CLINICAL INVESTIGATION

Overview on clinical trials in Waldenstrom's macroglobulinemia

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Waldenstrom's macroglobulinemia is characterized by lymphoplasmocytic cells accumulation predominantly in bone marrow, secreting immunoglobulin M monoclonal protein. There is not a standard of care as disease is rare and there are no large randomized trials to address treatment. Asymptomatic patients should be observed. In symptomatic patients treatment should be individualized considering patient fitness and disease characteristics. In elderly unfit patients single agent treatment may be still considered an option. In younger and fit patients immunochemotherapy should be considered the standard of care as recent data showed an improvement in quality of responses, progression-free and overall survival. In this overview are reported the most significant clinical trials that may help in treatment decision.

Keywords: bendamustine • immunochemotherapy • immunomodulatory drugs • monoclonal antibodies • proteasome inhibitors • treatment • Waldenstrom's macroglobulinemia

The 2008 WHO classification of tumors of Hematopoietic and Lymphoid Tissues defines Waldenstrom's macroglobulinemia (WM) as: a type of lymphoplasmocytic lymphoma that involves bone marrow and is associated with a monoclonal immunoglobulin of the immunoglobulin M (IgM) class in the serum [1].

It is a very rare disease which comprises about 2% of all non-Hodgkin's lymphomas with about twofold higher in men compared with women. Data from the US Surveillance, Epidemiology and End Results Registry (SEER) estimate the US incidence of WM to be 3.8 cases per million people per year [2]. WM has a distinct pattern reflecting racial disparity; incidence is higher in whites compared with blacks or Asians. Like other lymphoproliferative disorders, WM is a disease of the older population with an incidence rate increasing with age, median age at diagnosis of 73 years.

At least 25% of patients are asymptomatic at diagnosis, some of them may remain asymptomatic and will never need specific treatment, 50% of asymptomatic patients who are observed will not require therapy within 3 years [3,4]. This highlights the importance of careful determination for the need of treatment.

There is not a standard of care in WM. As the disease is rare there are limited large Phase III randomized trials, and treatment decisions are made basically on results from Phase II trials and expert recommendations. Recently, SEER indicated an improvement in overall survival (OS) in patients of all ages [5], which may be related to a better understanding of disease biology translating in new therapeutic approaches, a better risk stratification of patients, and an improvement of supportive care. Even though, an outcome improvement has not been observed in all series [6,7].

Usually WM follows an indolent course. In different series, OS reported ranges from 60 to 120 months [4,6–8]. In some cases disease may be more aggressive, leading to treatment refractoriness and death within few months. Many studies analyzed clinical and

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disease characteristics associated with a worse prognosis. Several risk factors have been found to predict outcome, but all these studies should be critically analyzed. Results are not uniform depending on: study endpoint (OS or response to treatment), cut-off values considered and patients characteristics, as in some series both asymptomatic or symptomatic patients are considered. Furthermore, as we are dealing with an elderly population the poorest survival of patients over 65 years may be related to a higher number of non-WM related deaths [9]. The main adverse prognostic factors found to be significant are older age, low albumin, β2-microglobulin and cytopenias. In some studies adverse prognosis has been associated with high IgM concentrations, whereas in other studies adverse prognosis was related to low IgM concentration [6,10–11].

The update of the Southwest Oncology Group (SWOG) S9003 study indicated that lactate dehydrogenase (LDH) was an independent prognostic factor for survival after fludarabine therapy. The prognostic role of LDH for OS has also been confirmed by Kastritis *et al.* [12,13].

Other features found to be significant for prognosis are: constitutional symptoms, hepato-splenomegaly, hyperviscosity, cryoglobulinemia, male gender and urine monoclonal component [3,6,11]. Recently, an International Prognostic Scoring System for WM (IPSSWM) was designed only to predict survival after first-line therapy in symptomatic patients. This score has been initially validated for patients treated frontline with alkylating agents and purine analogs [4]. Subsequently, Dimopoulous *et al.* showed in a large series of 93 patients that IPSSWM is also applicable in patients who received primary treatment with rituximab-based regimens [14]. The scoring system stratifies patients into three different risks categories based on five adverse covariates: age >65 years, hemoglobin 11.5 g/dl, platelet count 100 × 10^9/l β2-microglobulin >3 mg/l and serum monoclonal protein concentration >70 g/l.

As the IPSSWM is designed only for symptomatic patients needing treatment, it should not be applied to determine whether a patient requires treatment.

Several studies in recent years have shown the importance of the immunoglobulin-free light chain assay in predicting response and survival, but its role remains to be defined [15,16].

For asymptomatic patients, follow-up is recommended and the watch and wait strategy is still considered a standard while treatment should be reserved only to symptomatic patients [17]. Symptoms may be either related to the IgM monoclonal component or to the expansion of the neoplastic clone resulting in tissue infiltration. Clinical manifestations induced by IgM monoclonal component are listed in Table 1.

Treatment should be individualized, and patient fitness and disease characteristics must be taken in account before initiating therapy. Most WM patients are aged >70 years with nonlymphoma-associated comorbidities. No studies have been addressed for this category of patients and they are clearly under-represented in clinical trials. In older and unfit patients intensive immunochemotherapy should be avoided, and single-agent treatment may still be a valid option.

The choice of treatment is dependent not only on patient age and comorbidities, but is also strictly dependent on the need of: rapid disease control, associated cytopenias, neuropathies, autoimmune phenomena, candidacy for autologous transplantation and long-term treatment toxicity. In patients presenting with hyperviscosity syndrome, plasmapheresis should be promptly instituted. Plasmapheresis exerts a transient effect and can promptly reverse most clinical manifestations [18] but does not affect the underlying disease process so that systemic treatment should be administered concomitantly.

