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Phase I/II Trial of Everolimus in combination with Bortezomib and Rituximab (RVR) in Relapsed/Refractory Waldenstrom Macroglobulinemia

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ABSTRACT

We examined the combination of the mTOR inhibitor everolimus with bortezomib and rituximab in patients with relapsed/refractory Waldenstrom Macroglobulinemia (WM) in a phase I/II study. All patients received 6 cycles of the combination of everolimus/rituximab or everolimus/bortezomib/rituximab followed by maintenance with everolimus until progression. Forty-six patients were treated; 98% received prior rituximab and 57% received prior bortezomib. No dose limiting toxicities were observed in the phase I. The most common treatment- related toxicities of all grades were fatigue (63%); anemia (54%); leucopenia (52%); neutropenia (48%) and diarrhea (43%). Thirty-six (78%) of 46 patients received full dose therapy (FDT) of the 3 drugs. Of these 36, two (6%) had CR (90% CI: 1 - 16). 32/36 (89%) of patients experienced at least a minimal response (90% CI: 76 - 96%). The observed PR or better response rate was 19/36 (53%, 90% CI: 38 - 67%). For the 36 FDT patients, the median progression-free survival was 21 months (95% CI: 12 – N/E). In summary, this study demonstrates that the combination of everolimus, bortezomib and rituximab is well tolerated and achieved 89% response rate even in patients previously treated, making it a possible model of non-chemotherapeutic based combination therapy in WM.

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Introduction

Waldenstrom's Macroglobulinemia (WM) is an indolent lymphoplasmacytic lymphoma characterized by the presence of lymphoplasmacytic cells in the bone marrow and an IgM monoclonal gammopathy(1, 2). Therapy is initiated when symptomatic disease occurs as in the presence of symptoms or signs of cytopenias, hyperviscosity or hepatosplenomegaly (3-6).

Treatment options for patients with WM include rituximab, alkylating agents, nucleoside analogues and proteasome inhibitors (7, 8). Indeed, bortezomib alone or in combination with rituximab has shown activity of about 80% and only 5% complete remission in relapsed WM (9-12). However, despite these advances, patients with WM eventually experience tumor progression or develop toxicities that require a change in treatment.

Recent studies have shown that about 80-90% of patients with WM have mutation in the single nucleotide change in the myeloid differentiation primary response (MYD88) gene with a non-synonymous change at amino acid position 265 from leucine to proline (L265P)(13, 14). MYD88 leads to activation of NF-kB and MAPKs signaling pathway mediated through toll-like receptors(15). MYD88 L265P can also activate the PI3K/AKT signaling pathway(16). In about 30% of patients, another mutation in the chemokine receptor CXCR4 also occurs and leads to activation of the PI3K/Akt/mTOR pathway along with other proliferation, survival and migratory pathways(17-19). Moreover, the PI3K pathway can also be activated by non-coding RNAs such as miR155 that is upregulated in many patients with WM (20-22). Together, these studies indicate that the PI3K/Akt/mTOR pathway is a critical target for therapy in WM(20-22).

Mammalian target of rapamycin (mTOR) inhibitors such as Everolimus (RAD001, Afinitor™, Novartis Pharmaceuticals) have shown activity in many cancers including those with specific mutations in the mTOR pathway such as neurofibromatosis and tuberous sclerosis or those with activation of this pathway such as in renal cell carcinoma and hematological malignancies(23-25). Everolimus specifically targets the TORC1 protein complex, which regulates metabolism, growth, cell survival and angiogenesis(26-29). The main mechanism of activation of TORC1 is through growth factor stimulation via the canonical PI3K-AKT-mTOR pathway(30). Indeed, we previously showed that everolimus activity in WM is partially regulated by miR155 levels in these cells(22).

Single agent everolimus has shown activity in patients with relapsed WM with a partial response (PR) rate of 42% and an overall response rate of 70%(31, 32). In vitro studies on WM cell lines demonstrated synergistic activity of everolimus, bortezomib and rituximab indicating that this combination could be active in patients with WM (22). Here, we describe the results of a phase I/II clinical trial testing the safety and activity of the combination of everolimus and

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rituximab or everolimus and bortezomib and rituximab and the data of the phase II trial of the combination of everolimus, bortezomib and rituximab (RVR) in patients with relapsed or refractory WM.

Study Design

This is a phase I/II study. The phase I portion of the study was to determine the maximum tolerated dose of everolimus, rituximab combination or everolimus, bortezomib, and rituximab combination, while the phase II portion was to evaluate the depth of responses to the everolimus, rituximab, and bortezomib combination. The study was conducted at Dana-Farber Cancer Institute, Boston, MA and Colorado Blood Cancer Institute, Denver, CO. The study enrolled patients between April 2010 and July 2013. The review boards of participating centers approved the study, which was conducted according to the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice. All patients provided written informed consent. This study is registered as a phase I/II study with ClinicalTrials.gov, NCT01125293.

Patients were eligible for this trial if they had relapsed or refractory WM. Patients were required to have symptomatic disease that warrants therapy based on the consensus panel recommendations for therapy in WM(33). Proof of relapse was required by IgM increase and a bone marrow biopsy within 28 days prior to enrollment.

There was no limit on the number of prior therapies. Subjects may have received prior rituximab but could not be refractory to rituximab (no response to rituximab for at least 3 months post last rituximab). Patients may have received prior bortezomib or everolimus. The patients could not have received cytotoxic chemotherapy $\leq$ 3 weeks prior or biological or targeted therapies $\leq$ 2 weeks prior to enrollment on the study. The last rituximab infusion must have been at least 3 months prior to the start of the protocol therapy. Patients were required to be ≥18 years old with lymphoplasmacytic CD20+ cells in the bone marrow, and measurable quantitative IgM monoclonal protein greater than normal value. Patients were to have a life expectancy of $\geq$ 3 months; Eastern Cooperative Oncology Group performance status of 0, 1, or 2; absolute neutrophil count (ANC) $\geq$ 1,000 x 10^6/L; platelets $\geq$ 75,000 x 10^6/L; hemoglobin $\geq$ 8 g/dL; serum creatinine $\leq$ 1.5x the upper limit of normal (ULN); serum bilirubin $\leq$ 2 ULN (if total bilirubin >2 then a direct bilirubin of <1.5 ULN was acceptable); AST ≤ 3 x ULN (≤ 5 x ULN if

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liver involvement is present); fasting serum cholesterol ≤ 300mg/dL, fasting triglycerides ≤ 2.5 ULN; fasting glucose ≤ 1.5 ULN, no severely impaired lung function with DLCO >50% of predicted value or 02 saturation of >88% on room air and no active bleeding. Patients with known HIV infection or active Hepatitis B infection or active infections or other active malignancies were not eligible.

