected by *MXD88* or *CXCR4* mutation status.

CR, complete response; nCR, near complete response (defined as fulfilling all CR criteria in the presence of a positive immunofixation study) [13]; ORR, overall response rate (minimal response or higher); PFS, progression-free survival; PR, partial response; TTP, time to progression; VGPR, very good partial response.

used in clinical practice for WM. When used as a monotherapy, rituximab is associated with a response rate of ~30%, increasing up to 50% with extended schedule [7, 9]. Rituximab is associated with a transient increase in the levels of serum IgM ('IgM flare') which may lead to clinical complications. It has also been administered as a single agent in the maintenance setting for newly diagnosed WM, prolonging progression-free survival (PFS) and overall survival (OS) in a retrospective analysis [10].

Rituximab shows improved depth of response in WM when combined with chemotherapy [6, 27]. Updated results from a 6-year follow-up of a phase II study showed that patients receiving dexamethasone, rituximab, and cyclophosphamide (DRC) had a median PFS of 35 months and 5-year OS of 62%; 82% of patients achieved at least minimal response (MR) [28]. Rituximab in combination with bendamustine, in a subgroup analysis of a phase III trial evaluating first-line treatment in patients with low grade lymphomas, including some patients with WM [from Study group Indolent Lymphomas (StiL)], was shown to prolong the median PFS versus cyclophosphamide, doxorubicin, vincristine, prednisone, and rituximab (CHOP-R) (median PFS not yet reached with rituximab/bendamustine versus 40 months for CHOP-R); with a similar response rate (96% rituximab/bendamustine versus 94% CHOP-R). Patients treated with bendamustine and rituximab experienced less grade 3 and 4 cytopenias, infectious complications, and alopecia [29]. In another study of 30 relapsed and refractory WM patients treated with rituximab and bendamustine, the overall response rate (ORR) was 83.3% (this included six patients

intolerant to rituximab who received bendamustine alone). The median PFS for all patients was 13.2 months [30]. Rituximab can also be safely and effectively combined with proteasome inhibitors (PIs), nucleoside analogs, or immunomodulatory agents for the treatment of WM [6, 7].

The activity observed with rituximab in hematologic malignancies including WM has supported the investigation of other monoclonal antibodies. In a phase II trial of the anti-CD20 monoclonal antibody ofatumumab in patients with WM (n = 37, including 9 newly diagnosed and 28 with relapsed or refractory WM), ORR was 67% (6/9) for previously untreated and for patients with relapsed WM ORR was 57% (16/28) [11]. Notably, the ORR in rituximab-exposed patients was 52% (13/25). The most common adverse events were infusion-related; others included low-grade infections. In addition, IgM flare was reported with ofatumumab [31].

Obinutuzumab, a more recently developed anti-CD20 monoclonal antibody, is currently being investigated in non-Hodgkin's lymphoma (including WM) in a phase III study (NCT01287741).

Because CD52 is highly expressed in lymphoplasmacytic cells, alemtuzumab, an anti-CD52 antibody indicated for the treatment of CLL [32], has also been investigated in patients with WM [33]. In a phase II study of 28 patients with lymphoplasmacytic lymphomas (27 with WM), the ORR was 75% [12]. However, alemtuzumab was associated with high incidence of grade ≥3 cytopenias, cytomegalovirus reactivation, and late immunologic disorders (such as immune thrombocytopenia) and the clinical activity of

Table 2. Safety summary based on phase II studies of investigational therapeutic regimens for WM

