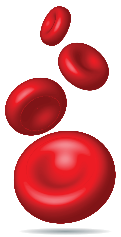
RESEARCH ARTICLE



Long-term results of the phase II trial of the oral mTOR inhibitor everolimus (RAD001) in relapsed or refractory Waldenstrom Macroglobulinemia

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Everolimus is an oral raptor mTOR inhibitor and has shown activity in patients with Waldenstrom's macroglobulinemia (WM). This study examines a large cohort of patients with relapsed/refractory WM with long-term follow up for survival. Patients were eligible if they had measurable disease, a platelet count >75,000 × 10^6/L, an absolute neutrophil count >1,000 × 10^6/L. Patients received everolimus 10 mg PO daily and were evaluated monthly. A success was defined as a complete or partial response (PR); minor responses (MR) were recorded and considered to be of clinical benefit. Sixty patients were enrolled and treated. The overall response rate (ORR) was 50% (all PR); the clinical benefit rate including MR or better was 73% (95% CI: 60–84%) with 23% MR. The median time to response for patients who achieved PR was 2 months (range, 1–26). The median duration of response has not been reached and median progression-free survival (PFS) was 21 months. Grade 3 or higher toxicities (at least possibly related to everolimus) were observed in 67% of patients. The most common grade 3 or 4 toxicities were anemia (27%), leukopenia (22%), and thrombocytopenia (20%). Other nonhematological toxicities were diarrhea (5%), fatigue (8%), stomatitis (8%) and pulmonary toxicity (5%). Everolimus has a high single-agent activity of 73% including MR, with a progression free survival of 21 months, indicating that this agent is active in relapsed/refractory WM.
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Introduction

Waldenstrom's Macroglobulinemia (WM) is an indolent lymphoplasmacytic lymphoma characterized by bone marrow infiltration with lymphoplasmacytic cells that produce an IgM monoclonal gammopathy. The clinical presentation is typically related to cytopenias and the consequences of serum hyperviscosity [1–4].

Although no mutations have been identified in the PI3K/mTOR pathway in WM, recent studies have shown that most patients harbor a a single nucleotide change in the myeloid differentiation primary response (MYD88) gene with a predicted nonsynonymous change at amino acid position 265 from leucine to proline (L265P) [5,6]. MYD88 is an adapter protein that mediates signal transduction for most toll-like receptors (TLRs) and leads to activation of NF-kB and MAPKs and production of proinflammatory cytokines [7]. TLR4-mediated signaling pathways including NFkB and MAP kinases also lead to rapid activation of PI3K which is involved in regulation of cell growth, apoptosis, and motility [7–9]. In addition, we, and others have shown that miR155 is upregulated in WM and can also lead to activation of the PI3K pathway [10–12]. Therefore, this pathway is highly activated in patients with WM [10–12].

Treatment options for patients with WM include rituximab, alkylating agents, nucleoside analogues and proteasome inhibitors [13,14]. However, despite these advances, patients with WM eventually experience tumor progression or develop toxicities that require a change in treatment. Everolimus (Afinitor™, Novartis Pharmaceuticals) is an mTOR (mammalian target of rapamycin) inhibitor that specifically targets the TORC1 protein complex. The mTOR pathway is critical for regulating cell metabolism, growth, cell survival, and angiogenesis [15–18]. mTOR kinase

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consists of two multiple protein complexes, TORC1 and TORC2. TORC1 is composed of three proteins, Raptor, PRAS40, and mLST8/GβL. The main mechanism of activation of TORC1 is through growth factor stimulation via the canonical PI3K-AKT-mTOR pathway [19]. TORC1 signals downstream to S6kinase and 4EBP:eIF4E leading to protein synthesis and cell cycle regulation.

Everolimus has shown activity in diseases that showed mutations in the mTOR pathway such as neurofibromatosis and tuberous sclerosis. This agent is also active in many carcinomas including renal cell carcinoma and hematological malignancies, specifically Hodgkin and non-Hodgkin lymphoma [20–22]. In 2009, we presented the results of a phase II study with single agent everolimus in 50 patients with relapsed or refractory WM. This trial showed a partial response (PR) rate of 42% (95%CI:26–55%) with a minimal response (MR) of 28% with overall response rate of MR or better of 70% (95%CI: 55–82%). Although the ORR to single-agent everolimus is impressive, the durability of these responses required long-term follow-up [23]. In this report, we present the results of 10 additional patients accrued to the trial and provide long-term follow for the 60 patients enrolled in this study.