Study treatment for the phase I and II studies

For the phase I, patients were assigned to a dose level in the order of study entry. In the dose-escalation scheme, everolimus was given at 5 or 10 mg with rituximab or with rituximab and bortezomib at 1.3 or 1.6 mg/m^2. Rituximab was given at a fixed dose of 375mg/m^2. In the phase II, patients received everolimus 10 mg flat dose PO daily, bortezomib IV 1.6mg/m^2 weekly on days 1, 8, 15 every 28 days and rituximab IV 375mg/ m^2 weekly on days 1, 8, 15 and 22 every 28 days on cycles 1 and 4 only. Treatment for 4 weeks (28 days) was considered one cycle. Patients received a total of 6 cycles followed by maintenance therapy with everolimus 10 mg PO daily until progression. Dexamethasone was not permitted. Patients were assessed every cycle while on the combination therapy and every 3 months while on maintenance therapy. Patients with stable disease (SD) or responding disease could continue therapy until progression. Patients discontinued therapy if they experienced PD, relapse, no further benefit, unacceptable toxicity, or by patient/investigator decision (see CONSORT diagram, Figure 1). Patients who came off study continued on event monitoring (every 3 months) until progression. Patients with rituximab-related IgM flare were not considered to have progressive disease.

Determination of the dose for the phase II study

Cohorts of three patients were sequentially enrolled at each dose level (everolimus and rituximab in dose levels 1 and 2 followed by the combination of everolimus, bortezomib, and rituximab in the next dose levels) with dose-escalation proceeding based on the dose-limiting toxicity (DLT) experienced during the first treatment cycle. At each cohort, three patients were treated. If one of the first three patients experienced a DLT, three additional patients were enrolled. Dose escalation occurred if none of the three patients in the cohort or one of the six patients in the cohort experienced DLT during the first treatment cycle if two or more patients experienced DLTs, dose escalation was halted. DLT was defined as any grade 3 or higher non-hematologic toxicity related to therapy or grade 4 hematological toxicity defined as platelets < 10x 10(9)/L on more than one occasion despite transfusion support, grade 4 neutropenia for

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more than 7 days and/or resulting in neutropenic fever (temperature ≥101°F), or inability to proceed to cycle 2 due to toxicity. Lymphopenia, a recognized side effect of bortezomib was not considered a DLT. The maximum tolerated dose (MTD) was defined as the dose level immediately below that at which two or more patients experienced DLT. Patients who experienced DLT could continue treatment if the toxicity resolved to grade 2 or lower. A 10-patient expansion cohort was included at the MTD.

Dose modifications

Dose modifications for attributable toxicities were allowed after the first cycle; a complete blood count was performed each week during the first cycle and with each subsequent cycle. If the platelet count was $\geq$40,000 x 10^6/L and the ANC $\geq$1000 x 10^6/L and there were no grade 3 or 4 non-hematological 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, and 5 mg every other day. Bortezomib could be reduced from 1.6 mg/m^2 to 1.3 mg /m^2 to 1.0 mg /m^2. No dose modifications were allowed for rituximab but it was omitted if there were significant rituximab reactions. No dose re-escalation was allowed. Patients received supportive therapy including erythropoietin and granulocyte colony-stimulating factor [G-CSF], as clinically indicated. For platelet count <10x10(9)/L or grade 3 thrombocytopenia with bleeding, both everolimus and bortezomib were omitted and platelet support was provided. If thrombocytopenia resolved to ≤ grade 2, that dose was held and treatment continued with the next planned dose, and both everolimus and bortezomib were resumed at the same dose. Varicella zoster prophylaxis with acyclovir or valacyclovir was recommended in all patients.

Efficacy and Safety Assessments

Toxicities were monitored throughout the study and for up to 30 days after the last dose of study drug. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0; Bethesda MD). Responses were determined at the end of every cycle using the International Waldenstrom Macroglobulinemia Workshop criteria and the new response criteria were adopted in the study in 2013. Therefore, the study was analyzed based on the original response criteria designed in the protocol and the new response criteria adopted in 2013(34).

MYD88L265P and CXCRWHIM mutation genotyping

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Genomic DNA was isolated from bone marrow smears obtained from patients with WM (n=20) using the QIAamp DNA Micro Kit (Qiagen, Valencia, CA, USA). All sample elutions were quantified using Quant-iT ™ PicoGreen® dsDNA Assay Kit (LifeTechnologies, Eugene, OR, USA) following manufacturer's protocol. Subsequently, allele-specific qRT-PCR for MYD88/L265P and CXCR4/C1013G was performed, as previously described(19). CT values ≥35 were considered as threshold for defining absence of the variant of interest. We did not perform sequencing for other CXCR4 mutations.

Statistical Analysis

The design of this phase I/II study is a standard 3+3 dose escalation design with 10 patients treated at MTD for the phase I (N=23) and a two-stage design for phase II with 23 patients treated on the first stage and additional 24 patients accrued if at least 2 response are observed among the first 23 patients. The design was selected to have high probability (0.90) of concluding the treatment to be effective when it is (defined as true VGPR or better response rate of 18%) and low probability (0.10) when not (true VGPR or better response rate of 5%). A total of 46 patients (23 phase I, 23 phase II) were eligible for analysis. Efficacy results are presented for all 46 patients, for the 36 patients who received the three-drug combination at the same dose (N=23 phase II, 10 phase I extension and 3 phase I dose escalation) and for the phase II patients only (N=23). Toxicity is reported for these groups as well as for the phase I patients who received the two-drug combination.

Exact binomial 90% confidence intervals (CIs) were reported. The Kaplan-Meier method was used to estimate distributions of DOR (time of first response to progression or death, censoring at the date patients were last known to be alive and disease-free for patients who had not progressed or died) and PFS (time of treatment initiation to progression or death, censoring as for DOR). At the data cutoff, 1 person has died and therefore, the overall survival is not reported. Patients were censored at the initiation of non-protocol therapy. The 95% CIs based on the log-log transformation were reported for TTE endpoints.

Post-hoc analyses were performed to evaluate response by prior treatment with everolimus or bortezomib and time to event endpoints by prior bortezomib among patients who received the three-drug combination. Statistical analyses were performed using R version 3.1.2. Data cut off was August 2014.

