

Combining Ixazomib With Subcutaneous Rituximab and Dexamethasone in Relapsed or Refractory Waldenström's Macroglobulinemia: Final Analysis of the Phase I/II HOVON124/ECWM-R2 Study

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PURPOSE Proteasome inhibitors are effective in Waldenström's macroglobulinemia (WM) but require parenteral administration and are associated with polyneuropathy. We investigated efficacy and toxicity of the less neurotoxic oral proteasome inhibitor ixazomib combined with rituximab, in patients with relapsed WM.

METHODS We conducted a multicenter phase I/II trial with ixazomib, rituximab, and dexamethasone (IRD). Induction consisted of eight cycles IRD wherein rituximab was started in cycle 3, followed by rituximab maintenance. Phase I showed feasibility of 4 mg ixazomib. Primary end point for phase II was overall response rate (ORR [\geq minimal response]) after induction.

RESULTS A total of 59 patients were enrolled (median age, 69 years; range, 46-91 years). Median number of prior treatments was 2 (range, 1-7); 70% had an intermediate or high WM-IPSS (International Prognostic Scoring System for WM) score. After eight cycles, ORR was 71% (42 out of 59) (14% very good partial response [PR], 37% PR, and 20% minor response). Depth of response improved until month 12 (best ORR 85% [50 out of 59]: 15% very good PR, 46% PR, and 24% minor response). Median duration of response was 36 months. The average hematocrit level increased significantly (0.33-0.38 L/L) after induction ($P < .001$). After two cycles of ixazomib and dexamethasone, immunoglobulin M levels decreased significantly (median 3,700-2,700 mg/dL, $P < .0001$). Median time to first response was 4 months. Median progression-free survival and overall survival were not reached. After median follow-up of 24 months (range, 7.4-54.3 months), progression-free survival and overall survival were 56% and 88%, respectively. Toxicity included mostly grade 2 or 3 cytopenias, grade 1 or 2 neurotoxicity, and grade 2 or 3 infections. No infusion-related reactions or immunoglobulin M flare occurred with use of subcutaneous rituximab. Quality of life improved significantly after induction. In total, 48 patients (81%) completed at least six cycles of IRD.

CONCLUSION Combination of IRD shows promising efficacy with manageable toxicity in patients with relapsed or refractory WM.

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ASSOCIATED CONTENT

Data Supplement Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Waldenström's Macroglobulinemia (WM) is an indolent B-cell lymphoma, characterized by bone marrow (BM) infiltration of lymphoplasmacytoid cells and plasma cells (PCs), producing immunoglobulin M (IgM) M-protein.¹

Anti-CD20 monoclonal antibodies–based combinations are used for the primary therapy of WM; however, management of relapsed or refractory (RR) disease

remains challenging. Several phase II studies have shown clinical activity of the proteasome inhibitor (PI) bortezomib in WM. However, bortezomib-associated peripheral polyneuropathy (PNP) occurs frequently, leading to treatment discontinuation in approximately 30% of patients with WM.²⁻⁷ The oral PI ixazomib is proven to be less neurotoxic and well tolerated in multiple myeloma (MM).^{8,9} A previous study of ixazomib, rituximab, and dexamethasone (IRD) in treatment-naïve WM

CONTEXT

Key Objective

What is the efficacy and safety of the novel less neurotoxic oral proteasome inhibitor ixazomib combined with subcutaneous rituximab and dexamethasone (IRD) in patients with relapsed or refractory Waldenström's Macroglobulinemia (WM)?

Knowledge Generated

Treatment with IRD achieved a major response rate of 51% including 14% very good partial response (PR) and 37% PR, which improved until month 12 to a major response rate of 61% with 15% very good PR and 46% PR with manageable toxicity. Use of SC rituximab did not result in infusion-related reactions or immunoglobulin M flare. Median progression-free survival and overall survival were not reached, and after median follow-up of 24 months, progression-free survival and overall survival were 56% and 88%, respectively.

Relevance

This phase I/II clinical trial demonstrates that the IRD regimen, with oral ixazomib and SC rituximab, provides an effective and well-tolerated treatment in patients with heavily pretreated WM. Larger randomized trials need to compare the efficacy of IRD to other regimens for relapsed or refractory WM.

patients demonstrated an overall response rate (ORR) of 96% and a median progression-free survival (PFS) of 40 months, with good tolerability and 20% incidence of grade 1 neuropathy.^{10,11} However, no data on the activity and toxicity of IRD in RR WM exist. In WM, *MYD88*^{L265P} and *CXCR4* mutations are present in > 90% and up to 40% of patients, respectively.¹²⁻¹⁴ Previous studies have demonstrated that PFS is unaffected by *CXCR4* status in patients treated with PIs in first line.¹¹ *CXCR4* mutations were, however, associated with lower very good partial response (VGPR) rates and increased time to response compared with *CXCR4* wild-type patients, but for relapsed patients, no data exist on the impact of *CXCR4* on PFS after treatment with PIs.^{10,11,15,16} Rituximab sensitization is observed in approximately 7% of patients with WM, often leading to treatment discontinuation.¹⁷ The use of subcutaneous (SC) rather than intravenous (IV) rituximab could result in less sensitization.

In this prospective, multicenter, phase I/II study performed by the Haemato Oncology Foundation for Adults in the Netherlands and European Consortium for Waldenström's Macroglobulinemia in collaboration with the Greek Myeloma Study Group (HOVON124/ECWM-R2), we establish the effective dose level for ixazomib in combination with SC rituximab and dexamethasone and demonstrate the feasibility and efficacy of this regimen in relapsed WM.

