blood

Prepublished online January 3, 2013;
doi:10.1182/blood-2012-06-439307

Results of the phase II trial of single agent histone deacetylase inhibitor panobinostat in patients with relapsed/refractory Waldenström macroglobulinemia

Irene M. Ghobrial, Federico Campigotto, Timothy J. Murphy, Erica N. Boswell, Ranjit Banwait, Feda Azab, Stacey Chuma, Janet Kunsman, Amanda Donovan, Farzana Masood, Diane Warren, Scott Rodig, Kenneth C. Anderson, Paul G. Richardson, Edie Weller and Jeffrey Matous

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

Results of the phase II trial of single agent histone deacetylase inhibitor panobinostat in patients with relapsed/refractory Waldenström macroglobulinemia

Irene M. Ghobrial, MD[1], Federico Campigotto, MS[2], Timothy J. Murphy, MD, FACP[3], Erica N Boswell, BS[1], Ranjit Banwait, MA[1], Feda Azab, PharmD[1], Stacey Chuma, BSN[1], Janet Kunsman, NP[1], Amanda Donovan, MS[1], Farzana Masood, PhD[1], Diane Warren, MS[1], Scott Rodig, MD, PhD[4], Kenneth C. Anderson, MD[1], Paul G. Richardson, MD[1], Edie Weller, PhD[2], and Jeffrey Matous, MD[3]

[1]Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA
[2]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA
[3]Rocky Mountain Cancer Center, Colorado Springs, CO
[4]Department of Pathology, Brigham and Women's hospital, Boston, MA

Corresponding author:
Irene M. Ghobrial, MD
Medical Oncology, Dana-Farber Cancer Institute
450 Brookline Ave, Boston, MA, 02215
Phone: (617)-632-4198
Fax: (617)-582-8608
Email: irene_ghobrial@dfci.harvard.edu

Running head: Panobinostat in Waldenstrom Macroglobulinemia

This study has been presented in the American Society of Hematology, 2011, San Diego, CA

KEY POINTS

- This study presents the data of a phase 2 clinical trial of panobinostat in patients with relapsed Waldenstrom Macroglobulinemia (WM).
- This study establishes a role for histone deacetylase inhibitors as an active class of therapeutic agents in WM.

ABSTRACT

This study aimed to determine the safety and activity of the histone deacetylase inhibitor panobinostat in patients with relapsed/refractory Waldenstrom Macroglobulinemia (WM). Eligibility criteria included patients with relapsed/refractory WM with any number of prior therapy. Patients received panobinostat at 30 mg three times a week; 12/36 (33%) patients were enrolled at 25 mg dose. Thirty-six patients received therapy. The median age was 62 years (range, 47-80), and the median lines of prior therapies were 3 (range, 1-8). All of the patients had received prior rituximab. Minimal response (MR) or better was achieved in 47% of patients (90%CI: 33-62), with 22% PR and 25% MR. In addition, 18 (50%) patients achieved stable disease and none showed progression while on therapy. Paraprotein response rate of MR or better was 20/36 (55%, 90%CI 41-70). The median time to first response was 1.8 months (range, 1.7-3.2). The median progression-free survival (PFS) was 6.6 months (90% CI 5.5,14.8). Grade 3 and 4 toxicities included thrombocytopenia (67%), neutropenia (36%), anemia (28%), leukopenia (22%), and fatigue (11%). Panobinostat is an active

therapeutic agent in patients with relapsed/refractory WM. This study establishes a role for histone deacetylase inhibitors as an active class of therapeutic agents in WM. (Registered at Clinical trials.gov, NCT00936611)

INTRODUCTION

Waldenström Macroglobulinemia (WM) is a low-grade lymphoplasmacytic lymphoma characterized by the presence of an IgM monoclonal protein in the serum(1-4). Patients typically present with symptoms related to cytopenias mainly anemia or symptoms related to hyperviscosity (1-4). Many patients have an indolent disease with a median overall survival of 5 years; however, some patients with high-risk features, only have a 5-year survival of 36% (2, 3, 5, 6).

Standard therapeutic agents currently used in the treatment of this disease include alkylating agents, rituximab, nucleoside analogues and stem cell transplantation. Many new agents have been recently tested in WM including bortezomib, lenalidomide, perifosine, everolimus, and bendamustine. However, most patients show disease relapse post-treatment with these agents or have significant toxicities including prolonged myelosuppression or neuropathy. Therefore, there is an urgent need to develop therapies that are beneficial for this unique population of patients.

Epigenetic alterations including methylation and histone acetylation are commonly deregulated in most cancers(7, 8). Histone acetylation is regulated by a tight balance

between histone deacetylase (HDAC) and histone acetyl transferases (HATs)(9). This balance is disrupted in many malignancies leading to increased expression of HDACs and a more open chromatin structure that induces activation of gene transcription.