Response criteria in WM

Before the second International Workshop in WM, held in 2002, response criteria had not been standardized. In most of the studies responses were generally based on monoclonal IgM reduction and/or improvement of nodal involvement. In a small number of studies marrow evaluation had been performed to assess response. Considering the heterogeneity of the disease determining categorical responses only on the basis of the change of M protein may not be appropriate. Furthermore not always IgM reduction correlates with symptoms improvement and often discrepancies between IgM and bone marrow responses are found. The consensus panel in 2002 proposed the first recommendations for specific tests to document response and guidelines for standardized response criteria. [19]. To better define responses and quality of responses recently an update of response assessment criteria has been published (Table 2) [20].

Single agents treatment

Alkylating agents in monotherapy and subsequently purine analogs have been extensively used. Single-agent chlorambucil may still be a valid option in non-fit patients [17]. An objective disease improvement, after chlorambucil treatment, has been observed in 50–80% of patients. Results of these studies should be carefully evaluated as most of the clinical trials are small Phase II studies with widely differing inclusion criteria; furthermore, they were performed before WM response criteria were standardized. No differences in outcome have been observed when comparing daily or

Table 1. Clinical manifestation related to IgM monoclonal component.

Condition	Clinical manifestations
Related to IgM structure	• Hyperviscosity syndrome: fatigue, headache, dizziness, blurred vision, easy oronasal bleeding, leg cramps, impaired mutation, ophtalmoscopic abnormalities (exudates, papilledema, segmented and dilated retinal veins) • Type I cryoglobulinemia coagulation abnormalities
Autoantibody reactivity	• Peripheral neuropathy: autoantibody activity to myelin-associated glycoprotein, ganglioside M1 and GM2 (GM1, GM2) • Hemolytic anemia cold agglutinin related Type II mixed cryoglobulinemia
IgM deposition in tissues	• Organ dysfunction: skin (papules, Schnitzler's syndrome) gastrointestinal (diarrhea, malabsorption, steatorrhea, bleeding) kidney (proteinuria) • AL amyloidosis (mostly involving heart, kidney, liver and nerves)

intermittent chlorambucil administration (0.1 vs 0.3 mg/kg/day for 7 days every 6 weeks) [21,22]. As time to response can take months, chlorambucil is not an appropriate option for patients needing a rapid disease control. Although the addition of corticosteroids does not improve response rates or survival, it may be useful in patients presenting with autoantibodies [23]. Both fludarabine and 2-chlorodeoxyadenosine (2-CdA) showed to be effective when used in monotherapy with objective responses ranging from 38 to 79% and 70 to 86%, respectively [24–31]. Responses after nucleoside analogs are more rapid than those observed after chlorambucil as they are observed within 3 months from the beginning of treatment.

The largest study on fludarabine initial therapy was performed by the South West Oncology Group and included 118 patients, results were subsequently updated in 2009 [10,12]. Responses (ORR 38%) occurred within 3–6 months of treatment initiation. A complete remission (CR) was observed in only 3%. Median event-free survival (EFS) and OS were 3.0 and 6.8 years, respectively. Recently results of a large randomized study on 339 treatment-naive WM patients in which chlorambucil was compared with oral fludarabine were reported [25]. Overall response rate (ORR) was similar in both arms but a significant advantage in terms of median response duration and progression-free survival was observed in patients receiving fludarabine with manageable toxicity. Furthermore, this is the first randomized trial in WM demonstrating an OS advantage (not reached in the fludarabine arm compared with 69.8 months in the chlorambucil arm).

Efficacy of 2-CdA is similar in terms of response and toxicity to that of fludarabine. A small study proved that there was no difference in outcome when 2-CdA was administered by continuous infusion or by 2-h iv. infusion for 5 days [29].

Although rituximab single-agent therapy is less effective in WM than in follicular lymphoma this treatment may be considered in patients with severe cytopenia in which chemotherapy should be avoided. Results obtained after rituximab alone are inferior to those observed after single-agent alkylating agents and purine analogs.

Studies using standard dose (4 weekly infusions at 375 mg/m^2) of rituximab demonstrated partial responses in approximately 27% of patients [32,33].

To ameliorate outcome an extended rituximab dose regimen was designed, wherein patients received rituximab at 375 mg/m^2 twice a week for 4 weeks, repeated at week 12 [34,35]. Response rates in these studies were higher (PR: 44–48%) than those reported with standard doses of rituximab.

The follow-up of the ECOG study of patients receiving only four doses of rituximab observed durable responses (median time to progression, 30 months; 5-year survival rate, 66%) [38]. Furthermore, patients achieving a minor response appeared to do as well as those achieving a major response, suggesting that there are categories of patients in which more aggressive or intensive therapy are not required. Responses after rituximab single agent are often slow so that it is considered generally a poor choice for patients in urgent need of therapy. Furthermore, an initial increase in the IgM level after rituximab infusion (IgM flare) has been reported as occurring in 54% of patients [39]. These levels may remain elevated for 3–12 weeks, and this does not indicate treatment failure. However, in patients with hyperviscosity related symptoms or with high IgM levels (>50 g/dl), plasmapheresis should be considered [40].

Table 2. Updated response criteria from the Sixth International Workshop on Waldenstrom's macroglobulinemia.

Categorization	Criteria
Complete response (CR)	Absence of serum monoclonal IgM protein by immunofixation. Normal serum IgM level. Complete resolution of extramedullary disease, for example, 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
	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
	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 but <25% increase in serum IgM level from baseline. No progression in extramedullary disease
	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

A predictive role for FcγRIIIA (CD16)-158 polymorphisms and responses to rituximab have been clearly demonstrated. Responses after the monoclonal antibody were significantly higher in cases presenting at least one valine in respect to phenylalanine (FcγRIIIA 158V/V (40.0%) V/F (35%) versus 158F/F (9.0%) [41]. Polymorphism determination is not possible in clinical practice and is not commonly performed before rituximab treatment. Disease biology is more and more important to understand treatment outcomes.