METHODS

Patients

Patients with progressive or relapsed WM after prior systemic therapy, requiring treatment based on consensus criteria, were enrolled.¹⁸ Patients had to have measurable disease (defined as IgM level > 1 g/dL). The Data Supplement (online only) shows the complete inclusion and exclusion criteria. Central pathology review was performed by K.A. and S.T.P.

All patients provided written informed consent. The study Protocol (online only) was approved by the Ethical Review Committee of all participating centers and was carried out in accordance with the principles of the Helsinki Declaration.

Study Design and Treatment

The HOVON124 study (<http://www.trialregister.nl> identifier: NL5025 [NTR5171]) is an international, multicenter, prospective, open-label phase I/II study conducted at 18 centers: 14 in the Netherlands, three in Belgium, and one in Greece. An independent Data Safety Monitoring Board evaluated the general progress and safety at predefined intervals.

Baseline assessment included protein electrophoresis, immunofixation, free light chain measurements, BM biopsy, molecular analysis for *MYD88* and *CXCR4* mutations, and computed tomography (CT) scan of neck, chest, and abdomen. Phase I study design is described in the Data Supplement.

For phase II, patients were treated with eight 28-day cycles of ixazomib at the recommended dose level (4 mg flat dose, orally, day 1, 8, and 15) and dexamethasone (20 mg orally, day 1, 8, 15, and 22). To avoid the risk of IgM flare and to assess the effect of ixazomib only, rituximab was added from cycle 3 onward; the first dose was given IV (375 mg/m² on day 1), and all subsequent doses were given at a flat dose of 1,400 mg SC.

After cycle 4, patients with progressive disease (PD) went off study. After cycle 8, patients with at least minor response (MR) continued to rituximab maintenance (rituximab SC 1, 400 mg every 3 months for 2 years).

Response Evaluation and End Points

Responses were determined using the International Workshop for WM-6 criteria.^{19,20} Definitions of complete response, VGPR, partial response (PR), and MR are provided in the Data Supplement. Cheson criteria were used to

asses CT scan results and are summarized in the Data Supplement.²¹ IgM flare is defined as a temporary IgM increase > 25% from baseline (with a minimum of 5 g/L) followed by an MR or better to treatment. Toxicity was reported according to the Common Terminology Criteria for Adverse Events version 4.03.^{2,22}

The primary end point of the study was ORR after eight cycles of IRD, based on IgM level. Secondary end points included the rate of complete response, VGPR, PR, and MR separately, the best responses and responses after cycles 2, 4, and 8, the increase in hematocrit and decrease in IgM level, time to first and best responses, duration of response (DOR), PFS, and overall survival (OS). Furthermore, toxicity profile of IRD and patient-reported outcome measures (PROMs) were studied with an emphasis on neurotoxicity, as well as quality of life. All end points are described in the Data Supplement. The efficacy analyses are performed in 59 patients, based on intention to treat.

Patient-Reported Outcome Measures

The European Organisation for Research and Treatment of Cancer (EORTC) Quality of Life (QOL) Questionnaire (QLQ-C30) is a cancer-specific multidimensional 30-item questionnaire containing functional, symptom, global health status (GHS), QOL, and single-item scales. The 30 question scores were converted to a 0-100 score according to the EORTC QLQ-C30 scoring manual. Higher scores on the GHS and functional scales represent better QOL, whereas higher scores on the symptom scales correspond to greater degree of symptom burden.²³ Neurotoxicity was assessed using the EORTC QLQ-CIPN20 questionnaire. This questionnaire is developed to assess chemotherapy-induced PNP and contains three subscales based on sensory, motor, and autonomous neuropathy complaints.²⁴ The subscales were transformed to a 0-100 score, with higher score representing more symptoms. Items 1-19 were analyzed. In addition, a neurotoxicity scoring tool directly linking complaints to CTC-AE grading (version 4.0) was used.²⁵

Assessment of Bone Marrow Response and Molecular Analysis

Paraffin-embedded BM biopsies performed at entry and after cycles 4 and 8 or at early withdrawal or progression or relapse were centrally reviewed. Infiltration percentage of BM biopsies was determined by immunohistochemical assessment of CD3, CD20, CD79a, CD138, κ , and λ . BM tumor populations were defined as follows: total tumor cells represented by CD79a⁺ cells, malignant B lymphocytes represented by CD20⁺ cells, lymphoplasmacytic cells represented by involved light chain (κ or λ) positive cells *minus* the CD138⁺ cells, and PCs by CD138⁺ cells. Two independent observers estimated the infiltration percentage of the populations, blinded for patient biopsy sample and time point.

For molecular analysis, genomic DNA was extracted from BM sections as well as BM aspirates of most patients using the QIAamp DNA Micro Kit (Qiagen, Santa Clarita, CA). Library preparation was carried out using the Ion AmpliSeq Library Kit 2.0 according to manufacturer's instructions. An overview of the 64-gene panel kit used and detailed description of sample processing for next-generation sequencing (NGS) is available in the Data Supplement.