Patients and Methods

This phase II study was conducted through a collaboration of the Mayo Clinic Cancer Center and Dana-Farber Cancer Institute and was approved by both Institutional Review Boards. All patients gave written consent. The trial was registered on clinicaltrials.gov NCT00436618. All authors had access to the primary clinical trial data. The data was analyzed by the department of Biostatistics at Mayo Clinic, Rochester, MN.

Patients were eligible for this trial if they had relapsed or refractory WM. Proof of relapse was required by a biopsy within 6 months prior to enrollment. Patients were required to have symptomatic disease that warrants therapy based on the consensus panel recommendations for therapy in WM [24,25].

There was no limit on the number of prior therapies. Patients were required to be ≥18 years old and have measurable disease. Measurable disease was defined as at least one lesion with a single diameter of >2 cm by computerized tomography or bone marrow involvement with >10% malignant cells and quantitative IgM monoclonal protein >1,000 mg dL^{-1}. Patients were to have a life expectancy of >3 months; Eastern Cooperative Oncology Group performance status of 0, 1, or 2; absolute neutrophil count (ANC) > 1,000 × 10^6/L; platelets > 75,000 × 10^6/L; hemoglobin > 8 g dL^{-1}; serum creatinine <2× the upper limit of normal (UNL); serum bilirubin < 2 UNL (if total bilirubin >2 then a direct bilirubin of <1.5 UNL was acceptable); AST ≤ 3 × ULN (≤ 5 × ULN if liver involvement is present). Patients could not have known HIV infection.

Patients were treated with a flat dose of 10 mg of everolimus orally in the fasting state. Treatment was daily and 4 weeks was considered one cycle. A complete blood count was performed each week during the first cycle and with each subsequent cycle. If the platelet count was >40,000 × 10^6/L and the ANC >1,000 × 10^6/L and there were no grade 3 or 4 nonhematological toxicities (NCI Common Toxicity Criteria version 3.0), the full dose of everolimus was prescribed for the next cycle. Patients who did not meet the re-treatment criteria had the dose held until recovery and followed by a stepwise dose modification to 5 mg daily, 5 mg every other day, and 5 mg every third day. Patients were not to receive prophylactic white blood cell growth factors to maintain dosing but could receive them at physician discretion if neutropenia developed. Erythropoietin treatment for anemia was also permitted at physician discretion.

Patients were restaged for tumor response after two and six cycles and every three cycles thereafter. Responses for WM were categorized using the Consensus recommendations for response [24,26]. However, progression was measured as a confirmed 25% increase in the monoclonal protein from baseline and not from nadir. Patients who progressed or had unacceptable toxicity at any time went off study. Patients with stable disease after six cycles continued treatment per MD discretion. Patients who had a CR on cycle 6 or later were to receive two cycles past CR and then could discontinue everolimus and be observed or could continue on drug per MD discretion. Patients with PR after six cycles continued until progression or toxicity.

Adverse events were graded using the NCI Common Toxicity Criteria (version 3.0). Toxicity was defined as an adverse event classified as being possibly, probably, or definitely related to study treatment.

Statistical design

This phase II study used a one-stage three-outcome design[28] to assess the efficacy and tolerability of everolimus in patients with WM. Although MR were to be reported, for statistical purposes a response was defined as either a CR or PR. Twenty-seven evaluable patients were required to test the null hypothesis that the true response rate for this regimen is at most 5% versus the alternative hypothesis that the true response rate is 20% or greater. The study had 82% power, with a 4% Type I error rate. A patient was considered evaluable for response if they were eligible and received treatment. At the time of the final analyses, a total of four or more responses were required in the first 27 evaluable patients to indicate that this regimen warrants further evaluation in this patient population. The response rate was estimated by the number of responses divided by the number of evaluable patients. A 95% exact binomial confidence interval for the true response rate was calculated assuming that the number of responses was binomially distributed.