Rituximab monotherapy also showed to be effective and should be considered in patients with peripheral neuropathy related to the IgM antimyelin associated activity [42].

More recently, bortezomib has been evaluated in monotherapy in the context of two prospective clinical trials. In the WMCTG trial 27, 26 pretreated patients received up to eight cycles of bortezomib 1.3 mg/m^2 on days 1,4,8 and 11 repeated every 21 days. Importantly, this study demonstrated the rapid disease control exerted by bortezomib as median time to 25% reduction in serum IgM was of only 1.4 months. No CRs were observed in this series of patients, 48.1% was the major response rate [36].

These results (major response rate of 44%, prompt IgM decrease) were confirmed in the pretreated and untreated group of 27 patients enrolled in the Study of the National Cancer Institute of Canada [37]. As expected, in both studies a low rate of hematological toxicity was observed, but peripheral neuropathy developed in a high percentage (74%) of patients although in most cases resolved or improved after treatment discontinuation. Single agents clinical studies are summarized in Table 3.

Immunochemotherapy

There is consensus that in symptomatic patients who are medically fit, the combination of rituximab with chemotherapy is among the most effective treatments and should be considered as the first option [43].

Rituximab administered with either alkylating agents, nucleoside analogs or bortezomib have significantly improved OS of patients when compared with the same regimens without monoclonal antibody [44]. Furthermore, immunochemotherapy leads to better quality of responses and this may may translate in a prolonged progression-free survival as observed by Treon *et al.* after the administration of fludarabine and rituximab [45].

Table 3. Single-agent treatments in Waldenstrom's macroglobulinemia.

Reference	Regimen	N° points (treated/ untreated)	ORR %	Major response (CR) %	Overall survival	Response duration	FU	Ref.
Kyle *et al.*, 1999	Chlorambucil 0.1 mg/kg/day continuous vs 0.3 mg/kg/day for 7 d, q 6w	24 vs 22 (0/46)	79 vs 68	75 vs 64	65 mo	26 vs 46 mo	n.s.	[22]
Leblond *et al.*, 2013	Chl 8 mg/m^2/day for 10 d vs F 25 mg/m2/d for 5 d iv. or 40 mg/m2/day for 5 d po.	170 (0/170) vs 169 (0/169)	35.9 vs 45.6	35.9 vs 45.6 (5)	68.9 mo vs Median not reached	21.3 vs 38.5 mo	27.1 vs 36 mo	[25]
Foran *et al.*, 1999	F 25 mg/m2 for 5 d iv. q28	20 (0/20)	79	79 (5)	>22.8 mo	41 mo	18 mo	[26]
Dhodapkar *et al.*, 2001	F 30 mg/m2/day for 5 d iv. q28	118 (0/118)	38	38 (3)	5 y OS, 62%	5 years PFS 49 mo	41 mo	[27]
Dimopoulus *et al.*, 1994	2-CdA 0.1 mg/kg/day for 7 d Cl	26 (0/26)	85	85 (5)	13 mo OS, 81%	13 mo 19% relapse rate	13 mo	[28]
Delannoy *et al.*, 1999	2-CdA 4 mg/m2/day for 5 d vs 5.6 mg/m2/day iv. for 5 d	11 (0/11)	73	73	n.s.	3 years probability of relapse 18%	33 mo	[29]
Hellman *et al.*, 1999	2-CdA 0.14 mg/kg/day iv. for 5 d, q28	22 (13/9)	86	40.9 (4.5)	n.s.	12 mo	n.s.	[30]
Liu *et al.*, 1998	2-CdA 0.12 mg/kg/day iv. for 5 d, q28	20 (13/7)	70	55 (5)	20 mo OS, 85%	n.s.	20 mo	[31]
Treon *et al.*, 2001	R 375 mg/m2/week for 4 w (>4 w in 7 responders)	30 (7/23)	60	27	n.s.	TTF 8 mo for PR	n.s.	[32]
Gertz *et al.*, 2004	375 mg/m2/week for 4 w	69 (35/34)	52.1	27.5	n.s.	27 mo	n.s.	[33]
Dimopoulus *et al.*, 2002	R 375 mg/m2 iv. for 4 w, repeated at 3 mo	27 (12/15)	55	44	n.s.	TTP 16 mo	15.7 mo	[34]
Treon *et al.*, 2005	R 375 mg/m2 iv. for 4 w, repeated at 3 mo	29 (12/17)	65.5	48.3	n.s.	19 mo; TTP 14 mo	29 mo	[35]
Treon *et al.*, 2007	B 1.3 mg/m2 d 1, 4, 8, 11	27 (26/1)	85	48.1	n.s.	TTP 6.6 mo	18.2 mo	[36]
Chen *et al.*, 2007	B 1.3 mg/m2 d 1, 4, 8, 11	27 (15/12)	78	44	n.s.	10 mo PFS 16.3 mo	n.s.	[37]

2CdA: Cladribine; B: Bortezomib; Chl: Chlorambucil; d: Daily; F: Fludarabile; FU: Follow up; iv.: Intravenous; mo: Months; n.s: Not specified; ORR: Overall response rate; PFS: Progression-free survival; po.: Per os; q: Every; R: Rituximab; TTF: Time to treatment failure; TTP: Time to treatment progression; w: Weekly.

Cyclophosphamide based

Several randomized trials in lymphoprolipherative disorders have shown that the inclusion of rituximab in cyclophosphamide-based regimens such as CVP (cyclophosphamide, vincristine and prednisone) and CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) improves response rate as well as response duration and OS [46,47].

Similar results have also been achieved in WM; in fact, in the German randomized trial in which untreated patients with WM were included, the addition of monoclonal antibody to CHOP schedule led to a higher objective response rate (91 vs 60%). Furthermore, R-CHOP induced a significantly longer time to treatment failure [48]. Although treatment was well tolerated R-CHOP may be considered to be too toxic because of the high incidence of myelosuppression.