Statistical Analysis

The statistical analysis plan for phase I/II is described in the Data Supplement. Using a Simon two-stage min-max design based on a historical response rate of 40% and an anticipated response rate of 60%, using an $\alpha = .05$ and a power of $(1-\beta)$ 90%, results in a sample size of 54 patients. Considering a putative 10% ineligibility rate, 60 patients were planned to be enrolled. Since 59 eligible patients were enrolled finally, we computed the point estimate for ORR, 95% CI, and *P* value for over-running Simon's two-stage design (Data Supplement). Time-to-event end points were estimated using the Kaplan-Meier method, and log-rank test was used to analyze group differences in PFS and OS. PROMs were analyzed with nonparametric statistics. A Wilcoxon matched-pairs signed rank test was used to analyze change over time. Correlations were carried out by Spearman's correlation. A *P* value < .05 was considered statistically significant. All statistical analyses were performed with Stata (v15.1, StataCorp LP, College Station, TX) and R (v3.6.1, R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patients

Between January 2015 and January 2019, 60 patients with RR WM were enrolled (*n* = 6 in phase I at the recommended dose level and *n* = 54 in phase II). One patient was ineligible (rituximab-refractory) and therefore 59 patients were included in the phase II analysis.

Table 1 summarizes patient's characteristics. A summary of prior treatments is included in the Data Supplement. The median age was 69 years (range, 46-91 years), 68% were males, and 21 (36%) patients were high risk based on the International Prognostic Scoring System for WM. Central pathology review confirmed the diagnosis of WM in all patients.

Dose level. During phase I, no dose-limiting toxicity during cycle 1 occurred and no serious adverse events (SAEs) were reported. Thus, the 4 mg dose was deemed feasible and the six patients treated during phase I were included in the interim efficacy analysis of phase II.

Efficacy

At the interim analysis, 24 of 29 (83%) patients treated in the phase II part (stage 1) achieved a response, which led to a positive advice from the Data Safety Monitoring Board to proceed to stage 2. Based on intention-to-treat, the ORR after eight IRD cycles was 71% (42 of 59; 95% CI, 60 to

TABLE 1. Patient Characteristics

Characteristic	Patients (N = 59)	After Cycle 8	P
Median age, years (range)	69 (46-91)	—	
Sex, No. (%)		—	
Male	40 (68)		
Female	19 (32)		
WM-IPSS, No. (%)		—	
Low risk	17 (29)		
Intermediate risk	20 (34)		
High risk	21 (36)		
WHO performance status, No. (%)		—	
0	38 (64)		
1	19 (32)		
2	2 (3)		
Median prior treatments, No. (%)	2 (1-7)	—	
Prior treatment with rituximab	37 (63)	—	
Prior treatment with PIs	4 (7)	—	
Prior treatment with BTK inhibitor	1 (2)	—	
Lymphadenopathy	53%	—	
Hepatosplenomegaly	17%	—	
Hemoglobin, g/dL (range)	10.6 (6.4-15.9)	12.6 (9.4-15.6)	< .001
IgM, mg/dL (range)	3,280 (1,000-9,100)	1,200 (800-4,400)	< .001
B2M, mg/L (range)	3.7 (1.8-25.1)	—	—
Involved sFLC: κ , mg/L (range), (n = 41)	30.7 (2-906)	19.9 (3-194)	.002
Involved sFLC: λ , mg/L (range), (n = 13)	10 (1-3,280)	9.2 (1-250)	.15

Abbreviations: BTK, Bruton's tyrosine kinase; IgM, immunoglobulin M; IPSS, International Prognostic Scoring System; PI, proteasome inhibitor; sFLC, serum free light chain; WM, Waldenström's macroglobulinemia.

79), including VGPR in 8 (14%), PR in 22 (37%), and MR in 12 (20%) patients. Two (3%) patients had stable disease and one (2%) had PD after eight cycles (Fig 1A). Responses continued to improve with therapy until month 12, with best ORR of 85% (50 of 59) with 15% (9 of 59) VGPR, 46% (27 of 59) PR, and 24% (14 of 59) MR. Median time to first and best responses was 4 and 5 months, respectively. Median DOR was 36 months. Average hematocrit level increased from 0.33 L/L at baseline to 0.37 L/L after four cycles ($P < .001$) and further increased to 0.38 L/L after eight cycles ($P < .001$; Fig 2A). After the first two cycles of single-agent ixazomib, IgM level decreased significantly (median 3,700-2,700 mg/dL, $P < .0001$), decreasing further to 1,200 mg/dL after eight cycles ($P < .001$; Fig 2B). In total, 48 of 59 patients (81%) completed at least six cycles of IRD. Reasons for earlier discontinuation of 14 patients were progression (n = 6), toxicity (n = 3), unrelated intercurrent death (n = 2), incompliance (n = 1), and other reasons (n = 2; Fig 3). Among the 14 patients who did not complete eight cycles of IRD, one had VGPR, 4 had a PR, 2 had an MR, three had stable disease, and four had a PD between cycles 2 and 7.

CT-confirmed lymphadenopathy and hepatosplenomegaly at baseline was present in 32 of 59 (54%) and 10 of 59 (17%) patients, respectively. On follow-up CT scan, lymphadenopathy and hepatosplenomegaly decreased or resolved in 14 of 32 (44%) and 5 of 10 (50%) patients and remained stable in 10 of 32 (31%) and 2 of 10 (20%) patients, respectively. Progression of lymphadenopathy occurred in 2 of 32 (6%) patients.

Survival

Median PFS and OS were not reached, and after a median follow-up of 24 months (range, 7.4-54.3 months), PFS was 56% (95% CI, 40 to 67; events = 23 out of 59) and OS was 88% (95% CI, 75 to 95; events = 6 out of 59; Figs 4A and 4B). Six patients died during the study period: two died of PD, one of progressive multifocal leukoencephalopathy, one of graft-versus-host-disease following subsequent allogeneic stem-cell transplantation after PD, and two patients with cardiac comorbidities died of sudden death. These were all considered unrelated to study treatment (the patient with progressive multifocal leukoencephalopathy, in hindsight, already had symptoms at baseline).