We, and others have shown that miRNAs are also deregulated in WM(10, 11). Our data indicated that aberrant increased expression of miRNA-206 and reduced expression of miRNA-9* leads to activation of HDACs in WM. Our data showed that HDACs and HATs are unbalanced in WM, with a decreased acetylated-histone-H3 and -H4, and increased HDAC activity in primary tumor cells compared to their normal cellular counterpart. Using the novel cinnamic hydroxamic acid HDAC inhibitor panobinostat (LBH589, Novartis, MA), we confirmed that this agent induces significant cytotoxicity in vitro and in vivo in WM cells.

Based on these promising preclinical studies, we designed a phase II clinical trial of single agent panobinostat to examine the response rate, toxicity, and progression-free survival in patients with relapsed or relapsed/refractory WM.

MATERIALS AND METHODS

Eligibility

Study participants were at least 18 years of age and had symptomatic disease requiring therapy for WM according to the consensus recommendations for WM(12). Patients had measurable monoclonal IgM immunoglobulin concentration on serum electrophoresis and IgM immunoglobulin protein twice the upper limit of normal by

nephlometry and evidence of lymphoplasmacytic cells in the bone marrow. Patients who had at least one previous therapy for WM and were relapsed or refractory were eligible. Any number of prior therapy was permissible. Patients must have had symptomatic disease requiring therapy to be entered on the study. Eligibility criteria also included a serum concentration of AST or ALT < 3 times the upper limit of the normal range, a serum total bilirubin <2 mg/dL, a measured creatinine <3.5 times the upper limit of the normal range, a platelet count of ≥75,000/mL, and an absolute neutrophil count of at least ANC ≥ 1.0 x 10^9/L. All patients gave written informed consent before entering the study, which was performed in accordance with the Declaration of Helsinki; approval was obtained from the institutional review board at each of the participating centers, (clinical trials.gov, NCT00936611).

Study Design and Treatment

Patients received single agent oral panobinostat 30 mg three days a week (Mondays, Wednesdays and Fridays). A cycle was 28 days. Patients with progressive disease after two cycles were taken off therapy. Patients with stable or responsive disease continued on therapy, (See Consort diagram). Restaging with a bone marrow biopsy and CT scans were performed at the end of cycle 6.

Dose modifications for attributable toxicities were allowed. Panobinostat could be reduced to 25 mg, 20 mg three times a week every week, or 20 mg three times a week every other week. No dose re-escalation was allowed. Dexamethasone was not allowed on this protocol. The protocol was amended because of concerns of toxicity to

allow a starting dose of 25 mg; 12/36 (33%) patients were enrolled on the 25 mg dose.

Efficacy and Safety Assessments

Tumor assessment was performed using the consensus panel recommendations(13). Patients were assessed every 28 days during the 6 cycles of therapy and every 3 months thereafter. Patients who came off therapy were monitored every 3 months until they progressed, were treated with another therapy, or died.

Response was calculated from the M spike measurement (or IgM measurement) of cycle 1 day 1 prior to therapy. Response was determined based on the IWMG response criteria (13) including CT scan and bone marrow assessment. All responses were confirmed. PR was assessed as patients having 50% reduction in pathological lymph nodes. Adverse events were assessed at each visit and graded according to the National Cancer Institute Common Toxicity Criteria (version 3.0) from the first dose until 30 days after the last dose of therapy.

Statistical Analysis

The primary endpoint was the overall response rate (defined as MR or better) and secondary endpoints included safety, duration of response (DR), time to progression (TTP) and progression-free survival (PFS). This study used a two-stage design (14 eligible on the first stage, 3 responses required to continue to the second stage with 23 eligible patients) with at least 11 MR or better observed among the 37 eligible

patients for the study drug to be considered promising. This study design has 0.93 probability of concluding that the drug is promising assuming true underlying MR or better response of 40% and less than 0.10 probability assuming 20% promising rate. . Patient baseline characteristics were summarized as number (%) of patients for categorical endpoints or median (range) of values for continuous endpoints. Exact two-stage binomial 90% confidence intervals (CI) were reported for response. Exact binomial 90% confidence intervals were reported for toxicity percentages. Comparisons between groups were performed using Fisher's exact test for binary endpoints and Wilcoxon rank sum test for continuous endpoints. Median time to event endpoints was estimated using the Kaplan-Meier method. Duration of response was measured from response to first PD or death, censored at the date patients were last known to be event-free and alive for those who have not failed. Time to progression (TTP), progression-free survival (PFS) and event free survival are measured from the time of treatment initiation to event (PD for TTP, PD or death for PFS, PD, death or non-protocol therapy for EFS). Patients without the event are censored at the date they were last known to be progression-free for TTP, and progression-free and alive for PFS and EFS. In addition, patients who start non-protocol therapy prior to progression are censored at the date of non-protocol therapy initiation for TTP and PFS and are events for EFS. All P-values are two-sided. Statistical analyses were performed using SAS statistical software (version 9.2, SAS Institute).