The importance of doxorubicin and vincristine inclusion in the chemotherapeutic regimen since the introduction of rituximab has not yet been still clarified. Furthermore, the use of anthracyclines is associated to adverse events such as alopecia, cardiopathies and cytopenia while vincristine should be avoided in patients presenting with neuropathies.

A non-randomized comparison of patients treated at the Dana Farber Institute showed that omitting doxorubicin or doxorubicin plus vincristine did not significantly decrease response rate with outcomes very similar to those observed after R-CHOP in the German study. Furthermore, these schedules were much less toxic compared with R-CHOP [49].

To avoid toxicity related to anthracyclines and vincristine, Dimopoulos *et al.* designed a regimen consisting of dexamethasone 20 mg followed by rituximab 375 mg/m^2 intravenously on day 1 and cyclophosphamide administered orally for 5 days at the dosage of 200 mg/m^2 (DRC) [50]. Only untreated patients were included in the study, major response rate was 83%, including 7% with CR. Median time to response was 4.1 months.

Importantly, treatment was very well tolerated as only 7 of the 72 enrolled patients (9%) developed grade 3–4 neutropenia. The updated results of this trial showed a favorable median time to progression of about 3 years, most patients with progression responded again to rituximab based regimens. After a minimum follow-up >6 years long-term toxicity was limited [51].

Currently in clinical practice, cyclophosphamide-based regimens and in particular DRC, which avoid unnecessary toxicity, are considered the standard of treatment for untreated patients. These regimens may also be preferable in younger patients eligible for stem cell collection [40,43].

Nucleoside analogs based

In vitro studies demonstrated strong evidence of synergy between nucleoside analog and alkylating agents. This translated in increase of ORR and response duration when fludarabine or cladribine were administered in combination in untreated or pretreated patients [52,53].

Furthermore, preclinical data indicated that rituximab sensitized cells to both fludarabine and cyclophosphamide (FC); thus enhancing their cytotoxic activity [54,55].

The addition of riruximab to nucleoside analog-based chemotherapy allowed to obtain an amelioration of quality of response with a prolonged progression-free survival (Table 4).

The results of a study on 43 patients demonstrated that the combination of rituximab and fludarabine (FR) is highly active leading to an ORR of 95.3% and median TTP of 51.2 months being longer in previously untreated patients and in those achieving at least a very good partial response [45]. Favorable responses and rapid disease control have also been obtained with the combination of rituximab and fludarabine plus cyclophosphamide (FCR) [56]. Authors conclude that although FR and FCR are highly effective, short- and long-term toxicities should be carefully considered. In both studies myelosuppression rate was high, leading to treatment dose reduction or discontinuation; long-lasting episodes of neutropenia were observed after the end of FCR treatment. Furthermore, in both studies during follow-up cases of myelodipslastic syndromes (MDS) and acute myeloid leukemia (AML) were reported (three in both studies), and three cases of diffuse large cell lymphoma after FR.

The safety of nucleoside analogs treatment in WM was the subject of investigation in a metanalysis by Leleu *et al.* [58,59].

The analysis of data showed a crude incidence of 6.6–10% for the development of disease transformation and an incidence of 1.4–8.9% for the development of MDS or AML in patients treated with fludarabine or cladribine, including patients who had previously received purine analogs. These results were not confirmed in the randomized study comparing the efficacy of fludarabine alone with that of chlorambucil [26]. In fact, in this study after 6 years the incidence of disease transformation was 7.7% in the fludarabine arm versus 11.1% in the chlorambucil arm, and MDS/AMLs were observed only in patients treated with the alkylating agent. These data suggest that the risk of these long-term complications are more frequent in patients treated with fludarabine–alkylator combinations.

The first experience with cladribine, cyclophosphamide and rituximab combination was reported by Weber *et al.* in 2003 in a small series of 17 patients [53].

Table 4. Immunochemotherapy in Waldenstrom's macroglobulinemia.

Reference	Regimen	Patients (treated/ untreated)	Response (%)	Survival	Duration of response	FU	Ref.
Buske C *et al.*, 2009	R-CHOP (R 375 mg/sqm, C750 mg/m2, Dx 50 mg/ m^2, V 1.4 mg/m^2 d1, P 100 mg/m^2 d1–5)	23 (0/23)	ORR: 91 CR: 9	n.s.	TTF 63 mo	n.s.	[48]
	CHOP (C750 mg/m^2, Dx 50 mg/m^2, V 1.4 mg/m^2 D1, P 100 mg/m^2 d1–5)	25 (0/25)	ORR: 60 CR: 4	n.s.	TTF 22 mo		
Ioakimidis L *et al.*, 2009	R-CHOP (R 375 mg/m^2, C750 mg/m^2, Dx 50 mg/m^2, V 1.4 mg/m^2 D1, P 100 mg/m^2 d1–5)	23 (0/23)	ORR: 96 CR: 17 VGPR: 9 PR: 44 mR: 26	n.s.	TTP 18 mo	25 mo	[49]
	R-CVP (R 375 mg/m^2, C750–1000 mg/m^2, V 1.4 mg/m^2 d1, P 100 mg/m^2 d1–5)	16 (0/16)	ORR: 88 CR: 12 VGPR: 7 PR: 44 mR: 25	n.s.	TTP median NR	15 mo	
	R-CP (R 375 mg/m^2, C 1000 mg/m^2 d1, P 100 mg/m^2 d1–5)	19 (0/19)	ORR: 95 PR: 74 mR: 21	n.s.	TTP median NR	9 mo	
Dimopoulos M *et al.*, 2007	DRC (D 20 mg, R 375 mg/m^2, C 100 mg/m^2 d1)	72 (0/72)	ORR: 83 CR: 7 VGPR: 67 PR: 9	Median NR	PFS median NR	23.4 mo	[50]
Treon S *et al.*, 2009	FR (F 25 mg/m^2, R 375 mg/m^2 d1)	43 (16/27)	ORR: 95 CR: 4 VGPR: 32 PR: 48		TTP 51,2 mo	40.3 mo	[45]
Tedeschi *et al.*, 2012	FCR (F 25 mg/m^2, C 250 mg/m^2, R 375 mg/m^2)	43 (15/28)	ORR: 79 CR: 12 VGPR: 21 PR: 42 mR: 4	Median NR	EFS 50 mo	37.2 mo	[56]
Weber *et al.*, 2003	2CDA-CR (2CDA 1.5 mg/m^2 × 3, C 40 mg/m^2 × 2 d1–7, R 375 mg/m^2 weekly for 4 week)	17	ORR: 93	Median NR	PFS 60	n.s.	[53]
Laszlo *et al.*, 2011	2CDA-R (2CDA 0,1 mg/kg d1–5, R 375 mg/m^2 d1)	29 (13/16)	ORR: 90 CR: 24 PR: 55 mR: 11	n.s.	TTF median NR	49.8 mo	[57]