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Results

Patient Characteristics, Disposition and Determination of Maximum Tolerated Dose

From April 2010 to July 2013, a total of 46 patients were enrolled on this trial; of these, 23 patients were in the phase I study and 23 patients in the phase II study. Twenty-eight (61%) of the patients were intermediate or high risk based on the International Prognostic Scoring system for WM (IPSSWM) (35).

Baseline patient characteristics are detailed in Table 1; the median number of prior treatments in all the patients was 2 (range 1-9), with 37% of patients receiving 3 or more lines of therapy. Prior therapies received at any time point included: rituximab based therapy in 45/46 (98%) of patients, bortezomib-based therapy 26/46 (57%) and prior bortezomib/rituximab combination in 24/46 (54%). The median time from diagnosis to study entry was 50 months (range, 5-163).

The median IgM at the time of study entry was 3180 mg/dL (255-9020), with a median IgM of 3130 mg/dL (range, 289-6200) in the phase II patients. The median beta 2 microglobulin for all patients was 3.6 mg/L (range, 1.8 - 9.8).

Patient disposition is detailed in Figure 1. No DLTs were observed and therefore no expansion of cohorts occurred. The MTD was, therefore, determined to be everolimus 10 mg, bortezomib 1.6 mg/m^2 and rituximab 375 mg/m^2. Ten more patients were enrolled on the dose expansion of the MTD in the phase I trial. Another 23 patients were enrolled on the phase II portion of the protocol. A total of 36 patients received full dose therapy of all three agents (13 in the last dose escalation and the expanded phase I trial and 23 in the phase II trial). These 36 patients are termed Full dose therapy (FDT) in further analysis described below.

Drug Exposure and Patient Disposition

Per protocol, all patients on study were to receive six cycles of the combination of everolimus/bortezomib/rituximab (or everolimus/rituximab alone in stage A phase I patients) followed by maintenance with everolimus alone until progression. As of August 2014, the median duration of therapy received for all patients was 10 months (1 – 41), for patients who received FDT (N=36) was 10 months (1 - 37) and for phase II patients was 7 months (1 – 24).

Among the patients who received the two-drug combination the median number of cycles was 26 (3 - 44). Among the patients who received the three-drug combination the median

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number of cycles was 23 (1 – 39). For bortezomib, 25/40 (63%) of patients received all 6 cycles of bortezomib, with 16/23 (70%) of patients in the phase II study. Of the 40 patients who received bortezomib, there were 19 patients who had doses missed and a total of 14 who had dose reductions. Most cases of dose reductions were due to sensory peripheral neuropathy except for 3 patients: 1 for neutropenia, 1 for tinnitus, and 1 for nausea. For rituximab, a total of 36/46 (74%) patients received 2 cycles of rituximab per protocol with 27/36 (75%) of the patients at FDT. There were 15 patients (6 phase I and 9 phase II patients) who had rituximab doses held/interrupted. Most cases of dose interruption for rituximab were due to allergic rituximab reactions except for one case of infection and one for generalized fatigue/malaise. For everolimus, a total of 38/46 (82%) patients completed all 6 cycles of combination therapy with 29/36 (80%) of patients at FDT. There were 24/46 (52%) cases of dose reductions or dose interruption for everolimus. There were 7 (15%) patients who came off study due to toxicity, 6 (17%) of those were in the 36 FDT.

Safety and Tolerability

There were no DLTs observed on this study and no deaths occurred related to study drugs. Treatment-related AEs (≥25% frequency for grade 1 and 2, and all grade 3-5) are shown in Table 2. The most common toxicities in all patients on study were fatigue (29 patients, 63%); anemia (25 patients, 54%); leucopenia (24 patients, 52%); neutropenia (22 patients, 48%) and diarrhea (20 patients, 43%). Sensory peripheral neuropathy was reported in 19/46 (41%) patients, 11 in the phase I, and 8 in the phase II. Of these, only 2/46 (4.3%) cases were reported to be Grade 3 neuropathy; none occurred in the Phase II patients, and only one occurred in FDT patients. No grade 4 sensory peripheral neuropathy was observed.

In the phase II study, the most common treatment-related toxicities included cytopenias, specifically anemia and leucopenia that occurred in 10/23 (43%) and 11/23 (48%) patients, respectively; 7 (30%) patients experienced G3-4 anemia and 3 (13%) leucopenia. Other non-hematological treatment-related toxicities included 4% G3-4 hyperglycemia; G1-2 toxicities included diarrhea in 43%; hypertriglyceridemia in 39%; fatigue and sensory neuropathy in 34%; taste disturbance, edema and hyperglycemia in 30% and pneumonitis in 26%.

In patients who received the FDT, the most common treatment-related toxicities were cytopenias, specifically anemia and leucopenia, which occurred in 18/36 (50%) and 19/36 (53%), respectively, with 10 (28%) experiencing G3-4 anemia and 9 (25%) experiencing G3-4

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leucopenia. Other non-hematological treatment-related toxicities included upper airway infections in 21 patients (58%), fatigue (20, 55%), and neutropenia (17, 47%).

Response.

Responses are summarized in Table 3. The primary outcome of VGPR and CR response rate for all patients and for FDT patients was 2/46 (4%. 90% CI: 1 - 13) and 2/36 CR (6%, 90% CI: 1 -16) respectively. One of 23 phase II patients had CR (4%, 90% CI: 0 - 19).

Based on the new response criteria(34), among FDT patients, 32/36 (89%) of patients experienced at least a minimal response (90% CI: 76 - 96%). The observed PR or better response rate was 19/36 (53%, 90% CI: 38 - 67%). There were 2 CR, 17 PR, 13 MR, 3 SD, and one PD. For the phase II patients, the rate of MR or better occurred in 20/23 (87%, 90% CI: 70 - 96) with 1 CR, 13 PR, 6 MR with only 2 stable disease and one progression while on therapy. Overall 40/46 patients had a minimal or better response (87% 90% CI: 76 - 94) with 2 CR, 21 PR, 17 MR, 5 SD, and one PD. Similar results were observed when we analyzed the data based on M spike response with serum protein electrophoresis (Figure 3B).

All patients had a decrease in their serum IgM (Figures 2A and B). The hemoglobin initially decreased, likely due to the myelosuppressive effect of everolimus and bortezomib, but then increased steadily with subsequent cycles after the anti-tumor effect became evident (Figure 2C).