Correlative studies

Peripheral blood mononuclear cells were obtained from patients prior to therapy (at screening or on day 1 cycle 1), n=11 samples. In addition, samples were also

obtained after cycle 3 or cycle 6 on some patients (n=3). The level of acetylated Histone 3 was measured using ELISA assay (Cell Signaling, MA) in these samples as previously described(10).

Bone marrow biopsies from 14 patients at pre-treatment or after therapy were fixed in Zenker's formalin, embedded in paraffin blocks, and sectioned. Sections were stained for CD20, kappa and lambda light chains (Cell Signaling Technology Inc, MA).

RESULTS

Patients and Treatment

From July 2009 to March 2011, 39 patients were enrolled; 36 started on active therapy in 2 centers, and 3 patients were found to be ineligible and were not initiated on therapy (Figure 1, Consort Diagram). Table 1 shows selected characteristics of all 36 patients. The median age at enrollment was 61.6 years (range, 47-79.8). The median IgM level at enrollment was 3240 mg/dL (range, 804-10,300), and the median M spike by serum protein electrophoresis was 2.06 gm/dL (range, 0.63-5.1). The median hemoglobin at enrollment was 10.7 gm/dL (range, 8.2-14.5), with 58.3% (n=21) of the patients with anemia (<11 gm/dL). Median Beta- 2 microglobulin at enrollment was 3.3 mg/dL (range, 1.5-7.4), with 47.2% (n=17) of the patients with beta-2 microglobulin > 3.5 mg/dL. The median percent of bone marrow involvement was 70% (range, 5-100), with 10 (27.8%) having over 70% involvement in the bone marrow. Twenty-one (58%) of the patients were intermediate or high risk by the ISS-WM staging system at the

time of enrollment. All patients had symptomatic disease requiring initiation of therapy based on consensus recommendations for WM.

The median number of lines of prior therapies was 3 (range 1-8). Prior therapies included rituximab in all 36 (100%) patients, nucleoside analogues including fludarabine or cladribine in 11 (31%) patients, alkylating agents including chlorambucil and cyclophosphamide alone or in combination with other agents in 10 patients (28%), bortezomib containing regimen in 20 (56%) patients, and other therapies including thalidomide, imatinib mesylate, everolimus, and radiation therapy in 20 (56%) patients.

Among the 36 patients, the median number of completed cycles of therapy was 5 (0-32). There are 7 patients still on active therapy to this date of analysis in March 2012. Of the 36 patients, 26 (72%) received at least one dose modification. Among the 36 patients, the median (range) number of cycles without dose modifications was 1 (0-11 cycles). The protocol was amended to allow a starting dose of 25 mg; 12/36 (36%) patients were enrolled on the 25 mg starting dose.

Of the 12 patients who were treated on 25 mg, 7/12 (58%) had dose reductions, while of those who received 30 mg dosing (n=24), 19/24 (79%) had dose reductions. The median number of days that patients remained on 30 mg dosing schedule was 61 days (range7-321), while the median number of days that patients who were started on the 25 mg dosing stayed on 25 mg dosing was 38 (range, 11-97). The median number of dose reductions for those started on 30 mg was 2 (range, 0-3), while that of

those started on 25 mg was 1 (0-2). The median total amount of drug received for those who were started on 30 mg dosing was 1263 mg (range, 210-1185), while the median total amount of drug received for those who started at 25 mg was 740 mg (range, 175-3520).

At the time of this analysis, 8 patients remain on study follow up (7 on active therapy), and the remaining 28 patients are off study due to progressive disease in 3 (11%), new systemic therapy in 22 (61%), patients lost to follow up in 1 (3%) and patient withdrawal from study in 2 (5%).

Efficacy

Using the uniform WM response criteria (13) that includes monoclonal M spike and CT scans for measuring organ and lymph node involvement, the following responses were observed: Of the 36 patients, 8 achieved a partial response (PR) (22%), and 9 achieved minor response (MR) (25%), with an overall response rate of 17/36 (47% (90% CI, 33-62), (Table 2). Stable disease was achieved in 18 (50%) of the patients and 1 (3%) patient was unevaluable because he did not complete the first cycle and did not have M spike measurements post initiation of therapy. The median time to first response (N=17) was 1.7 months (0.9-1.8) and time to best response (N=17) was 1.8 months (1.7-3.2). The duration of response (MR or better) was 18.9 months (4.8, -).