2CDA: 2-Chloro-2′-deoxyadenosine; C: Cyclophosphamide; CR: Complete remission; D: Dexamethasone; Dx: Doxorubicin; EFS: Event-free survival; F: Fludarabine; FU: Follow up; mo: Months; mR: Minor response; NR: Not reached; n.s.: Not specified; ORR: Overall response rate; P: Prednisone; PFS: Progression-free survival; PR: Partial remission; R: Rituximab; TTP: Time to progression; TTF: Time to treatment failure; V: Vincristine; VGPR: Very good partial remission.

Although combination treatment did not improve response rate the median remission duration was longer (23 months for cladribine alone versus not reached after a median follow-up of 21 months in patients treated with immunochemotherapy).

The efficacy of rituximab and subcutaneous cladribine combination was evaluated in a larger study of 29 newly diagnosed/pretreated WM patients. ORR rate observed was 89.6% and was not influenced by previous treatment. Interestingly in this series after a median follow-up of 43 months none of the patients developed MDS/AML or transformation to aggressive lymphoma [57].

Although purine analogs-based immunochemotherapy of WM allows rapid and durable responses, studies are still needed to optimize dosage, drug combinations and treatment duration. In fact, these regimens induce a prolonged immunosuppression and a sustained depletion of CD4+ and CD8+ T-lymphocytes that may translate in increased number of infections. There is a general consensus to avoid purine analogs-based treatment in first-line treatment and in younger patients not only due to the risk of MDS/AML development but also because they may hamper the ability of a subsequent stem cells collection for an autologous stem cell transplant [60].

Bendamustine based

Bendamustine is effective in the treatment of chronic lymphocytic leukemia and other lymphoprolipherative disorders. A randomized trial comparing R-CHOP versus R-bendamustine (BR) in untreated low-grade non-Hodgkin's lymphoma patients showed that the two regimens induce comparable response rates; however, PFS is significantly longer after BR (54.8 vs 31.2 months) [61]. The PFS benefit was confirmed also after the analysis of the subgroup of 40 WM enrolled patients (not reached vs 35 months after CHOP). Furthermore, BR regimen was better tolerated with significantly lower rates of hematological toxicity infections and peripheral neuropathy [62].

The first experience of bendamustine treatment in the relapsed/refractory patients was published in 2011 by Treon *et al.* Overall, 30 patients received bendamustine-based treatment, 24 in combination with rituximab [63]. Overall and major response rate was 83.3%. Treatment was well tolerated, and dose reduction and/or truncation of intended therapy was needed in 8/30 (26.6%) patients (with no difference in toxicity development when comparing younger to older patients). A retrospective Italian study on 54 patients showed similar results in terms of responses, major responses 83.4%, confirming also good tolerability. Longer follow-up and prospective trials are needed to evaluate the long-term safety of this combination [64].

Proteasome inhibitors based

Considering the favorable results obtained with bortezomib monotherapy several studies aimed to evaluate the efficacy of the combination bortezomib and rituximab (Table 5).

Bortezomib administered at the dose of 1.3 mg/m^2 in combination with dexamethasone 40 mg on days 1, 4, 8 and 11, and rituximab 375 mg/m^2 led to a higher major response rate, 78%, compared with bortezomib monotherapy [65]. However, after this schedule the development of grade 3 peripheral neuropathy was very high (30%), suggesting that in WM the weekly bortezomib administration would be preferred. Similar results with manageable toxicity, 5% of grade 3–4 neutropathy and 78% of patients concluding the intended therapy, were achieved after the weekly bortezomib administration at the higher dosage of 1.6 mg/m^2 (days 1, 8,15 in a 28-day cycle for six cycles) in combination with rituximab [66]. Median time to progression in this population of 37 heavily pretreated patients (3 median number of prior lines of treatment) resulted of 16.4 months. The same regimen administered to untreated patients led to a 100% of at least minor response or better, with a major response rate of 66%. Again with the weekly administration of bortezomib none of the patients developed grade 3 or 4 neuropathy [67].

Recently, a larger study of the European Myeloma Network confirmed the efficacy and low toxic profile of weekly bortezomib 1.6 mg/m^2 (from the second course) followed by dexamethasone (40 mg) and iv. rituximab (375 mg/m^2) in cycles 2 and 5 [68]. Major response rate resulted 68% with a median progression-free survival of 42 months and a 3-year duration of response for patients obtaining at least a PR of 70%. Even in this case peripheral neuropathy grade 3–4 developed in 7% of patients.