Common AEs (grade 3/4, unless stated)	Hematologic				Nonhematologic			Other reported AEs	
	Anemia	Leukopenia	Neutropenia	Thrombocytopenia	Peripheral neuropathy	IgM flare ^a	Pneumonitis/pneumonia		Rash
Rituximab [9, 10]	–	–	Up to 2%	–	–	–	–	–	One patient developed cytokine release syndrome, 3% grade 3 upper respiratory tract infections
Ofatumumab [11]	3%	–	3%	–	–	5%	–	–	Grade 1/2 infusion reactions and infections
Alemtuzumab [12]	11%	57%	54%	25%	–	–	–	11%	Grade 3 fatigue, infection, allergic reaction, hyponatremia, transaminitis, abdominal pain, late onset immune thrombocytopenia
Bortezomib twice per week/ dexamethasone/rituximab [13]	4%	–	30%	9%	30%	–	4%	0	Grade 3 hypotension, myopathy
Bortezomib induction followed by weekly bortezomib/ dexamethasone/rituximab [14]	0%	–	15%	5%	7%	11%	5%	–	Grade \geq 3 diarrhea, fatigue, constipation, fever, hypotension, respiratory infection, increased creatinine
Weekly bortezomib/rituximab [15,16]	8%–11%	4%–14%	12%–16%	8%–14%	0%–5%	22%	– ^b	–	Grade 3/4 nausea, vomiting, lymphopenia, dizziness, syncope, grade 3/4 lymphopenia
Carfilzomib/rituximab/ dexamethasone [17]	3%	–	10%	0%	0%	23%	–	0%	Grade 3 cardiomyopathy, grade 1/2 rash, peripheral neuropathy (grade 1), hyperamylasemia, hyperbilirubinemia, Grade 3 hyperglycemia, hyperlipasemia,
Thalidomide/rituximab [18]	–	–	–	–	28%	29% ^d	–	8% ^c	Grade \geq 2 somnolence, confusion, tremor, brachycardia, palpitations
Lenalidomide/rituximab [19]	6%	0%	31%	6%	0%	75%	–	–	Grade 2 fatigue, Grade 3 chest pain, arrhythmia, pleural effusion
Everolimus [20, 21]	27%; 39% ^c	22%	18% ^c	12% ^c –20%	–	–	15% ^c	27% ^c	Grade \geq 2 hyperglycemia, mucositis, fatigue, diarrhea, nausea, cellulitis
Ibrutinib [24]	2%	–	14%	13%	0%	–	2%	0%	Grade \geq 2 post-procedural bleeding, epistaxis, atrial fibrillation

^a \geq 25% rise in IgM.
^bGrade 5 pneumonia was reported in one patient [16].
^cGrade \geq 2.
^dIn 5 out of 17 patients without prophylactic plasmapheresis.
 AE, adverse event; igM, immunoglobulin M; NOS, not otherwise specified.

alemtuzumab in WM must be considered in the context of these toxicities.

proteasome inhibitors

PIs can affect cell proliferation and survival in B-cell malignancies such as WM [34]. Bortezomib is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome and is cytotoxic to cancer cells. Bortezomib was approved in the United States in 2003 for the treatment of multiple myeloma [35]. Preclinical studies showed that bortezomib had activity in WM [36], and in a phase II trial of patients with newly diagnosed WM ($n = 23$), bortezomib combined with dexamethasone and rituximab was associated with an ORR of 96% [13]. However, 39% and 30% of patients had grade 2 and 3 PN, respectively, which is a common adverse event associated with bortezomib. In another phase II study of bortezomib/dexamethasone/rituximab in 59 patients with previously untreated WM [14], the ORR was 85%. PN occurred in almost half of patients, but only 17% had grade 2, 7% had grade ≥ 3 , and only 8% discontinued treatment as a result of neurotoxicity. A third phase II trial in patients with newly diagnosed WM ($n = 26$) administered bortezomib weekly combined with rituximab [15]. The ORR was 88%. With this regimen, no grade ≥ 3 PN was observed (54% of patients had grade 1–2 PN).

In a phase II trial of patients with relapsed/refractory WM ($n = 37$), weekly bortezomib and rituximab treatment was associated with an ORR of 81%. The most common grade ≥ 3 adverse events were neutropenia, anemia, leukopenia, and thrombocytopenia. Forty-three percent of patients experienced grade 1–2 PN, and two patients (5%) experienced grade 3 neuropathy [16].

Carfilzomib is an irreversible tetrapeptide epoxyketone PI that binds to active sites of the 20S proteasome, but is structurally distinct from bortezomib. Carfilzomib was approved in 2012 by the FDA for the treatment of relapsed and refractory multiple myeloma [37]. Carfilzomib/rituximab/dexamethasone combination was assessed in a phase II trial of 31 patients with newly diagnosed WM [17]. The ORR was 87% and responses were not affected by *MYD88* and *CXCR4* mutation status. The most common grade ≥ 3 adverse events were dexamethasone-related hyperglycemia (77%) and carfilzomib-related hyperlipasemia (42%); hyperlipasemia was also accompanied by asymptomatic hyperamylasemia. There is no clear explanation for this increase; however, carfilzomib was temporarily held or the dose was modified in 11 patients. Low-grade rituximab-related infusion reactions were reported in 19% of patients. Unlike bortezomib, carfilzomib is associated with a low risk of neurotoxicity [7, 17]. Treatment-related grade 2 PN occurred in only one patient with disease-related grade 1 PN at baseline.