The median time to MR or better for 32/36 responding FDT patients was 64 days (95% CI: 28 – 399) and for 20/23 responding phase II patients was 60 days (95% CI: 28 – 211). Overall the median time to response for all 40 responders was 68 days (95% CI: 28 - 318). The median duration of MR or better response for both FDT and phase II patients has not been reached. For all 40 responding patients the median duration of response was 16 months (95% CI: 9 – N/E).

Time to event analysis

The median follow up for all patients was 15 months (range: 2 months-3.5 years). The total number of PFS events was 22 for all patients, 16 for FDT patients, and 9 for phase II patients.

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At data cut-off in August 2014, 9 patients remain on treatment having received a maximum of 42 cycles of therapy, 42 in the phase I study and 26 in the phase II study. In the phase I/II study, treatment was discontinued in the remaining patients due to PD (n=22, 48%), unacceptable toxicity (determined by the physician and the patient, n=7, 15%), withdrawal of consent (in which patients did not wish to continue on therapy for personal reasons that are not due to toxicity, n=3, 7%), death due to unrelated cardiac event (n=1, 2%), symptomatic deterioration (n=2, 4%), non-protocol therapy (n=1, 2%), and unspecified (n=1, 2%).

For all 46 patients on study, the median progression-free survival was 18 months (95% CI: 12 – N/E). For the 36 FDT patients, the median progression-free survival was 21 months (95% CI:12 – N/E), Figure 3. The median PFS time for phase II patients has not been reached. Overall, the one- and two-year probabilities of PFS were 65% (95% CI: 48 - 78) and 42% (95% CI: 26 - 58).

In a post-hoc analysis, we evaluated the outcome among patients who received prior bortezomib (refractory or not refractory). The median follow up of patients who have received prior bortezomib was 12 months (range, 2 months – 36 months). Among the 26 patients who received prior bortezomib, there were 13 PFS events with an estimated median PFS of 18 months (95% CI: 12 – N/E). One of 46 patients (2%) died while on study due to an unrelated cardiac event. This patient was on study for 24 months and had maintained stable disease while on treatment.

MYD88 and CXCR4 mutation analysis

Of the patients enrolled, there were 20 patient samples available for analysis. Of those, 16 (80%) were shown to have MYD88/L265P mutation only 1 (5%) had the CXCR4/C1013G mutation. The 4 wild type MYD88 patients had a CR, 2 MR and 1 SD. The patient with CXCR4/C1013G mutation also had MY88/L265P mutation and showed SD at best response. No correlation to response with MYD88/L265P was observed. Correlation with CXCR4/C1013G was not performed given that there was only 1 case with the mutation.

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Discussion

This study was designed to evaluate the safety and response rate of a 3-drug combination of targeted agents that are active in WM to develop a platform for a non-chemotherapy-based approach for patients with WM. This study was based on the single agent activity of everolimus, bortezomib and rituximab in patients with WM and based on the preclinical mechanisms of activity of these agents and synergistic activity in preclinical models allowing for the therapeutic targeting of both the PI3K and NF-kB pathways in WM(22).

The study proved to be safe with no DLTs observed with full dosing of the combination of everolimus, bortezomib and rituximab in patients with relapsed or refractory WM. The major grade 3 and 4 hematological toxicities were anemia and leucopenia (with neutropenia), while grade 3-4 thrombocytopenia was present in 11% of patients in both Phase I and II trials, indicating that the combination of agents that induce thrombocytopenia such as bortezomib and everolimus is achievable and manageable. Non-hematological toxicities were most significant for upper respiratory infections, diarrhea, pneumonitis and grade 1-2 peripheral neuropathy. Hyperglycemia and hypertriglyceridemia were also seen but most cases were grade 1 and 2 and related to everolimus. These were all expected toxicities of bortezomib or everolimus therapy.

Overall, the response rate for the 3-drug combination was 89% indicating that this combination is highly effective in achieving a response despite that 98% of patients received prior rituximab therapy and 57% received bortezomib-based therapy with 54% receiving prior bortezomib/rituximab combination. Potentially, the addition of everolimus can be useful in enhancing response or overcoming resistance to prior therapy with bortezomib or rituximab. In addition, everolimus can cross the blood brain barrier and therefore may be useful in combination therapy in patients with systemic and central nervous system disease with Bing-Neel syndrome. However, further studies recruiting patients with Bing-Neel syndrome on this combination are required to further prove this hypothesis.

Recent studies using whole genome sequencing have shown that MYD88 and CXCR4 mutations are critical regulators of response to novel therapeutic agents in WM. Previous studies using the BTK inhibitor Ibrutinib have shown that patients with nonsense and frameshift CXCR4 mutations are more likely to be resistant to single agent Ibrutinib(36). Here, we show that patients with and without MYD88 mutation might respond similarly to the combination of 3-targeted agents. There was only once case of CXCR4/C1013G mutation and therefore, correlation with response was not performed. We did not perform CXCR4 sequencing to identify

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other CXCR4 mutations and therefore cannot determine whether a higher frequency of CXCR4 mutations could have correlated with response/resistance in this study.

Novel agents that have been developed since the initiation of this study and include the BTK inhibitor Ibrutinib(36, 37). Future studies to examine whether everolimus should be combined with ibrutinib or whether ibrutinib should be added to this 3 drug-combination to enhance response and overcome resistance could be examined. In addition, everolimus is a TORC1 inhibitor and does not inhibit TORC2 even at high concentrations, indicating that Akt activation can still occur in these cells and can be a mechanism of resistance(22). Potentially, agents that target the PI3K pathway upstream of TORC1 or inhibit both TORC1 and 2 might show better activity and deeper responses in patients with WM. These include new PI3K inhibitors and TORC1 and 2 dual inhibitors among others.

In summary, this study demonstrates that the combination of everolimus, bortezomib and rituximab is well tolerated and achieved a high response rate even in patients previously treated with rituximab or bortezomib. This may be considered one of the models of non-chemotherapeutic based combinations in WM. Further studies to confirm those results in larger trials or in the upfront setting are warranted.