Carfilzomib is a 'second generation' proteasome inhibitor that specifically irreversibly binds the chymotripsine-like site of the proteasome and is associated with lower rates of polyneuropathy when compared with bortezomib [71]. An *in vitro* model of neurodegeneration demonstrated that bortezomib, but not carfilzomib, reduced neurite length and neuronal cell survival despite equivalent levels of proteasome inhibition with both agents. A nonproteasomal mechanism has been suggested; in fact, in cell lysates bortezomib, in contrast to carfilzomib, significantly inhibited the serine proteases cathepsin G (CatG), cathepsin A, chymase, dipeptidyl peptidase II and HtrA2/Omi at potencies near or equivalent to that for the proteasome [72].

Carfilzomib has been approved for the treatment of relapsed/refractory multiple myeloma, and its activity has been examined in combination with rituximab and dexamethasone (CARD) in WM patients in a

prospective Phase II trial [73]. Thirty-one patients, most of them untreated, received an induction treatment consisting of six courses of carfilzomib 20 mg/m^2 cycle 1 then 36 mg/m^2 from cycle 2 and beyond) with iv. dexamethasone 20 mg given on days 1, 2, 8, 9 and rituximab 375 mg/m^2 on days 2, 9 of each 21 day cycle. The best ORR obtained was 81% with 21 patients achieving a major response, responding patients subsequently received eight maintenance cycles. Median time to response was very short (2.1 m). As major toxicity of grade >2 was an asymptomatic increase in elevation of lipase, authors conclude that CARD is highly active and is a neuropathy sparing approach as no grade 2 or greater neuropathies were recorded.

Immunomodulatory agents based

Considering the *in vitro* synergistic effect of rituximab with immunomodlatory agents, two clinical trials were designed by the Waldenstrom's Macroglobulinemia Clinical Trials Group (WMCTG) with either thalidomide or lenalidomide [69,70]. The intended therapy of thalidomide consisted of: 200 mg thalidomide for 2 weeks, followed by 50 weeks of 400 mg thalidomide, rituximab 375 mg/m^2 was combined intravenously from weeks 2–5 and 13–16. Even if responses in the 25 symptomatic enrolled patients were encouraging, with a major response of 64% and median time to treatment failure of 34.8 months, high doses of thalidomide were poorly tolerated. All patients needed a dose reduction, and in 11 it was necessary to discontinue treatment. The poor tolerability to high doses of thalidomide had also been reported by Dimopoulous when the drug was administered in monotherapy [74]. Furthermore, thalidomide did not prevent the rituximab flare. Interestingly, responses were unaffected by FcγRIIIA-IgM levels. Lenalidomide was administered at the dosage of 25 mg in combination with rituximab in 16 patients. The ORR in the 12 evaluable patients was 67% most characterized by a minor response (four PRs). During the study an acute decrease of hematocrit, without any signs of hemolysis, was observed in 81% of cases resulting in hospitalization in four patients. The underlying mechanism for anemia development is not completely known but it persisted despite reducing the dosage to 5 mg/daily. Thus, the use of this agent among WM is considered still investigational [17].

While there is a consensus of the role of adding rituximab to chemotherapy or novel agents, the use of rituximab maintenance therapy in WM is controversial. In follicular lymphomas rituximab maintenance in randomized trials determined a prolongation of PFS, longer time to next treatment translating also in a longer OS [75]. There are no randomized maintenance trials designed for WM patients. A retrospec-

Table 5. Proteasome inhibitors and immunomodulatory agents in Waldenstrom's macroglobulinemia.

Reference	Regimen	Patients (treated/untreated)	Response (%)	Survival	Duration of response	FU	Ref.
Treon *et al.*, 2009	BDR (B 1.3 mg/m^2 d 1–4–8–11, D 40 mg/d 1–4–8–11, R 375 mg/m^2 d 11)	23 (0/23)	ORR 96 CR: 13 nCR: 9 VGPR: 13 PR: 48 MR: 13	n.s.	TTP median NR	22.8 mo	[65]
Ghobrial *et al.*, 2010	BR (B 1.6 mg/m^2 d1–8–15, R 375 mg/m^2 weekly cycle 1 and 4)	37 (37/0)	ORR 81 CR: 3 nCR: 3 PR: 45 mR: 30	Median NR	19.5 mo PFS 15.6 mo TNT 17.6 mo	16 mo	[66]
Ghobrial *et al.*, 2010	BR (B 1.6 mg/m^2 d 1–8–15, R 375 mg/m^2 weekly cycle 1 and 4)	26 (0/26)	ORR 88 CR: 4 nCR: 4 PR: 58 mR: 22	Median NR	Median NR PFS median NR	14 mo	[67]
Dimopoulos *et al.*, 2013	BDR (B 1.3 mg/m^2 d 1–4–8–11 1° cycle B 1.6 mg/m^2 d 1–8–15–22 cycles 2–5 - D 40 mg d 1–8–15–22 cycles 2–5, R 375 mg/m^2 d 1–8–15–22 cycles 2–5)	59 (0/59)	ORR 85 CR: 3 VGPR: 7 PR: 58 mR: 17	n.s.	PFS median 42 mo	42 mo	[68]
Treon *et al.*, 2008	TR (T 50 200 mg/d for 2 w, 400 mg/day for 4 w, R 375 mg/m^2 weekly on w 2–5, 13–16)	25 (5/20)	ORR: 72 CR: 4 MR: 64 MR: 8	n.s.	PFS median 34.8 mo	47.1 mo	[69]
Treon *et al.*, 2009	LR (L 25 mg/d d 1–21, R 375/m^2/weekly on w 2–5, 13–16)	16 (12/4)	ORR: 50 CR: 0 MR: 25 mR: 25	n.s.	PFS median 17.1 mo	31.3 mo	[70]

B: Bortezomib; CR: Complete remission; d: Day; D: Dexamethasone; FU: Follow up; L: Lenalidomide; mo: Months; MR: Major response; mR: Minor response; NR: Not reached; ORR: Overall response rate; PFS: progression-free survival; R: Rituximab; T: Thalidomide; TNT: Time to next treatment; TTP: Time to progression; w: Week.

tive analysis showed an amelioration of PFS and OS in the group of patients treated with rituximab maintenance over patients who were not selected for maintenance treatment [76]. Even though treatment was very well tolerated the group of patients receiving further monoclonal antibody treatment showed lower normal Ig levels translating in an increase of infections. Although infections were mostly non severe the role of rituximab in WM should be better clarified possibly in prospective randomized trials.