Oral PIs (ixazomib, oprozomib) are currently under investigation in patients with WM and may have promising activity and may be convenient alternatives to parenteral PIs [38].

However, no randomized prospective data exist for the use of PIs in WM or to evaluate the role of primary therapy with PIs. A randomized phase III trial of DRC with or without bortezomib for patients with WM is currently recruiting patients (NCT01788020).

immunomodulatory agents

Thalidomide and lenalidomide are immunomodulatory agents with proven efficacy in hematologic malignancies, including

multiple myeloma. In an early phase II trial, single-agent thalidomide was investigated in 20 patients with relapsed/refractory WM and the ORR was 25% [39]. Common adverse events included constipation, somnolence, fatigue, and mood changes. Thalidomide was subsequently assessed in combination with rituximab in a phase II trial of 25 patients with WM [18]: 20 had newly diagnosed WM, 4 had relapsed disease, and 1 was refractory to prior therapy; all patients were naive to both thalidomide and rituximab. The ORR was 72% for all patients, and among the 20 previously untreated patients, the ORR was 80%. However, grade 3 PN was observed in 28% of patients and toxicity led to discontinuation of therapy for most patients.

In a phase II study of lenalidomide/rituximab [19], the ORR was 50%, but 13 of 16 patients developed an acute (median 5%) decrease in hematocrit within the first 2 weeks and discontinued treatment, leading to the premature discontinuation of the study. A recent study indicated that lower doses of lenalidomide (15 mg/day on a 21-of-28-days schedule) could be effective with manageable toxicity [40]. Due to its association with hematologic toxicity, the updated The International Workshop on WM (IWWM)-7 guidelines advise that the use of lenalidomide in WM is restricted to clinical trials.

phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin pathway inhibitors

The phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway regulates cell survival and the migration of lymphocytes in WM [41]. Several agents that inhibit this pathway have shown to be effective in the clinical trial setting. In a phase II trial of 33 patients with newly diagnosed WM, everolimus, an oral mTOR inhibitor, was administered until disease progression or unacceptable toxicity [20]. The ORR was 72% and grade ≥ 2 adverse events at least partially everolimus-related included anemia, rash, oral ulcerations, and neutropenia. In a phase II study of 60 patients with relapsed/refractory WM, the ORR was 73% [21]. However, two-thirds of patients experienced grade ≥ 3 adverse events possibly related to everolimus, most commonly anemia, leukopenia, and thrombocytopenia. Pulmonary toxicity was reported in 5% of patients.

Although not currently endorsed by treatment guidelines for WM, perifosine, an inhibitor of Akt, has shown antitumor activity in preclinical and clinical studies of WM [42]. In a phase II study of 37 patients with relapsed and/or refractory WM, perifosine monotherapy was associated with an ORR of 35%. The most commonly observed adverse events were gastrointestinal disorders, fatigue, and cytopenias [23].

Bruton's tyrosine kinase inhibitors

Bruton's tyrosine kinase (BTK) is a component of the B-cell receptor signaling pathway and has been implicated in WM tumorigenesis. The *MYD88*^{L265P} mutation has been shown to act upstream of BTK and increase the activity of BTK signaling [43]. In a phase II trial of patients ($n = 63$) with relapsed or refractory WM, patients received the BTK inhibitor ibrutinib until disease progression or unacceptable toxicity [24]. Patients achieved an ORR of 90.5% and a major response rate [partial response (PR) or better] of 73%. Drug-related grade ≥ 2 adverse events included thrombocytopenia and neutropenia; atrial fibrillation occurred in

5% in patients with history of arrhythmia. Based on these data, the US Food and Drug Administration approved ibrutinib for the treatment of WM and the European Commission approved ibrutinib for the treatment of patients with WM who have received at least one prior therapy, or as first-line treatment for patients unsuitable for chemo-immunotherapy [44, 45]. Enrollment is ongoing in a randomized, placebo-controlled phase III study of ibrutinib/rituximab versus placebo/rituximab in patients with previously treated or untreated WM (NCT02165397).