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Author contribution:

Irene M. Ghobrial: Initiated and wrote the clinical trial and was the principal investigator at DFCI. She conducted the clinical trial, analyzed the data and wrote the manuscript, Robert Redd: performed the statistical analysis; Philippe Armand: enrolled patients and reviewed the manuscript; Erica Boswell: entered the data and analyzed the data and reviewed the manuscript; Stacey Chuma: was the research nurse of the study; Antonio Sacco: performed the mutation studies for MYD88 and CXCR4, Kimberly Noonan: assessed patients during therapy and follow up; Ranjit Banwait: entered the data and analyzed the data and reviewed the manuscript; Courtney Hanlon: entered the data and analyzed the data and reviewed the manuscript; Houry Leblebjian: pharmacist of the study and reviewed treatment and dose modifications of the study; Diane Warren: project manager of the clinical study; Megan MacNabb: assistant project manager, managed the regulatory components of the study; Aldo Roccaro: performed the mutation studies for MYD88 and CXCR4; Daisy Huynh: performed the mutation studies for MYD88 and CXCR4; Adriana-Perilla-Glenn performed the mutation studies for MYD88 and CXCR4; Patrick Henrick: entered the data and analyzed the data and reviewed the manuscript; Jorge Castillo: cared for patients on the study and reviewed the manuscript; Jacob Laubach: enrolled patients and reviewed the manuscript; Claudia Paba-Prada: enrolled patients and reviewed the manuscript; Kenneth C. Anderson: input in the design and conduct of the study reviewed the manuscript; Paul G. Richardson: input in the design and conduct of the study and reviewed the manuscript; Jeffrey Matous enrolled patients and reviewed the manuscript; Edie Weller: performed the statistical analysis ; Steven P. Treon: input in the design and conduct of the study, enrolled patients and reviewed the manuscript.

Conflicts of Interest Disclosure:

Irene M.Ghobrial: Consultant for Novartis, Millennium, Celgene and BMS.

Philip Armand: funding BMS, Merck, Otsuka, Sigma Tau, Sequenta; consultancy Merck

Paul Richardson: Consultant for Novartis, Millennium and Celgene

The rest of the authors declare no conflicts of interest.

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Figure legends:

Figure 1: Consort diagram showing the distribution of patients in the phase I and II trials.

Figure 2:

A: Maximum percent decrease from baseline in IgM over all cycles in response to therapy per patient. **B**: Median and interquartile range for IgM values in response to RVR per each cycle in the FDT patients, N=36**. C:** Median and interquartile range for hemoglobin (Hb) values in response to RVR per each cycle in the FDT patients, N=36. The lowest hemoglobin value per patient for each cycle was used for this analysis.

Figure 3: Kaplan Meier curve of progression free-survival (PFS). Events are defined as first progression or death from any cause. For the 36 FDT patients, the median progression-free survival was 21 months (95% CI: 12 – N/E). Patients are censored (vertical tick mark) otherwise.

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Table 1: Patient baseline characteristics for all patients on study and by phase.

Patient baseline characteristics for all patients on study, full dose therapy (FDT), phase II.

		Sub-population	
	All patients	**FDT**	**Phase II**
	n = 46	**n = 36**	**n = 23**
Age at registration			
Median (range)	64 (48 - 84)	65 (48 - 84)	66 (49 - 84)
Sex			
Male	26 (57%)	20 (56%)	12 (52%)
Race			
Black or African American	1 (2%)	0 (0%)	0 (0%)
Other	1 (2%)	1 (3%)	1 (4%)
White	44 (96%)	35 (97%)	22 (96%)
Disease Status			
Refractory	2 (4%)	2 (6%)	2 (9%)
Relapsed	15 (33%)	14 (39%)	6 (26%)
Relapsed and Refractory	29 (63%)	20 (56%)	15 (65%)
ECOG PS			
0	34 (74%)	24 (67%)	13 (57%)
1	9 (20%)	9 (25%)	7 (30%)
2	3 (7%)	3 (8%)	3 (13%)
IPSSWM			
Low	18 (39%)	14 (39%)	6 (26%)
Intermediate	21 (46%)	18 (50%)	13 (57%)
High	7 (15%)	4 (11%)	4 (17%)
No. of prior therapies			
Median (range)	2 (1 - 9)	2 (1 - 9)	2 (1 - 9)
1	12 (26%)	11 (31%)	8 (35%)
2	17 (37%)	14 (39%)	7 (30%)
3	5 (11%)	3 (8%)	3 (13%)
4 +	12 (26%)	8 (22%)	5 (22%)
Prior rituximab			
Yes	45 (98%)	35 (97%)	22 (96%)
Prior bortezomib			
Yes	26 (57%)	20 (56%)	13 (57%)
Prior rituximab/bortezomib combination			
Yes	25 (54%)	19 (53%)	12 (52%)
Time from initial diagnosis to study entry (months)			
Median (range)	63 (5 - 251)	63 (5 - 251)	64 (5 - 251)

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Table 1-Baseline Patient Characteristics-Continued

Table 1: Baseline patient characteristics-continued.

	All patients	Sub-population	
		FDT	Phase II
	N = 46	N = 36	N = 23
IgM (mg/dL)			
Median (range)	3,180 (255 - 9,020)	2,980 (255 - 6,200)	3,130 (289 - 6,200)
Electrophoresis, M-spike			
Median (range)	2.0 (0.3 - 6.8)	1.9 (0.3 - 3.7)	1.9 (0.3 - 3.7)
Kappa (mg/L)			
Median (range)	31.6 (1.2 - 1470.0)	32.0 (1.2 - 1470.0)	32.0 (1.2 - 668.0)
Lambda (mg/L)			
Median (range)	4.0 (1.0 - 395.0)	6.2 (1.2 - 395.0)	4.8 (1.4 - 395.0)
Kappa/lambda ratio			
Median (range)	7.6 (0.0 - 1176.0)	7.6 (0.0 - 1176.0)	7.8 (0.0 - 160.6)
Albumin (g/dL)			
Median (range)	3.8 (2.8 - 4.7)	3.8 (3.0 - 4.7)	3.7 (3.0 - 4.7)
β-2 microglobulin (mg/L)			
Median (range)	3.6 (1.8 - 9.8)	3.6 (1.8 - 9.8)	3.7 (2.1 - 9.8)
Hemoglobin (g/dL)			
Median (range)	11.1 (7.3 - 15.6)	11.1 (7.3 - 15.6)	10.7 (7.3 - 14.4)
LPL cell in bone marrow (%)			
Median (range)	70 (5 - 95)	60 (10 - 95)	70 (20 - 95)

No. of patients with missing values: kappa (n=1), lambda (n=2), β-2 microglobulin (n=1), LPL (n=5).

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Table 2: All toxicities of Phase I and phase II. of toxicity. All G 3 and 4 toxicity and 10% or more G1 and 2. Highest grade for each toxicity for each patient is reported.

Toxicities for all patients on study, full dose therapy (FDT), phase II. Highest grade for each toxicity for each patient is reported.