Duration of response (DR) was defined as the time from the date of documented response to the date of progression. Time to progression (TTP) was defined as the time from the date of registration to the date of progression. Patients without disease progression were censored at the date of their last evaluation. If a patient died without documentation of disease progression, the patient was considered to have had disease progression at the time of death unless there was sufficient documented evidence to conclude that progression did not occur prior to death. Progression-free survival (PFS) was defined as the time from the date of registration to the date of progression or death due to any cause. Time to discontinuation of active treatment was defined as the time from the date of registration to the date the decision was made to take the patient off active treatment. Overall survival (OS) was defined as the time from the date of registration to the date of death resulting from any cause. Patients who were still receiving treatment at the time of these analyses were censored at the date of their last evaluation. The distributions of these time-to-event endpoints were each estimated using the Kaplan–Meier method [27]. The Wilcoxon rank sum test was used to evaluate the relationship between response status and patient characteristics (age, baseline hemoglobin, etc.).

Results

Patient characteristics

A total of 61 patients were enrolled on this trial from April 2006 to November 2009. One patient never received treatment and was classified as a cancel leaving 60 patients eligible for analysis (Table I). The patients were a typical population of relapsed WM with a median age of 63 years (range, 43–85) and a median number of 3 prior therapies (range, 1–11). All but two patients (97%) had received prior rituximab based therapy and 60% of patients had received prior alkylator based therapies. Fifty-nine percent of the patients were intermediate or high risk based on the international scoring system for WM (ISSWM) [28].

Clinical outcomes

Of the 60 patients who received therapy, 50% (30/60; 95% CI: 37–63%) of patients achieved a PR; there were no CR. In addition, 23% (14/60) of patients achieved an MR for an overall clinical benefit rate of 73% (95% CI: 60–84%). Stable disease occurred in 17% (10/60) patients and 5% (3/60) progressed on therapy without response (primary progression). Three patients went off study before the first response evaluation and were considered non-responders. One of these three patients died of disease and two refused further therapy. The median TTP, PFS, and OS for the entire study population are 25 months (95% CI: 13-not reached (NR)), 21 months (95% CI: 12–41), and median not reached (NR) (95% CI: 46-NR), respectively (Fig. 1). There was no difference in PFS between patients who achieved MR (median PFS 20 months, 95% CI: 10, 41) and those who achieved PR (median PFS not reached, 95%CI: 18, not reached), $P = 0.11$.

The median time to response for patients who achieved PR ($N = 30$) was 2 months (range, 1–26). The median duration of response for these patients has not yet been reached and 18 of these patients remain in response after a median follow up of 38 months (range, 3–68). There was no association between response status and age, hemoglobin level at baseline, or IgM level at baseline. Responders had lower beta 2 microglobulin values at baseline compared to nonresponders ($P = 0.04$).

All but two patients had a decrease in their serum IgM (Fig. 2A,B). The hemoglobin initially decreased, likely due to the myelosuppressive

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TABLE I. Baseline Characteristics of the 60 Patients

Characteristic	Number (%)
Age in years, median (range)	63 (43–85)
Sex, male	50 (83%)
Performance status	
0	36 (60%)
1	19 (32%)
2	5 (8%)
International scoring system for WM[a]	
Low	11 (41%)
Intermediate	8 (30%)
High	8 (30%)
Baseline IgM level in mg dL^{-1}, median (range)	3510 (323, 7670)
Baseline Serum M-Protein in g dL^{-1}, median (range)	2.0 (0.2, 10.5)
Baseline Hemoglobin in g dL^{-1}, median (range)	11.4 (8.1, 17.4)
Grade 0	10 (17%)
Grade 1 ($\geq$10 g dL^{-1} but less than normal)	36 (60%)
Grade 2 (8–<10 g dL^{-1})	14 (23%)
Baseline platelet count in 10^9/L, median (range)	242 (75, 494)
Bone marrow percent involvement, median (range)[b]	50 (0, 90)
Beta 2 microglobulin >3.0 mg dL^{-1}[a]	17 (63%)
B-Symptoms	15 (25%)
Nodal disease	43 (72%)
Number of extranodal sites	
0–1	51 (78%)
$\geq$2	9 (22%)
Number of prior therapy treatments	
Median (range)	3 (1–11)
1	15 (25%)
2	12 (20%)
3	6 (10%)
4	11 (18%)
$\geq$5	16 (27%)
Type of prior therapy	
Rituximab	58 (97%)
Alkylator (including cyclophosphamide, chlorambucil CHOP, CVP)	36 (60%)
Purine nucleoside analog (including Fludarabine, cladribine, pentostatin)	17 (28%)
Bortezomib	11 (18%)
Others (including thalidomide, sildenafil, imatinib, interferon, alemtuzumab, radioimmunotherapy, dexamethasone)	20 (33%)
Stem cell transplant	4 (7%)

[a]Thirty three patients are missing beta 2 microglobulin and international scoring system for WM.
[b]Two patients are missing bone marrow percent involvement.