histone deacetylase inhibitor

Increased expression of micro-RNAs has been shown to deregulate expression of histone deacetylases (HDACs) in WM, increasing HDAC activity [46]. In preclinical studies, the HDAC inhibitor panobinostat had cytotoxic activity against WM cells [46]. In a phase II trial of panobinostat, the ORR was 47% among 36 patients with relapsed and/or refractory WM. The most common grade 3–4 adverse events included thrombocytopenia, neutropenia, and anemia [25]. Panobinostat is not currently endorsed by treatment guidelines for WM.

novel WM agents in development

Ongoing early phase clinical trials for WM include those evaluating oprozomib, an orally administered epoxyketone PI (phase I/II, relapsed WM, NCT01416428); ixazomib, an orally administered boronate PI, in combination with dexamethasone and rituximab (phase II, previously untreated WM, NCT02400437 and phase I/II in previously treated patients); ACP 196, a novel BTK inhibitor (phase I, NCT02180724); IMO-8400, an oligonucleotide specifically designed to inhibit toll-like receptor

signaling pathways, for which MYD88 is a key linker protein (phase I/II, relapsed/refractory WM, NCT02092909), the phosphoinositide 3-kinase inhibitor idelalisib (phase I, relapsed/refractory WM, NCT02242045; phase II, relapsed/refractory WM; NCT02439138), and the glutaminase inhibitor CB-839, based on the knowledge that glutamine is required for cell growth and survival (phase I relapsed/refractory WM, NCT02071888) [47].

guideline recommendations for WM

Treatment recommendations produced during IWWM and the National Comprehensive Cancer Network (NCCN) (Table 3) have been recently updated in accordance with emerging clinical data [7, 8]. IWWM-7 recommendations are based on the assessment of patient characteristics (such as age or comorbidities) and the clinical presentation of the disease, including eligibility for autologous stem cell transplantation (ASCT), and the type and severity of symptoms [7]. Recommended primary therapies include rituximab-based combinations with chemotherapy (such as DRC) or bortezomib/rituximab for most patients or for patients with mild cytopenias, or rituximab with bendamustine, especially for patients with high tumor bulk. Patients treated with rituximab may have a transient surge in IgM levels (i.e. IgM flare). Plasmapheresis should be considered for patients with high levels of IgM or hyperviscosity. Immediate initiation of systemic therapy is crucial and primary therapy with bortezomib, in order to avoid an IgM flare, followed by bortezomib/rituximab or rituximab with bendamustine may be considered. If an IgM flare occurs in response to rituximab, plasmapheresis should be initiated immediately. For patients with neuropathy, rituximab (monotherapy or combined with chemotherapy) may be

Table 3. Recommendations based on IWWM-7- and NCCN-listed therapeutic options for WM [7, 8]

Newly diagnosed WM	Relapsed/refractory WM
<p><i>Patients with WM-related cytopenias or organomegaly:</i> rituximab-based combination: DRC (low toxicity) or bendamustine/rituximab (fast acting), alternatively bortezomib/rituximab ± dexamethasone (non-stem cell toxic, fast-acting, potential neurotoxicity)</p> <p><i>Patients with symptomatic hyperviscosity, cryoglobulinemia, or cold agglutininemia:</i> bortezomib induction followed by bortezomib/rituximab ± dexamethasone, or bendamustine/rituximab (fast acting, less data on IgM flare)</p> <p>Alternatively: fludarabine/rituximab ± cyclophosphamide (stem cell toxic, potential long-term toxicity)</p> <p><i>Patients with paraprotein-related neuropathy:</i> primary options: rituximab monotherapy or DRC (low toxicity, risk of IgM flare)</p> <p>Alternatively: fludarabine/rituximab (more toxic, probably for selected patients with rapidly deteriorating neuropathy) or bendamustine/rituximab (less data in patients with neuropathy)</p> <p><i>Young patients eligible for ASCT:</i> DRC or bortezomib/rituximab ± dexamethasone</p> <p>Alternatively: bendamustine/rituximab (probably does not compromise stem cell collection)</p> <p><i>Elderly patients with poor PS:</i> DRC (low toxicity) or oral fludarabine (if suitable for single-agent oral therapy)</p> <p>Alternatively: rituximab monotherapy or chlorambucil</p> <p><i>Elderly patients not eligible for systemic intravenous therapy:</i> oral fludarabine (alternatively chlorambucil)</p>	<p>As listed for primary therapy, taking into account:</p> <ul style="list-style-type: none"> • Can consider same regimen in patients who achieved responses that lasted at least 12 months • For patients with short-lasting remissions (<12 months) or with progressive disease/resistance to a first-line regimen, select agents of a different class (as monotherapy or in combination) • Avoid stem cell toxic agents in ASCT candidates • ASCT probably beneficial in patients with three or less lines of prior therapy and chemosensitive disease • Enrollment in clinical trials preferable