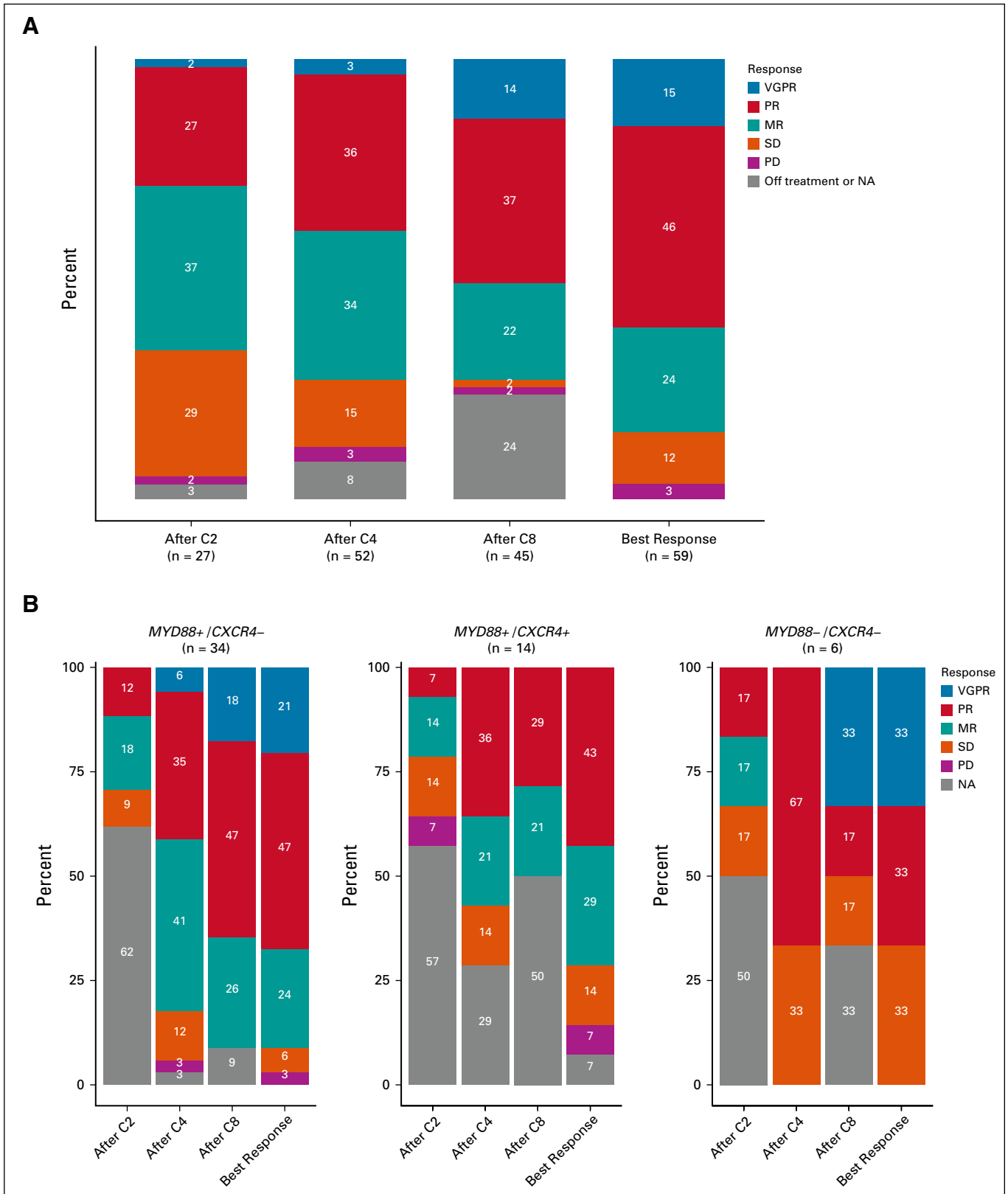


FIG 1. (A) Responses during induction cycle 1-8 of 59 enrolled patients and (B) responses during induction cycle 1-8 by mutation status. C, cycle; MR, minor response; NA, not available; PD, progressive disease; PR, partial response; SD, stable disease; VGPR, very good partial response.