	All patients (N=46)				FDT (N=36)				Phase II (N=23)			
	Grade 1/2	Grade 3	Grade 4	All grades	Grade 1/2	Grade 3	Grade 4	All grades	Grade 1/2	Grade 3	Grade 4	All grades
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Worst overall grade across all toxicities	*13*	*19*	*14*	*46*	*11*	*16*	*9*	*36*	*9*	*11*	*3*	*23*
Fatigue	29 (63%)	-	-	29 (63%)	20 (56%)	-	-	20 (55%)	8 (35%)	-	-	8 (35%)
Upper airway/sinus and lung	26 (57%)	3 (7%)	-	29 (63%)	18 (50%)	3 (8%)	-	21 (58%)	12 (52%)	2 (9%)	-	14 (61%)
Hemoglobin	12 (26%)	12 (26%)	1 (2%)	25 (54%)	8 (22%)	9 (25%)	1 (3%)	18 (50%)	3 (13%)	6 (26%)	1 (4%)	10 (43%)
Leukocytes	10 (22%)	11 (24%)	3 (7%)	24 (52%)	10 (28%)	7 (19%)	2 (6%)	19 (53%)	8 (35%)	2 (9%)	1 (4%)	11 (48%)
Neutrophils	9 (20%)	7 (15%)	6 (13%)	22 (48%)	9 (25%)	4 (11%)	4 (11%)	17 (47%)	7 (30%)	3 (13%)	-	10 (43%)
Diarrhea	20 (43%)	-	-	20 (43%)	16 (44%)	-	-	16 (44%)	10 (43%)	-	-	10 (43%)
Neuropathy-sensory	17 (37%)	2 (4%)	-	19 (41%)	15 (42%)	1 (3%)	-	16 (44%)	8 (35%)	-	-	8 (35%)
Pneumonitis/pulmonary infiltrates	18 (39%)	1 (2%)	-	19 (41%)	12 (33%)	1 (3%)	-	13 (36%)	6 (26%)	-	-	6 (26%)
Cough	15 (33%)	1 (2%)	-	16 (35%)	8 (22%)	1 (3%)	-	9 (25%)	4 (17%)	-	-	4 (17%)
Hyperglycemia	13 (28%)	2 (4%)	-	15 (33%)	13 (36%)	1 (3%)	-	14 (39%)	7 (30%)	1 (4%)	-	8 (35%)
Dyspnea	13 (28%)	-	-	13 (28%)	11 (31%)	-	-	11 (31%)	4 (17%)	-	-	4 (17%)
Muco/stomatitis	13 (28%)	-	-	13 (28%)	8 (22%)	-	-	8 (22%)	2 (9%)	-	-	2 (9%)
Edema	12 (26%)	-	-	12 (26%)	11 (31%)	-	-	11 (31%)	8 (35%)	-	-	8 (35%)
Hypertriglyceridemia	11 (24%)	-	1 (2%)	12 (26%)	11 (31%)	-	-	11 (31%)	9 (39%)	-	-	9 (39%)
Taste disturbance	12 (26%)	-	-	12 (26%)	11 (31%)	-	-	11 (31%)	7 (30%)	-	-	7 (30%)
Platelets	4 (9%)	1 (2%)	6 (13%)	11 (24%)	4 (11%)	1 (3%)	4 (11%)	9 (25%)	3 (13%)	1 (4%)	2 (9%)	6 (26%)
Allergic reaction	10 (22%)	-	-	10 (22%)	10 (28%)	-	-	10 (28%)	7 (30%)	-	-	7 (30%)
Head/headache	10 (22%)	-	-	10 (22%)	5 (14%)	-	-	5 (14%)	1 (4%)	-	-	1 (4%)
Hypercholesterolemia	9 (20%)	-	-	9 (20%)	9 (25%)	-	-	9 (25%)	8 (35%)	-	-	8 (35%)
Hypophosphatemia	7 (15%)	2 (4%)	-	9 (20%)	7 (19%)	2 (6%)	-	9 (25%)	7 (30%)	2 (9%)	-	9 (39%)
Fever w/o neutropenia	8 (17%)	-	-	8 (17%)	6 (17%)	-	-	6 (17%)	3 (13%)	-	-	3 (13%)
Nausea	8 (17%)	-	-	8 (17%)	6 (17%)	-	-	6 (17%)	3 (13%)	-	-	3 (13%)
Rash/desquamation	8 (17%)	-	-	8 (17%)	5 (14%)	-	-	5 (14%)	2 (9%)	-	-	2 (9%)
Pruritus/itching	6 (13%)	-	-	6 (13%)	5 (14%)	-	-	5 (14%)	2 (9%)	-	-	2 (9%)
Urinary tract	6 (13%)	-	-	6 (13%)	4 (11%)	-	-	4 (11%)	2 (9%)	-	-	2 (9%)

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Table 3: Categorical response for phase I, phase II patients and the 36 patients with Full dose therapy (FDT)

Table 3A: Response for all, 36 with full dose treatment (FDT), and phase II patients.

	All patients*	**FDT**	**Phase II**
	n = 46	**n = 36**	**n = 23**
CR	2 (4%)	2 (6%)	1 (4%)
PR	21 (46%)	17 (47%)	13 (57%)
MR	17 (37%)	13 (36%)	6 (26%)
SD	5 (11%)	3 (8%)	2 (9%)
PD	1 (2%)	1 (3%)	1 (4%)
VGPR or better	2 (4%, 90% CI: 1 - 13)	2 (6%, 90% CI: 1 - 16)	1 (4%, 90% CI: 0 - 19)
PR or better	23 (50%, 90% CI: 37 - 63)	19 (53%, 90% CI: 38 - 67)	14 (61%, 90% CI: 42 - 78)
MR or better	40 (87%, 90% CI: 76 - 94)	32 (89%, 90% CI: 76 - 96)	20 (87%, 90% CI: 70 - 96)

***** These include patients treated with everolimus and rituximab alone (N=6)

** Response assessed by Owen et al, 2013 (35)

Table 3B: M-spike response by SPEP for all, 36 with full dose treatment (FDT), and phase II patients.