New monoclonal antibodies

Ofatumumab is a fully human monoclonal antibody targeting both the large and small extracellular loops of CD20. Considering the promising results obtained in chronic lymphocytic leukemia and other lymphoproliferative disorders, a Phase II study for WM has been designed. Preliminary data on 37 patients showed that in WM ofatumumab has an acceptable toxicity and a low incidence of IgM flare (5%) (Table 6) [77]. The monoclonal antibody showed to be effective (ORR 59%) even in those patients relapsing after rituximab treatment.

An alternative target in the treatment of WM may be CD52. CD52 is expressed on WM mast cells which are typically increased in WM and support the growth and survival of the neoplastic clone through CD40 ligand [83]. Alemtuzumab is a fully humanized IgG1 monoclonal antibody that targets CD52 inducing antibody-dependent cell-mediated cytotoxicity againts mast cells. In 28 WM symtomatic patients alemtuzumab led to an ORR of 76% with 32% achieving a major response [78]. The median time to progression was 14.5 months. As expected with alemtuzumab a high rate of neutropenia, infections and CMV reactivation were observed being more common in heavily pretreated patients. Authors conclude that despite the fact that alemtuzumab may be considered active, short- and long-term toxicities should be weighed against other available treatment options.

Signal transduction inhibitors

It is well known that lymphoma cells' survival and growth are strictly dependent on signal transduction pathways. Furthermore, there is strong evidence of the role of the tumor microenvironment in supporting the expansion of the malignant clone [84]. In recent years, better understanding of disease biology has led to the development of new agents that specifically target some of these signal transduction pathways leading to apoptosis and inhibition of proliferation. Results obtained with this new compounds are summarized in Table 6.

MYD88 L265P is a common recurring mutation among patients with WM which has been rarely observed in other lymprolipherative disorders [85]. Normally MYD88 is directly activated after Toll-like receptor or IL-1 receptor binds to its ligand. Dimerization of MYD88 triggers autoplhosphorilation of IL-1 receptor associated kinase and bruton tyrosine kinase (BTK), resulting in a signal propagation that determines the activation of NF-κB [86]. L265P mutation exerts an oncogenic effect as it determines a constitutively activating signal resulting in survival and proliferation of the malignant clone. BTK is highly expressed in cells from patients with WM and moreover overexpression of L265P leads to more robust BTK activation [87]. Considering that ibrutinib inhibits BTK activity there is a strong rationale for investigating its role in WM.

The administration of 420 mg of ibrutinib to 63 pretreated patients including 17 with refractory disease determined a rapid decrease in IgM level [79]. After a median follow-up of six cycles the best ORR resulted in 81% with a PR or better in 57%. The rate of >2 grade recorded is low and consisted mostly of neutropenia (19%), thrombocytopenia (14%). Interestingly a higher response rate was observed in patients with wild-type CXCR4 (77%) when compared with patients showing WHIM-like CXCR4 mutation (30%).

Akt, which is upregulated in patients with WM, plays an important role in lymphomagenesis as it regulates multiple signaling pathways controlling, proliferation, cell cycle and apoptosis [88]. Perifosfine is a novel Akt inhibitor, preclinical studies demonstrated that is effective in inhibiting Akt in WM primary cells and cells line [89]. A Phase II clinical trial in which perifosfine was administered orally 150 mg/daily was conducted in 37 heavily pretreated patients. At least a minor response was rapidly obtained in 35% of patients while the majority of them (54%) remained in stable disease [80]. The median PFS was 12.6 months superior to other targeted agents used in monotherapy such as bortezomib. The main toxicity was gastrointestinal of grade 1 and 2, neutropenia grade 3–4 was only 11%. The good tolerability of treatment and the *in vitro* evidence of a synergistic effect of perifosfine with rituximab warrants further studies using combination treatment.

Everolimus (RAD001) is a TORC 1 inhibitor that is effective in tumors that are dependent from PI3K/AKT/mTOR/ pathway. Everolimus induces direct cell cytotoxicity with induction of caspase cleavage and cell cycle arrest and furthermore inhibits angiogenesis [90]. RAD001 cytotoxicity has also been demonstrated in WM cell lines even if there are no reported specific mutations in the PI3K/mTOR pathway [91]. The administration of RAD001 in monotherapy as salvage regimen in 50 pretreated patients with a median of three lines of therapy led to an ORR of 70% with 42%

PRs and 28% of minor responses [81]. Grade 3–4 toxicity recorded was mostly hematologic. At the time of publication median PFS was not reached and 62% of patients were alive and progression free at 12 months. In the setting of first-line treatment everolimus showed to be active in 22 patients allowing to achieve an OR rate of 77% [92]. As observed in pretreated patients the drug induced a rapid reduction in serum IgM levels with a discordance to underlying bone marrow disease burden. This suggests that serial bone marrow evaluations are necessary for response assessments in patients treated with everolimus. Considering the high effectiveness of this drug administered as single agent everolimus is considered a potential new therapeutic strategy. Preliminary results of a Phase I/II study including 46 heavily pretreated patients showed that everolimus given in combination with weekly bortezomib and rituximab is well tolerated with a low rate of grade 3–4 adverse events, and no grade 3–4 neuropathies. Major responses were recorded in 50% of patients [82].