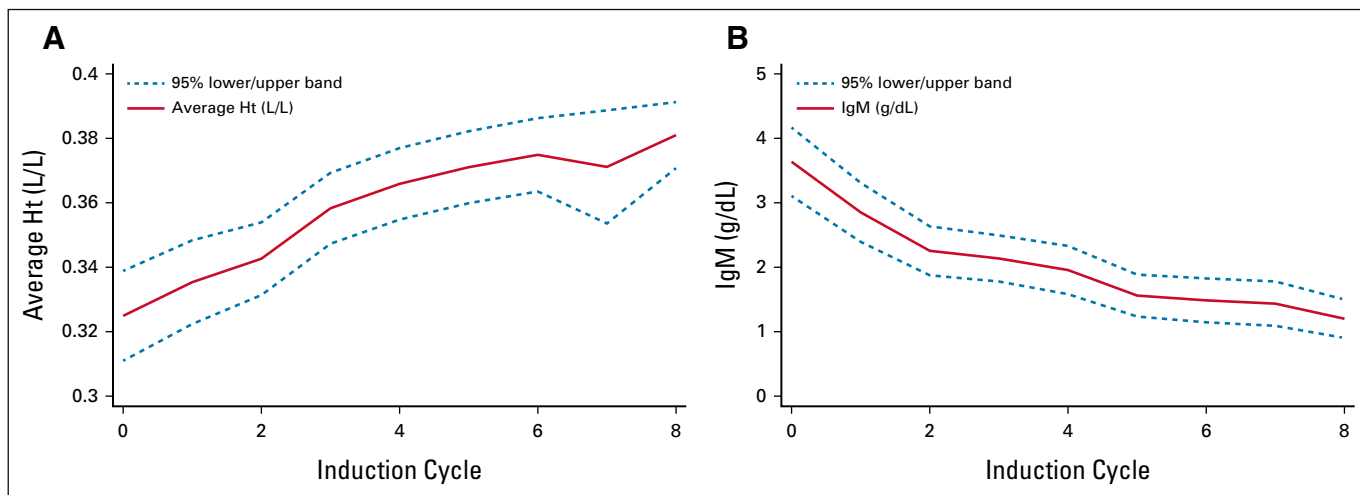


FIG 2. (A) Ht (L/L) profile over the first eight induction cycles for the included patients and (B) total serum IgM (g/dL) profile over the first eight induction cycles for the included patients. Ht, hematocrit; IgM, immunoglobulin M.

No statistically significant differences were found for PFS in the univariable analysis for baseline risk factors (ie, International Prognostic Scoring System for WM score; Data Supplement).

Safety

During induction, none of the patients experienced an IgM flare. In 34 patients, a cycle of IRD was delayed because of hematologic toxicity ($n = 6$), infusion-related reactions (IRRs) to IV rituximab ($n = 2$), neurotoxicity ($n = 5$), or other toxicity ($n = 21$). Grade 1 neurotoxicity and grade 2 infections, gastrointestinal disorders, and local reactions were common. Anemia grade 3 ($n = 4$), thrombocytopenia grade 2 ($n = 11$), grade 3 ($n = 4$), and grade 4 ($n = 3$), and neutropenia grade 3 ($n = 7$) and grade 4 ($n = 4$) were seen. SAEs occurring in $\geq 4\%$ of patients were infections ($n = 8$) and other conditions ($n = 7$) like dehydration, subarachnoid bleeding (because of trauma), and secondary malignancy. A complete overview of adverse events and SAEs is provided in the Data Supplement.

Patient-Reported Outcome Measures

Neuropathy. The QLQCIPN20 and Common Terminology Criteria for Adverse Events grading were obtained at baseline ($n = 57$), after cycle 4 ($n = 46$), and after cycle 8 ($n = 47$). Outcomes for the EORTC QLQ-CIPN20 are summarized in the Data Supplement. Mean scores at baseline were 10.2, 9.2, and 14.7 for the sensory, motor, and autonomic domains, respectively. When compared to scores at the end of induction for all subscales, the average change in means was not statistically significant ($P > .05$ for sensory, motor, and autonomic scales), demonstrating no increase in neuropathy-associated symptom burden during treatment.

Quality of life. A total of 57, 46, and 41 patients completed the EORTC QLQ-C30 questionnaire at baseline, after cycle 4, and after cycle 8, respectively. The mean scores from the

EORTC QLQ-C30 scales and items are summarized in the Data Supplement. Patients reported a significant improvement in all items of the functional scales ($P < .05$ for role, emotional, and social functioning) at the end of induction except for physical and cognitive functioning when compared with baseline. Overall, GHS significantly increased at the end of induction ($P = .01$), suggesting improvement in QOL.

Post Hoc Analyses: Assessment of BM Response and Molecular Analysis

Median BM involvement at baseline was 35% and after eight cycles decreased significantly to 12% ($P < .001$; Table 2 and Data Supplement). NGS was performed on BM biopsies and BM aspirates of 23 and 24 patients, respectively. In the BM biopsies, NGS demonstrated a *MYD88*^{L265P} in 23 of 26 patients (88%), with a median variant allele frequency (VAF) of 20.4% (range, 1.4%-46.5%) at baseline. In BM aspirates, a *MYD88*^{L265P} was present in 39 of 42 patients (93%) with a median VAF of 5.7% (range, 0.3-43.6%) at baseline. In the whole study group, *MYD88* and *CXCR4* mutations were present in 51 of 55 (93%) and 14 of 52 (27%) patients, respectively. *MYD88* and *CXCR4* mutation status was undetermined in four and seven patients, respectively. Median BM involvement in *MYD88*^{WT} versus *MYD88*^{L265P} patients was 10% versus 35%.

After eight cycles of IRD, the *MYD88*^{L265P} median VAF decreased from 20.4% to 8.0% ($P = .03$) and from 5.7% to 0% ($P = .05$) for BM biopsies and BM aspirates, respectively (Table 3). *MYD88*^{L265P} VAF determined by NGS on BM biopsies correlated strongly with the immunohistochemically estimated BM involvement (CD79⁺) at baseline ($r = 0.85$; $P < .001$), after cycle 4 ($r = 0.93$; $P < .001$), and after cycle 8 ($r = 0.97$, $P < .001$; Data Supplement). Patients with *MYD88*^{L265P}/*CXCR4*^{WT} and *MYD88*^{WT}/*CXCR4*^{WT} had the highest rates of VGPR and PR (47% and 33%) while no patient with *MYD88*^{L265P}/*CXCR4*^{MUT} achieved VGPR (Fig 1B).