	All patients*	**FDT**	**Phase II**
	n = 46	**n = 36**	**n = 23**
CR	2 (4%)	2 (6%)	1 (4%)
PR	22 (48%)	21 (58%)	15 (66%)
MR	12 (26%)	7 (19%)	3 (13%)
SD	9 (20%)	5 (14%)	3 (13%)
PD	1 (2%)	1 (3%)	1 (4%)
VGPR or better	2 (4%, 90% CI: 1 - 13)	2 (6%, 90% CI: 1 - 16)	1 (4%, 90% CI: 0 - 19)
PR or better	24 (52%, 90% CI: 39 - 65)	23 (64%, 90% CI: 49 - 77)	16 (70%, 90% CI: 50 - 85)
MR or better	36 (78%, 90% CI: 66 - 88)	30 (83%, 90% CI: 70 - 92)	19 (83%, 90% CI: 65 - 94)

***** These include patients treated with everolimus and rituximab alone (N=6)

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Figure 1

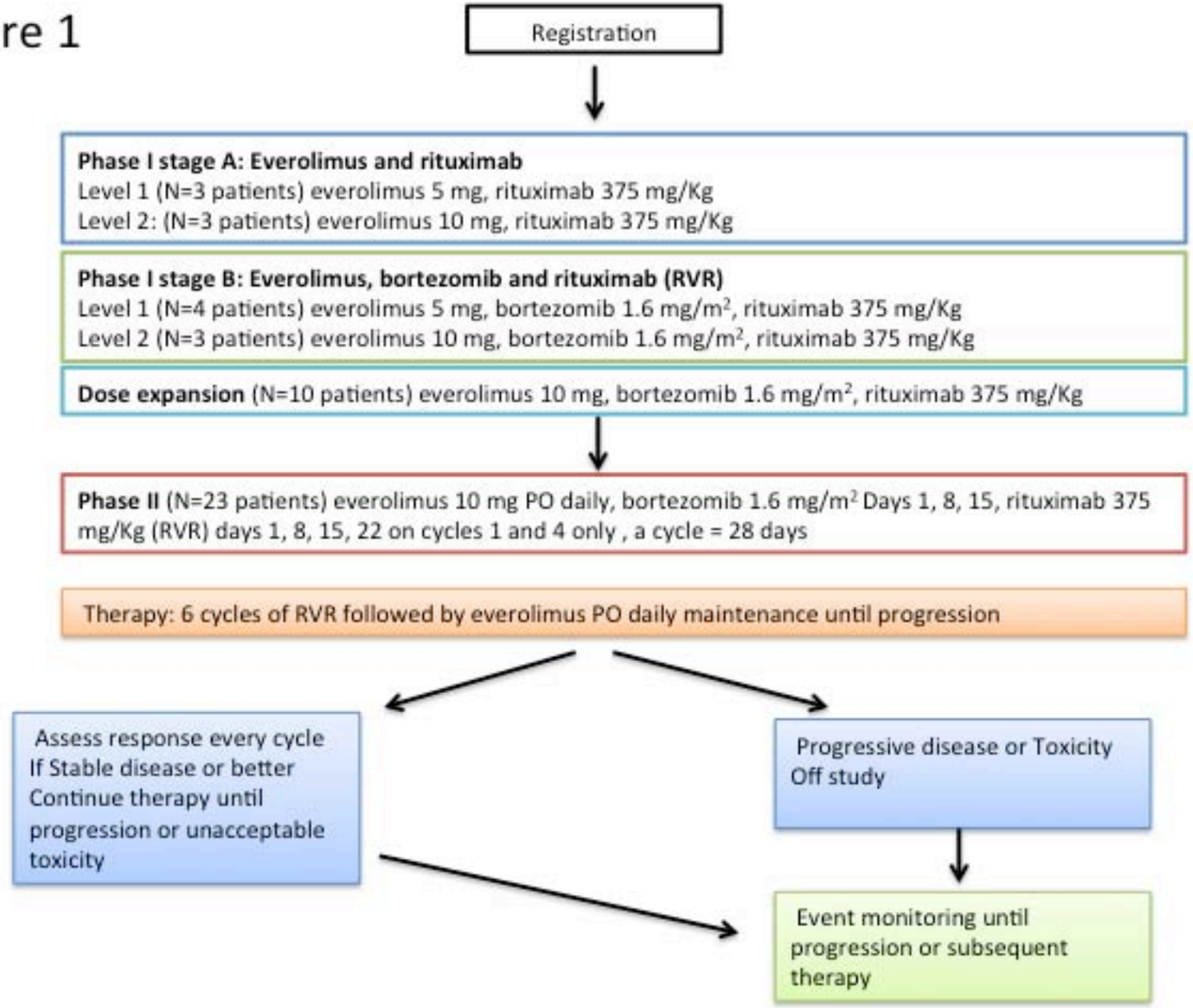

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Figure 2A

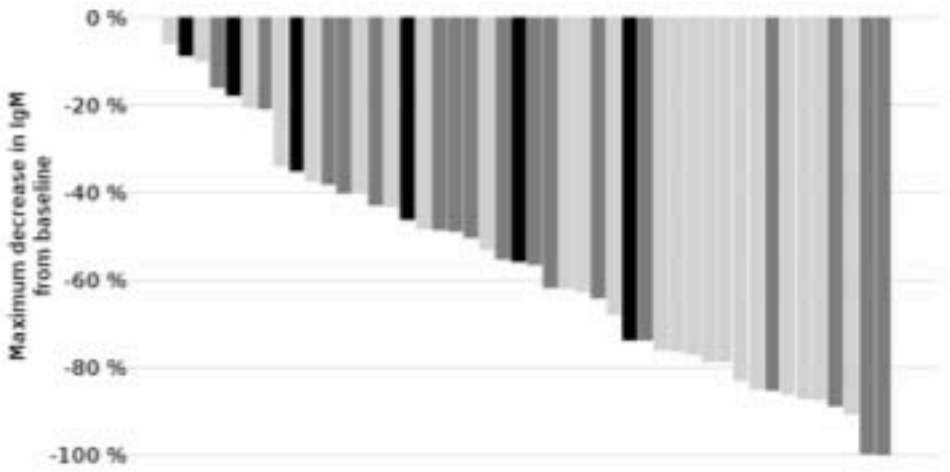
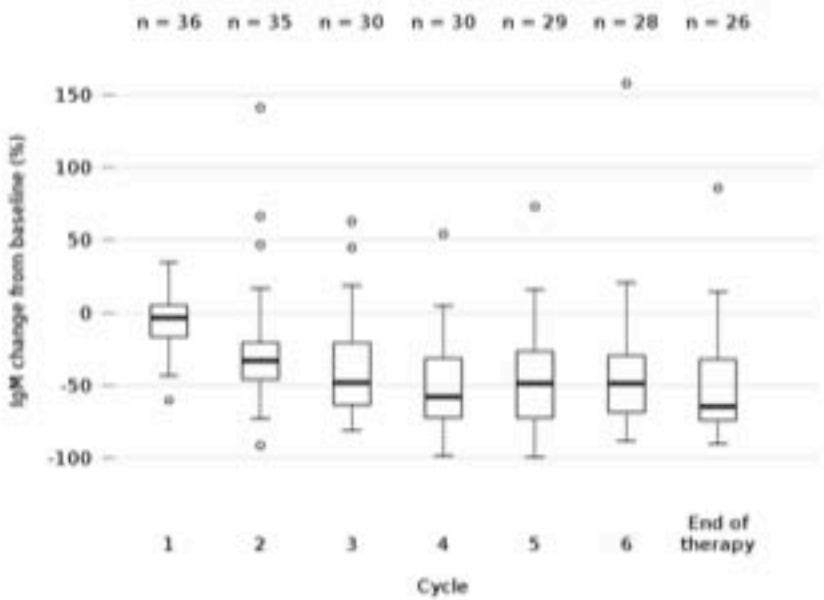