Conclusion & future perspective

Treatment options for WM are derived from other lymphoprolipherative disorders. As there is not a standard of therapy in this disease, randomized controlled trials to assess the efficacy and toxicity of the different therapeutic options should be recommended. Most of the studies published up to now are based on Phase II studies on small series of patients so that many issues still remain open.

Phase II studies revealed that immunochemotherapy led to the achievement of better quality of responses; however, randomized trials are warranted to determine whether the higher response rate will translate into survival improvement and to determine late treatment toxicities. Nucleoside analogs are effective in WM treatment even though there is a general consensus to avoid their use in younger patients on the basis of retrospective data demonstrating an increased incidence of disease transformation to high-grade non-Hodgkin's lymphoma and the potential development of tMDS/AML [58]. Optimal dosage and duration of fludarabine treatment has not yet been established.

BR demonstrated a better toxic profile in respect to R-CHOP in first line treatment in WM but there is not a general consensus to consider BR as the standard front line treatment as data published in literature specifically for WM are scanty (22 patients) [62]. Studies are needed to better understand the role of bendamustine treatment.

Rituximab has become part of treatment in combination with chemotherapy in most patients with WM allowing to obtain benefits in response and progression-free survival [44]. Results of the efficacy of ritux-

Table 6. New agent treatments in Waldenstrom's macroglobulinemia.

Reference	Regimen	Patients (treated/untreated)	Response %	Duration of response	Survival	FU	Ref.
Furman R *et al.*, 2011	Ofatumumab 300 mg w1--> 1000 mg w 2-4 vs 2000 mg w 2-5 iv. Possible further 5 w therapy in SD or MR	37 (28/9)	ORR: 59 PR: 35 mR: 24 (all) ORR: 47 vs 68 (ARM a vs B)	n.s.	n.s.	n.s.	[77]
Treon *et al.*, 2011	Alemtuzumab 30 mg iv. three-times/w up to 12 w	28 (23/5)	ORR: 75 CR: 5 MR: 31 mR: 39	TTP 14.5 mo	n.s.	64 mo median	[78]
Treon *et al.*, 2013	Ibrutinib 420 mg/d po.	63 (17 rel; 46 refr)	ORR: 81 VGPR: 6 PR: 51 Mr: 24	n.s.	n.s.	6 mo median	[79]
Ghobrial et al, 2010	Perifosfine 150 mg/d po.	37 (18 rel; 19 refr)	ORR: 35 PR: 11 Mr: 24	TTP, PFS 12,6 median	26 mo	19.5 mo median	[80]
Ghobrial *et al.*, 2010	Everolimus 10 mg/d po.	50 (0/50)	ORR: 70 PR: 42 mR: 28	Median NR Estimated PFS 62% at 12 mo	Median NR	11.5 mo Median (alive points only)	[81]
Ghobrial *et al.*, 2013	Everolimus + bortezomib + rituximab	46 (0/46)	ORR: 87 CR: 13 PR: 68 mR: 6 (Phase II, 16 evaluable pts)	n.s.	n.s.	n.s.	[82]

CR: Complete remission; d: day; FU: Follow up; iv.: Intravenous; mo: Months; mR: Minor; n.s: Not specified; ORR: Overall response rate, po.: Per os; PR: Partial remission; q: Every; refr: Refractory; rel: Relapsed; VGPR: Very good partial remission; w: week.

imab in maintenance treatment have been reported by Trreon *et al.* in a retrospective study and are consistent with that observed in other lymphoproliferative disorders. A prospective trial to better understand the role of maintenance treatment and late toxic effects should be considered in the future.

Considering the high efficacy obtained with bortezomib second generation proteasome inhibitors are under investigation in WM [73]. Studies with carfilzomib are ongoing and other proteasome inhibitors showing a synergistic effect with bortezomib are in development.

The understanding of the disease biology at the molecular and cellular level, the discovery of MYD88 L265P mutation, allowed the development of new pharmacological compounds. Results that will be obtained from the ongoing and future clinical trials on PKC inhibitors, histone deacetylase inhibitors, new anti-CD20 monoclonal antibodies will revolutionize the options for patients with WM. Preliminary studies showed that these target therapies have a lower toxic profile when compared with chemotherapy allowing the possible use in combination treatment to obtain higher and durable responses.

Multilevel genetic characterization of WM will provide in the near future the development of new targeted therapies. Whole genome sequencing revealed activating somatic mutations in MYD88 (L265P) and CXCR4, both are important determinants of clinical presentation and impact OS [93,94]. Targeted therapies directed against MYD88 and/or CXCR4 signaling may provide a personalized treatment approach to WM. Furthermore, microRNAs have shown to play an important in supporting WM pathogenesis and represent an important prognostic marker. In particular, miRNA-155 levels showed to be elevated in stromal cells from WM patients compared with control samples and stromal cells from miRNA-155-knockout mice led to significant inhibition of WM tumor growth [95,96]. These data indicate the potential role of miRNA-155 inhibition for WM treatment.

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Executive summary

- Not all patients with Waldenstrom's macroglobulinemia (WM) require therapy.
- Asymptomatic patients with WM should be observed and monitored and do not require treatment until symptoms develop.
- There are not randomized trials to define which first-line treatment should be considered as standard therapy.
- Treatment should be individualized and patient fitness and disease characteristics must be taken into account before initiating therapy.
- Rituximab-based therapies should be considered as initial treatment for most patients with WM.
- Long-term complications after nucleoside analogs therapy need to be better evaluated in large prospective studies especially in younger patients. Considering the expanding options treatment risk versus benefit should be carefully evaluated when administering nucleoside analogs in first line treatment.
- Although a retrospective study demonstrated a better outcome in patients receiving rituximab as maintenance there is still no clear evidence for supporting its use.
- The introduction of novel agents for multiple myeloma such as proteasome inhibitors provided benefits in the treatment of WM.
- A better understanding of disease biology determined the use of small targeted molecules that are currently tested in Phase II studies.

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