considered. Oral fludarabine (or chlorambucil, if fludarabine is unavailable) is recommended for elderly patients for which oral single-agent therapy may be considered as suitable. DRC [27] or bortezomib/rituximab combinations are the preferred choices for patients eligible for ASCT, although bendamustine/rituximab may also be considered, especially where there is high tumor bulk [7].

NCCN guidelines for WM list treatment regimens for newly diagnosed and relapsed disease according to the risk of stem cell toxicity [8]. Generally, rituximab combination regimens involving cyclophosphamide-based chemotherapy, PIs, and steroids dominate treatment choices. Bendamustine, cladribine, fludarabine, and thalidomide are alternative combination partners. While the NCCN guidelines do not indicate preferred treatments, bortezomib followed by bortezomib/rituximab or bortezomib/dexamethasone/rituximab in addition to plasmapheresis are suitable for patients who have symptomatic hyperviscosity; bortezomib ± rituximab and bortezomib/dexamethasone/rituximab are also suitable for patients who require rapid IgM reduction [8]. For relapsed disease, primary therapeutic options for WM still apply. However, for patients who had short remissions (<12 months duration), guidelines note they should be switched to a different class of agent to that administered as frontline therapy [7, 8]. Alemtuzumab, ofatumumab (in patients intolerant or resistant to rituximab), everolimus, and ibrutinib (NCCN guidelines only) are suggested for relapsed/refractory patients with few remaining alternatives, although alemtuzumab is less preferred to the other agents listed [7, 8].

practical considerations for treatment selection in WM

Treatment recommendations are guided by response rates and toxicity of available regimens. The IWWM-6 consensus criteria (based on serum IgM measurements and signs/symptoms of active disease) are utilized to categorize responses in WM [48]. The best ORR is defined by the percentage of patients who achieve a complete response (CR; absence of serum monoclonal IgM protein by immunofixation, normal serum IgM, resolution of extramedullary disease, and normal bone marrow aspirate), very good partial response (VGPR; detectable IgM protein but $\geq 90\%$ reduction in serum IgM, resolution of extramedullary disease, no new signs or symptoms of WM); PR (50–90% reduction in serum IgM, reduction in extramedullary disease, no new signs or symptoms), and MR (25–50% reduction in serum IgM, no new signs or symptoms) [48]. A retrospective study has shown that achieving deeper responses in WM could be associated with improved PFS [49]. Many investigational regimens have been shown to improve ORR for WM patients in clinical trials (Table 1), although there have been no prospective phase III data. However, some responses are more durable than others and establishing the true therapeutic effects of some agents may require analysis of response beyond that defined by IWWM-6 criteria. For example, patients who receive rituximab can experience an IgM flare following treatment, but this is not necessarily correlated with treatment failure, and may resolve. In patients treated with bortezomib, everolimus, or ibrutinib, a reduction in tumor burden in the bone marrow is not always accurately reflected in IgM levels, and overall differences in response

kinetics between agents make it prudent to confirm response using sequential bone marrow assessments [6, 7, 24].

Between-regimen differences in safety profiles (Table 2) must be balanced against treatment benefit and alternative options for WM [7], particularly as many patients are elderly and may have poor performance status. Comorbidities lower the threshold at which side-effects appear acceptable to the patient or clinician, but also directly influence drug choice.