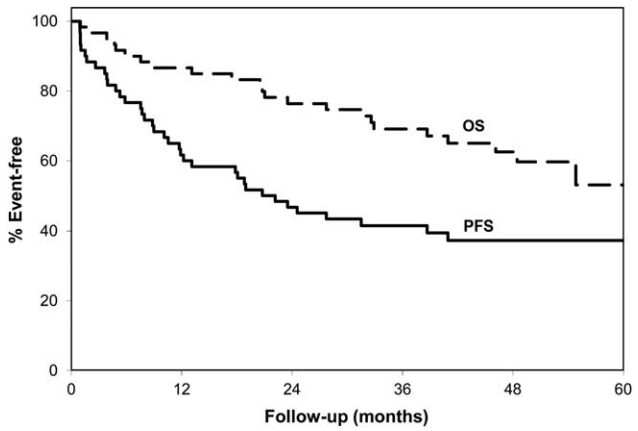

Figure 1. Kaplan–Meier curve of progression free-survival (PFS) and overall survival (OS) in 60 patients with relapsed Waldenstrom's macroglobulinemia treated with single-agent everolimus.

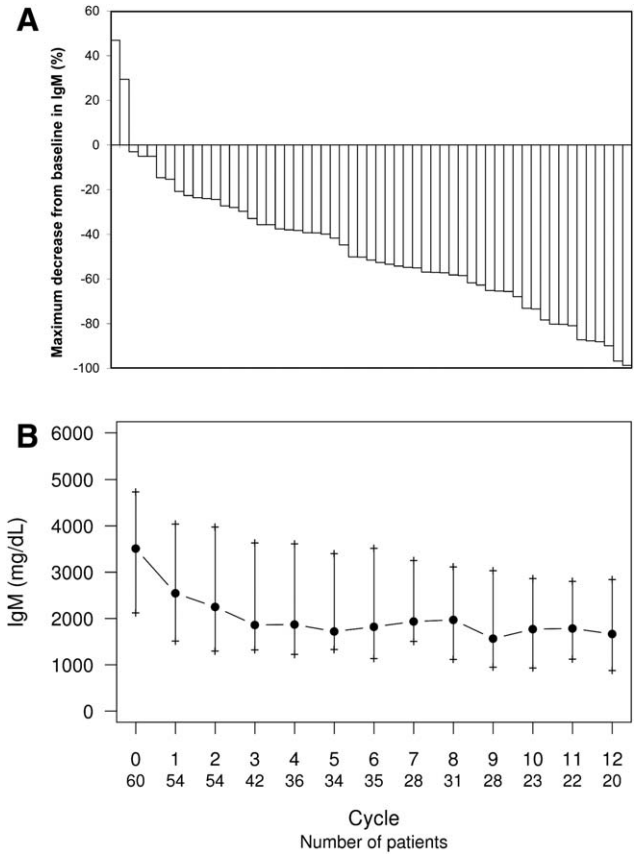

Figure 2. A: Maximum percent decrease from baseline in IgM over all cycles in response to everolimus per patient. B: Median and interquartile range for IgM values in response to everolimus per each cycle.

effect of everolimus, but then increased steadily with subsequent cycles after the anti-tumor effect became evident (Fig. 3).

Six patients remain on therapy after a median of 55 months (range, 49–70) of treatment; 54 patients have discontinued therapy with a median time to discontinuation of active treatment of 7 months (range: 0.4–57). To date, 53% (32/60) of patients have progressed and 38% (23/60) have died. Fifteen of the deaths were due to progressive WM. Other causes of death included sepsis (one patient), pneumonia (two patients), congestive heart failure (two patients), and intracranial hematoma (1 patient), which were felt to be all unrelated to everolimus. Two additional patients had an unknown cause of death. The median follow-up for the patients who remain alive is 49 months (range, 8–161).