Figure 2B



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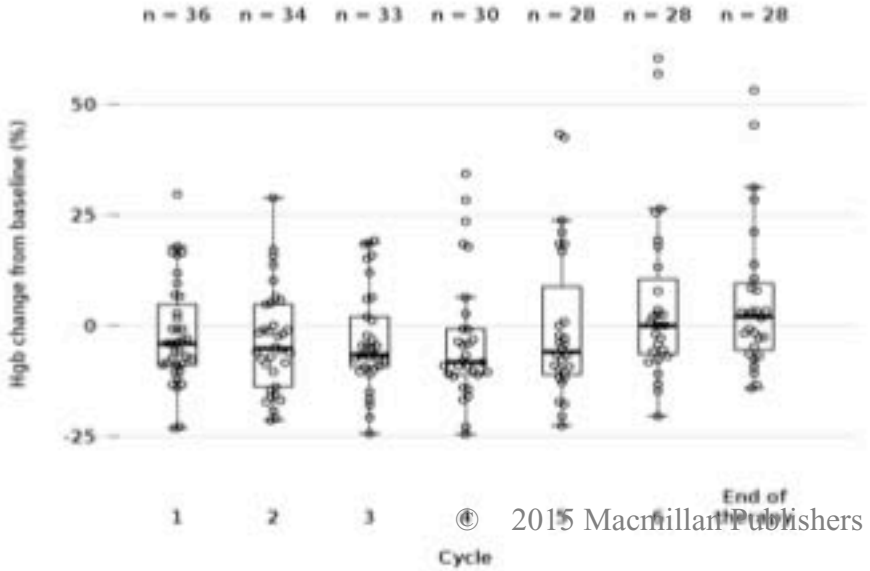

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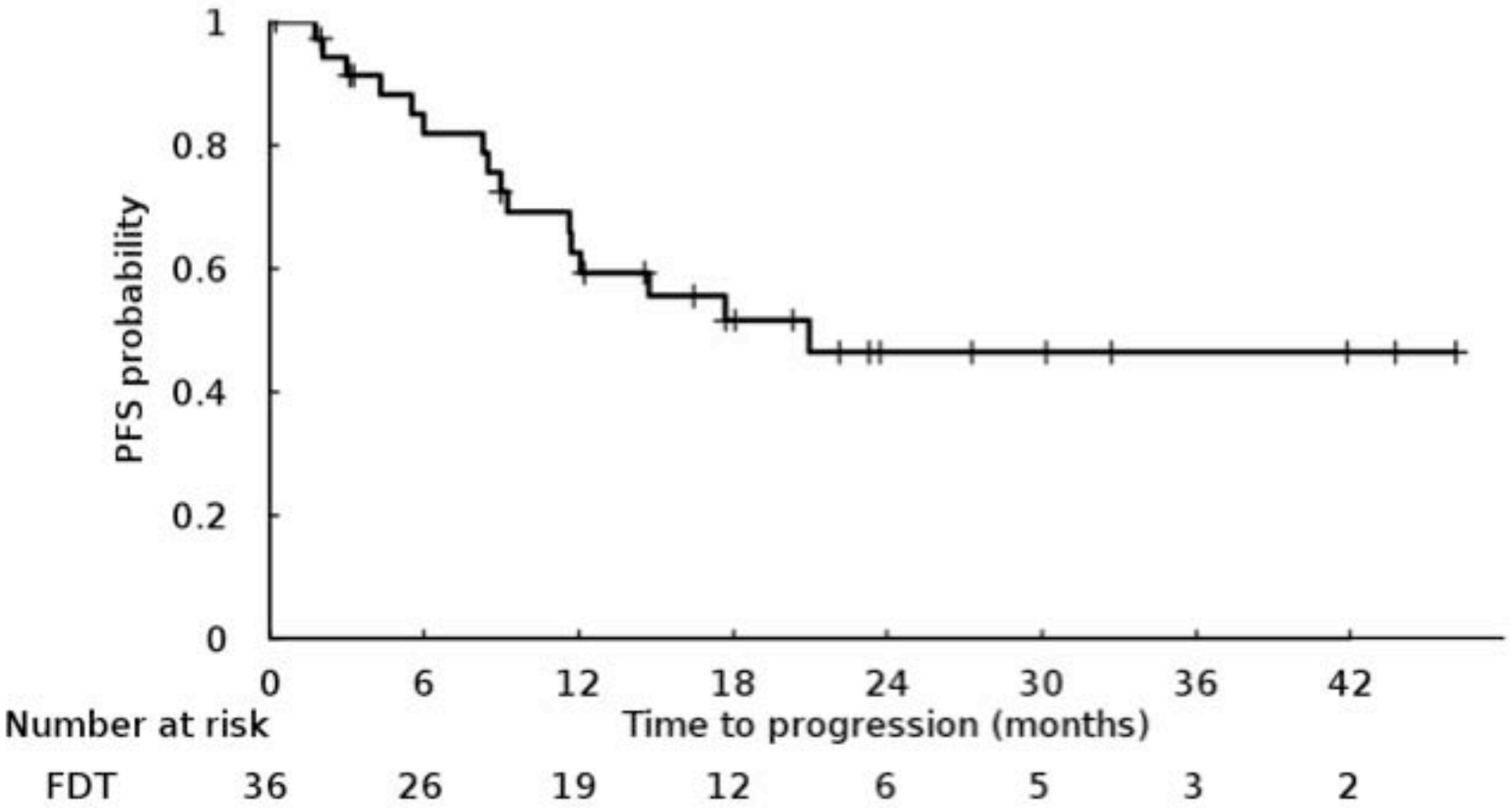
Figure 3



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