Specific clinical conditions may require special actions. For example, elevated serum IgM arising from the growing number of IgM-secreting clonal cells in the bone marrow of patients with WM often results in hyperviscosity syndrome with visual disturbance, mucosal bleeding, and neurological symptoms. Therapeutic plasma exchange can alleviate symptoms either before or during primary treatment, and rituximab should be avoided unless pre-emptory plasmapheresis and/or bortezomib treatment is given to mitigate IgM flare [7, 8]. Monoclonal IgM can also cause disease-related PN, cryoglobulinemia, cold agglutininemia, and renal insufficiency. Bortezomib, thalidomide, and vincristine should be avoided where PN (a known side-effect of these agents) is present in WM, and choosing alternatives (e.g. carfilzomib or combinations such as DRC or bendamustine–rituximab) may avoid symptom exacerbation. However, bortezomib remains an option for patients with other IgM-related comorbidities, where rapidly reducing IgM levels will be beneficial [7, 8].

Clonal expansion of WM cells in the bone marrow may interfere with the development of other types of blood cells, resulting in cytopenias. For patients with cytopenias, regimens with a lower risk of hematologic toxicity, such as rituximab, bortezomib–rituximab, or DRC, are advisable [7]. Thalidomide is another suitable choice for patients with poor bone marrow reserve, given its low propensity for myelotoxicity [7]; however, non-hematologic toxicity is of concern.

Further patient management considerations are specific to particular drugs, and include the need to monitor IgA and IgG levels (which can be depleted) during carfilzomib therapy, and to provide herpes prophylaxis in patients receiving carfilzomib or bortezomib-based regimens [8].

In patients eligible for ASCT, it is important to avoid regimens that are stem cell toxic. High-dose therapy with ASCT is a salvage option for patients with WM that is still responsive to chemotherapy, although the benefit of this approach wanes with advancing lines of therapy [7].

conclusion

Treatment options for WM are rapidly expanding, with investigational agents and novel regimens being explored in clinical trials with varying levels of success. There is no standard of care yet, and only ibrutinib has been filed for regulatory approval in WM. However, treatment guidelines provide suggestions for appropriate therapies in the newly diagnosed and relapsed/refractory settings. Utilizing available information on patient characteristics, disease presentation, and the efficacy/safety profiles of alternative regimens help inform individual management decisions. In all cases, optimizing response is the key to maximizing patient survival, and avoiding treatment-related toxicities is important because of the symptoms and complications of WM.

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Beyond the exome: the role of non-coding somatic mutations in cancer

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The comprehensive identification of mutations contributing to the development of cancer is a priority of large cancer sequencing projects. To date, most studies have scrutinized mutations in coding regions of the genome, but several recent discoveries, including the identification of recurrent somatic mutations in the *TERT* promoter in multiple cancer types, support the idea that mutations in non-coding regions are also important in tumour development. Furthermore, analysis of whole-genome sequencing data from tumours has elucidated novel mutational patterns and processes etched into cancer genomes. Here, we present an overview of insights gleaned from the analysis of mutations from sequenced cancer genomes. We then review the mechanisms by which non-coding mutations can play a role in cancer. Finally, we discuss recent efforts aimed at identifying non-coding driver mutations, as well as the unique challenges that the analysis of non-coding mutations present in contrast to the identification of driver mutations in coding regions.

Key words: whole-genome sequencing, driver mutations, non-coding DNA, mutational signatures

introduction

The Catalogue of Somatic Mutations in Cancer (<http://cancer.sanger.ac.uk/cosmic>) contains over 21 000 genomes or exomes from cancer patients [1]. Many of these sequences come from large multi-institution consortia whose aim is to comprehensively characterize the molecular variations that occur in human cancers by identifying genes containing somatic mutations [2]. This task is complicated by the fact that most mutations within

the genome of a cancer cell are 'passenger' mutations which are not directly implicated in tumour development [3, 4]. Hence, it is not always clear whether a given mutation in a patient's tumour is a passenger mutation or a 'driver' mutation, which does confer a selective advantage to cancer cells and is therefore likely to be involved in pathogenesis. A major effort that has emerged in the field of cancer genomics is the systematic identification of cancer driver genes (genes that can contain driver mutations) [5, 6]. The identification of driver genes is critical both in understanding the molecular events that take place within cancer cells as well as for the prioritization of targets for therapeutic intervention. In addition, the sequencing of thousands of cancer exomes and genomes has allowed the inference

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