Per the original protocol, progression was defined as a >25% increase in IgM over baseline on two measurements within a 1-month period. We also examined an alternative definition of progression, based on more recent criteria that define progression as a >25% increase over the lowest recorded IgM value on two consecutive measurements [29]. There were 25 patients who fit this second definition for progression while on active treatment after a median of 8 months (range, 2–62). Three of these patients progressed on the same day by both definitions. Eight patients progressed a median of 387 days (range: 113–588) earlier using the lowest recorded value compared to baseline. Fourteen of these patients have not progressed by an increase from baseline a median of 30 months (range, 2–49) after having a >25% increase over the lowest recorded value.

Safety and tolerability

Grade 3 or higher toxicities (adverse events considered at least possibly related to everolimus) were observed in 67% (40/60) of patients (Table II) and were primarily hematologic toxicity. There were 29 patients with grade 3 and 11 patients with grade 4 toxicities. Twenty percent of patients developed thrombocytopenia that was either grade 3 or grade 4. It should be recalled that patients could enroll on the trial with grade 1 thrombocytopenia and patients continued full dosing as long as the platelet count was at least 40,000 $\times$ 10^6/L on day 1 of each cycle. This likely explains the level of thrombocytopenia observed on this trial. Pulmonary toxicity did occur on this trial and was manageable. Five patients (8%) experienced pulmonary toxicities including three patients (5%) who had grade 3 pulmonary toxicity (1 pleural effusion and dyspnea, 2 dyspnea) and two patients who experienced grade 2 pulmonary toxicity. Everolimus was held and patients had improvement of symptoms with the addition of steroids and reduction in the dose of everolimus.

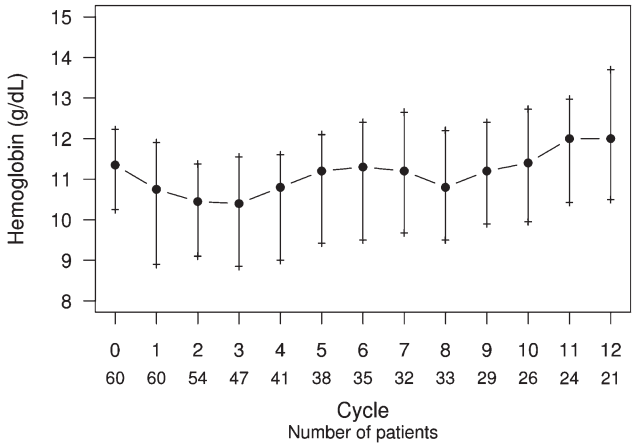

Figure 3. Median and interquartile range for hemoglobin values in response to everolimus per each cycle. The lowest hemoglobin value per patient for each cycle was used for this analysis.

Two thirds (40/60) of patients had dose reductions or treatment delays. Dose reductions due to toxicity occurred in 62% (37/60) of patients. Dose delays occurred in 40% (24/60) of patients and were mostly due to cytopenias. Patients were able to receive a median of two cycles of everolimus at full dose (range, 1–39). Seventy-eight percent (47/60) of patients received 10 mg daily for at least the first cycle of treatment; 12% (7/60) patients required dose reductions in cycle 1; and 7% (4/60) of patients went off study during cycle 1 (two patients refused further therapy due to toxicity; one refused without a reason; and one patient withdrew consent and elected hospice care). Two additional patients went off study after completing cycle one due to disease progression and death on study. Of the 45 patients who completed cycle one at the full dose level and continued treatment, 29 patients eventually required a dose reduction in subsequent cycles, and 19 patients had the treatment delayed due to either adverse events or hospitalization. Of the 37 patients who had dose reductions, 26 patients were reduced to 5 mg daily, 10 patients were reduced to 5 mg every other day, and 1 patient was reduced to 5 mg every third day. Responses were maintained after being dose reduced and in 10 patients a treatment response (4 PR and 6 MR) occurred after dose reductions.