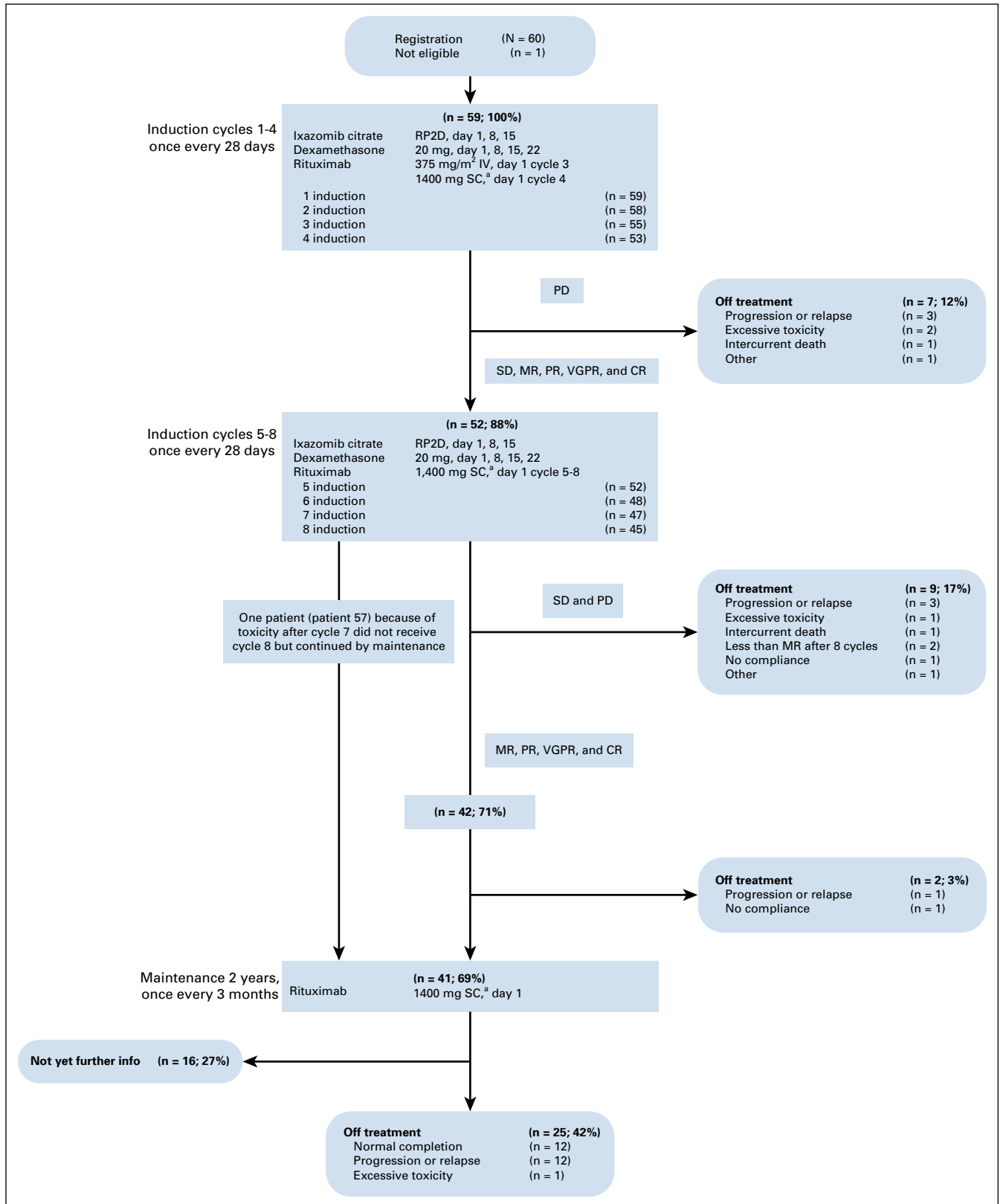


FIG 3. Flow diagram: number of patients going through the protocol treatment including reasons for exclusion. ^aPatients who for whatever reason do not tolerate the SC administration of rituximab can be treated with rituximab IV at the regular dose of 375 mg/m². CR, complete response; IV, intravenous; MR, minor response; PD, progressive disease; PR, partial response; RP2D, recommended phase II dose; SC, subcutaneous; SD, stable disease; VGPR, very good partial response.