Discussion

Significant advances have emerged in the understanding of the pathogenesis of WM in the last few years. Recent studies using whole

TABLE II. All Grade 3 and 4 Toxicities (Adverse Events at Least Possibly Related) to Everolimus

		Grade			
		3		4	
		N	%	*N*	%
Body system	Toxicity	15	25	1	2
Hematology	Anemia				
	Leukopenia	11	18	2	3
	Neutropenia	8	13	2	3
	Thrombocytopenia	4	7	8	13
Infection/febrile neutropenia	Febrile neutropenia	2	3		
	Pneumonia	2	3		
	Respiratory tract infection	1	2		
	Cellulitis infection	1	2		
	Upper airway infection	1	2		
	Sepsis	1	2		
Lymphatics	Edema: limb	1	2		
Metabolic/laboratory	Hypercholesterolemia	2	3		
	Hyperglycemia	2	3		
	Hypertriglyceridemia	1	2		
	Hypoglycemia	1	2		
	Hyponatremia	1	2		
Pain	Arthralgia	2	3		
	Headache			1	2
Pulmonary	Dyspnea	2	3		
	Pleural effusion with dyspnea	1	2		
Other	Squamous cell carcinoma malignancy	1	2		
Constitutional symptoms	Fatigue	4	7	1	2
Gastrointestinal	Diarrhea	3	5		
	Mucositis, oral ulcers	5	8		
	Mucositis, larynx	1	2		
Maximum overall grade		29	48	11	18

Maximal overall toxicity grade refers to the number of patients that had the respective grade toxicity across all toxicity types.

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genome sequencing as well as epigenetic studies have shown that the PI3K/mTOR pathway is highly activated in WM, possibly due to MYD88 mutation or miRNA-155 activation [5,6,10,12,30]. These results indicate that this rare lymphoplasmacytic lymphoma is dependent to the PI3K/mTOR pathway, despite the lack of specific mutations in the pathway itself. Preclinical studies confirmed indeed that mTOR inhibition with everolimus leads to significant cytotoxicity of WM cells even in the presence of the bone marrow microenvironment [12].

In this study, we examined the long-term progression-free survival and follow up of the patients on this study with a larger cohort of patients compared to the original report [31]. Overall, 50% of patients achieved a PR and 23% patients achieved an MR, with an overall clinical benefit rate of 73%. These results are significantly higher than other novel agents used to date as single agents in this disease type. The response rates observed with rituximab range from 30 to 50%, while those with bortezomib range from 22 to 48% partial responses.

The median TTP, PFS, and OS for the entire study population were 25 months (95% CI: 13-not reached; NR), 21 months (95% CI: 12–41), and NR (95% CI: 46-NR), respectively. The longest follow up of a clinical trial in WM was the updated follow-up of the Southwest Oncology Group Study 9003 (SWOGS9003), where the estimated 5 year progression free survival was 41% in patients who received fludarabine single agent [32]. In addition, 20% of patients had a 10-year event free survival. However, this was in previously untreated patients. In relapsed WM, our results with everolimus compare favorably with other single-agent trials. Rituximab produced a median TTP of 31 months in the study in ECOG, but only 14 months (range, 13–17) in the studies reported by Treon and Dimopoulos et al. [33–35]. The median TTP with bortezomib was short at a median of 6.6 months in the Waldenström Macroglobulinemia Clinical Trials Group (WMCTG) study [36]. The median PFS with perifosine in a similar patient population was 10.7 months [37], while most recently the median PFS with the pan-HDAC inhibitor panobionostat was 6.6 months (90%CI 5.5,14.8) [38]. Therefore, everolimus represents an agent that shows prolonged PFS comparable to single agent rituximab or other novel agents such as bortezomib or panobinostat in this patient population.

The overall tolerability of oral everolimus was acceptable. The primary toxicity was hematologic and a dose of 5 mg daily was the one most tolerable for patients to take on a long-term basis. Six patients remain on therapy after a median of 55 months (range, 49–70) of treatment. Pulmonary toxicity was typical for TORC1 inhibitors at a rate of 8% and responds to dose cessation or reduction.

Rapamycin analogues such as everolimus are TORC1 inhibitors and even at high concentrations do not completely inhibit TORC2 in most cells, and therefore cannot completely inhibit signalling downstream of this pathway, leading to loss of the feedback inhibitory circuit mediated by S6K, activation of Akt and enhanced survival and chemoresistance [39–48]. Indeed, 4EBP1 overexpression is a resistance factor in patients with mantle cell lymphoma treated with mTORC1 inhibitors [39–46]. New generations of TORC1 and 2 inhibitors are showing significant preclinical activity in WM and may prove to have higher responses in future clinical trials [49].

In summary, this study demonstrates that everolimus is an active therapeutic agent in patients with relapsed WM and produces durable responses. Further studies with this class of agents are warranted.

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