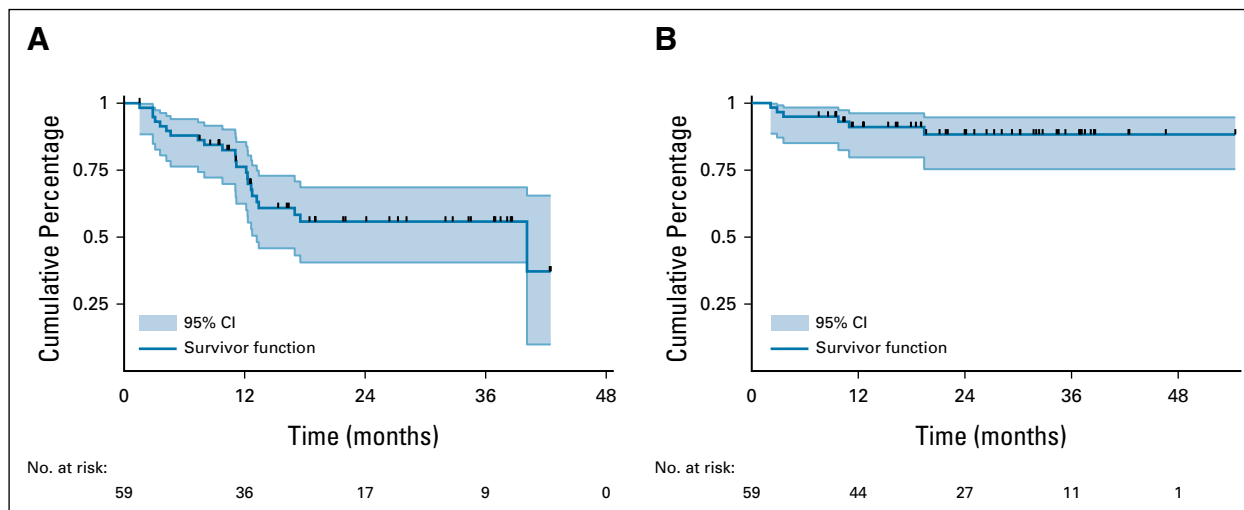


FIG 4. Kaplan-Meier curves for all 59 patients in the intention-to-treat analysis for (A) PFS and (B) OS measured from enrollment. OS, overall survival; PFS, progression-free survival.

Median PFS was not reached in both *MYD88^{L265P}/CXCR4^{WT}* and *MYD88^{WT}/CXCR4^{WT}* patients and was 36 months in *MYD88^{L265P}/CXCR4^{MUT}* patients. At 24 months, the PFS for the *MYD88^{L265P}/CXCR4^{WT}*, *MYD88^{L265P}/CXCR4^{MUT}*, and *MYD88^{WT}/CXCR4^{WT}* patients was 75% (95% CI, 61 to 92), 57% (95% CI, 36 to 90), and 67% (95% CI, 30 to 100), of which 9 (26.5%), 8 (57%), and one patients progressed, respectively (log-rank $P = .19$; Data Supplement). Although these results suggest an inferior outcome for *CXCR4^{MUT}* patients, statistical significant difference was not reached since the study was underpowered to detect such a difference.

DISCUSSION

In this international, prospective phase I/II study, we investigated the efficacy and safety of the IRD regimen in patients with RR WM. The current study is the first reporting on the use of ixazomib in RR WM and SC rituximab in WM. We observed a high ORR of 71% after eight cycles of IRD, with further improvement of response until month 12 (best ORR 85%) and a median DOR of 36 months. Median time to minor and major response was 4 and 5 months, respectively. In a previous phase II study of IRD in treatment-naive WM patients, a higher ORR of 96% was achieved, but with similar VGPR rates and DOR.^{10,11}

TABLE 2. Immunohistochemical Assessment of Bone Marrow Biopsies

Immunohistochemical Assessment	Median (range)			P^a
	Baseline	After Cycle 4	After Cycle 8	
Tumor cells (CD79a), %	35 (0-80)	20 (0-85)	12 (1-80)	< .001
		%Δ after cycle 4 -20 (-160 to 100)	%Δ after cycle 8 -45 (-50 to 95)	< .0001
B cells (CD20), %	30 (0-80)	8 (0-70)	1 (0-80)	< .001
		%Δ after cycle 4 -60 (-400 to 100)	%Δ after cycle 8 -85 (-14.3 to 100)	< .0001
PCs (CD138), %	8 (1-30)	8 (1-25)	5 (1-12)	.03
		%Δ after cycle 4 -25 (-233.3 to 83.3)	%Δ after cycle 8 -10 (-400 to 70)	.7
Plasmacytoid cells, %	7 (0-55)	5 (0-62)	5 (0-32)	.1
		%Δ after cycle 4 -33.3 (-500 to 100)	%Δ after cycle 8 -50 (-400 to 100)	< .0001
CD20/CD138 ratio	2.5 (0-80)	0.7 (0-23.3)	0.3 (0-23.3)	.07
		%Δ after cycle 4 -21.9 (-1,200 to +100)	%Δ after cycle 8 -83.5 (-242.9 to +100)	.002

Abbreviation: PC, plasma cell.

^a P value for cycle 8 compared with baseline.

TABLE 3. Molecular Analysis of Bone Marrow Biopsies and Bone Marrow Aspirates

Molecular Analysis Mutational Status	No. (%)
<i>MYD88</i> (L265P) mutation (n = 55)	51 (93)
<i>CXCR4</i> (n = 52)	14 (27)
Frameshift	1 (7)
Nonsense	13 (93)
<i>MYD88</i> ^{L265P} / <i>CXCR4</i> ^{WT}	34 (65)
<i>MYD88</i> ^{L265P} / <i>CXCR4</i> ^{MUT}	14 (27)
<i>MYD88</i> ^{WT} / <i>CXCR4</i> ^{WT}	4 (8)

NGS	No.	Median (range)			P ^a
		Baseline	After Cycle 4	After Cycle 8	
<i>MYD88</i> (L265P) VAF BM biopsy	23	20.4 (1.4-46.5)	18.1 (1.26-43)	8.0 (0-42.8)	.04
<i>MYD88</i> (L265P) VAF BM aspirate	24	5.7 (0.3-43.6)	2.2 (0.4-42.8)	0 (0-24.3)	.001
<i>CXCR4</i> load biopsy	24	21 (8-36)	9.6 (0-29.2)	12 (0-42)	.9
<i>CXCR4</i> load aspirate	24	3.9 (3.9-9.3)	6.9 (0.9-12.1)	0 (0-1.5)	.07
Cancer cell fraction	10	0.9 (0.4-1.8)	0.9 (0.3-1.2)	0.8 (0.3-1.1)	.3

Abbreviations: BM, bone marrow; NGS, next-generation sequencing; VAF, variant allele frequency.

^aP value for cycle 8 compared with baseline.

The study design also permitted evaluation of single-agent activity of ixazomib, an oral drug, being able to reduce IgM levels significantly after just two cycles, possibly contributing to the low rates of IgM flare after rituximab introduction.

We observed a 2-year PFS rate of 56% (95% CI, 41 to 69) and an OS of 88% (95% CI, 75 to 95), in a previously treated population with a median of two prior lines of therapy. Interestingly, 2-year PFS and OS rates were only slightly lower compared with the results of IRD in treatment-naïve WM patients. Similar to that study, we also found that PFS was not affected by *CXCR4* mutational status.¹⁰ Maintenance with ixazomib could be a promising approach to increase PFS, as indicated by a median PFS of 40 months after six IRD maintenance cycles in the aforementioned study.¹¹

Previous studies evaluating ixazomib in MM have shown low rates of PNP (12%-20%).^{8,26} In our study, new onset or worsening of pre-existing PNP occurred in 13 (22%) and 3 (5%) patients, respectively (Data Supplement), and recovered in most patients during follow-up. Using PROMs with a validated PNP-specific questionnaire, no increase in PNP-related symptoms was observed and thus ixazomib appears to compare favorably to bortezomib (incidence between 30% and 64%).²⁻⁷ We observed a relatively high incidence of grade 1 PNP, which was probably because of thorough and systematic evaluation of PNP using two different questionnaires at different time points. However, in contrast to bortezomib PNP, it did not lead to discontinuation of therapy or increase in symptom burden. The

improvement of QOL also underscores the tolerability of IRD. Nonetheless, the low rate of severe PNP-related symptom burden and improvement in QOL could potentially be biased because of patient selection as only 68% (40 of 59) and 69% (41 of 59) of patients completed the PNP and QOL questionnaires after eight cycles, respectively.

Patients with WM have a higher risk of sensitization to rituximab (up to 7%) than other lymphoma patients.¹⁷ In our study, two IRRs occurred after IV rituximab. During subsequent cycles, all patients received SC rituximab without IRRs. In addition, no patient developed rituximab intolerance. Thus, IRD proves a well-tolerated, convenient regimen for patients with RR WM as 81% of the patients completed at least six cycles.

Bruton's tyrosine kinase inhibitors have revolutionized the treatment of WM because of high response rates in both treatment-naïve and RR WM.^{27,28} However, in patients at risk for bleeding or cardiac complications, they may be poorly tolerated; long-term follow-up data of one of the pivotal studies demonstrated a 12.7% incidence of atrial fibrillation in relapsed WM.²⁹ Other retrospective studies outside clinical trials setting also indicated discontinuation rates for toxicity of about 15%.³⁰ Furthermore, 5-year PFS rate for all patients was 54%.²⁹ Therefore, there is a need for alternative chemotherapy-free fixed-duration regimens such as IRD.

The post hoc analyses comprised immunohistochemical and molecular evaluation. Using immunohistochemistry, we demonstrated that the PC population persisted in most patients at the end of induction, whereas the CD20⁺ B-cell

population had substantially decreased. Part of this decrease could, however, be a result of epitope masking by rituximab or internalization of the CD20:anti-CD20 complex.^{31,32} These findings are remarkable since PCs have been shown to be sensitive to ixazomib and other PIs.³³ A possible explanation for the lesser sensitivity of WM PC population, compared with MM PCs, is that WM PCs might have greater resemblance to normal B lymphocytes. This is supported by gene-expression studies indicating differences in WM PCs compared with MM and marginal zone lymphoma.^{34,35}

Our molecular analysis identified *MYD88*^{L256P} in 89% with a coexisting CXCR4 mutation in 26%, consistent with previous reports. We did not perform this analysis on CD19-selected cells but in DNA extracted from entire BM biopsies or aspirates.³⁶ The *MYD88* VAFs determined from analyzing BM biopsy extracted DNA strongly correlated with the

CD79a⁺ BM tumor infiltration but not when DNA derived from BM aspirates was used, presumably because of the varying composition of the aspirate. Our findings advocate for the use of DNA extracted from whole BM biopsies for mutational analysis in WM since it yields quantitative data concerning tumor load. This approach is more practical and feasible for most laboratories as it avoids the need for CD19 selection, which can only be done on fresh samples. However, a consistent decalcification method that is not impairing DNA quality of BM biopsies is imperative.

In conclusion, the IRD regimen with oral ixazomib and SC rituximab provides a patient-friendly and efficient treatment in patients with heavily pretreated WM, inducing high rates of response and respectable PFS with very good OS and, thus, could be an additional treatment option for patients with RR WM.

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DISCLAIMER

Study medication was provided by Takeda (ixazomib citrate), and Roche (rituximab). Takeda and Roche did not have any influence on the analysis of the data or the interpretation of the results.

EQUAL CONTRIBUTION

M.J.K. and K.A. are shared first authors.

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CLINICAL TRIAL INFORMATION

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DATA SHARING STATEMENT

The authors confirm that parts of the data supporting the findings of this study are available within the supplementary materials (eg, Study Protocol and Statistical Analysis Plan). The participant data that underlie the results reported in this study are available on request from the corresponding author, M.J.K. The data are not publicly available because of restrictions, for example, their containing information that could compromise the privacy of research participants.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**Combining Ixazomib With Subcutaneous Rituximab and Dexamethasone in Relapsed or Refractory Waldenström's Macroglobulinemia: Final Analysis of the Phase I/II HOVON124/ECWM-R2 Study**

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