Response using the monoclonal protein M spike level was as follows: 1 patient achieved a very good partial remission (VGPR) (3%), 10 patients achieved PR (27%),

9 patients achieved minor response (25%), 1 patient showed progressive disease (3%), and 1 was unevaluable, with a response rate of MR or better of 20/36 (56%, 90% CI 41-70) and of PR or better of 11/36 (31%, 90% CI 18-46).

Using IgM response evaluated by nephlometry, 19 patients (53%) achieved at least MR or better (90% CI: 38-67%), and 12 patients (33%) achieved PR or better (90%CI: 21-48), with 1 (4%) VGPR, 11 (31%) PR, and 7 (19%) MR. Figure 2 shows the maximum percent change from baseline in IgM over all cycles among all patients.

Bone marrow biopsies were performed, and results were available before and after therapy (after at least 6 cycles of therapy) in 20 (56%) patients. Of these 20 patients, 12/20 (60%) patients showed reduction in the % involvement of bone marrow after therapy, with a median % decrease of 63% (range, 20-94% decrease). These patients showed MR or better in 17/20 (85%) patients, and 3 patients showed stable disease by response criteria. Among the other 8 patients with no change in the bone marrow involvement or increase in BM involvement, the median % increase was 11%, with a range from 0-300%. The increase in 300% was in a patient with a percent increase from 5% to 20% in his pre and post-treatment samples respectively. The responses in these 8 patients included 2 PR, 3MR, and 3 stable disease by response criteria.

Given that 23 patients were initiated on therapy at 30 mg dosing and then the dose was reduced because of toxicity and another 12 patients were initiated on therapy at 25 mg starting dose, we reported on the response rates of the patients initiated on 30

mg or on 25 mg, tables 2A and B. The overall response rate and response rate by best paraprotein in all 36 patients as well as those patients treated with 30 mg or 25 mg dosing of panobinostat is reported in tables 2A and B.

Time to progression and progression-free survival

After a median follow up of 7.7 months (90%CI 6.8-9.5), 24 patients have progressed; of these, 1 subsequently died and 14 are alive. Among the other 12, 4 started a new therapy and one patient died without documented disease assessment of progression, measured as 25% progression in the paraprotein.

The two deaths occurred off therapy and after receiving subsequent therapy. Both patients died with large cell transformation and progressive disease. One patient received 16 cycles of therapy and then showed progression and went on to another therapy. The patient then developed infections and large cell transformation and died 6 months after being on panobinostat. The second patient received 2 cycles of therapy and then withdrew consent. The patient had another therapy and also showed large cell transformation and died 7 months after being on the clinical trial.

The median duration of response (MR or better, n=17) was 6.9 months (90%CI 3.1,-18.9), Figure 3A. The median time to progression (TTP) and progression free survival (PFS) were similar in the 36 patients who had follow up and was 6.6 months (90%CI

5.5,14.8), Figure 3B. In the TTP and PFS analysis, patients who started non-protocol therapy prior to progression (n=4) were censored at the initiation of non-protocol treatment. For the EFS analysis, in which the initiation of non-protocol therapy is counted as an event, the median EFS time is 5.8 (90% CI 4.6-7.7) months, Figure 3C. The estimated 1-year event free rate was 31% (90% CI 19-44%). The estimated event free rate at 2 years was 17% (90% CI, 7%-29%).

Safety

A summary of all toxicities related to therapy is shown in Table 3A. The most common grade 3 and 4 therapy related adverse events included thrombocytopenia in 67%, neutropenia in 36%, anemia in 28%, and leukopenia in 22%, fatigue in 11% and syncope in 6%. Grade 1 and 2 diarrhea occurred in 58%, nausea and anorexia in 44%, taste disturbance in 28%, lower extremity edema in 22%, weight loss in 22%, dizziness in 19%, dyspnea in 19%, and alopecia in 17%. Pneumonitis occurred in 5 patients, 4 grade 1 and 1 grade 2 toxicity. All of those were observed incidentally with CT scans performed during staging studies in follow up during therapy. Pneumonitis was treated with tapering doses of steroids as well as withholding therapy and re-initiating it at a dose reduction.

Grade 3 and 4 toxicities observed in patients who were initiated at 30 mg (N=23) or in those initiated at dose 25 mg (N=13) is shown in Table 3B. There was no difference in the rate of Grade 3 and 4 toxicities observed in these two groups.

Translational studies

The degree of involvement (% involvement) observed in bone marrow biopsies pre and post-treatment in patients who achieved MR or better to therapy is shown in Figure 4A. The level of acetylated H3 in the peripheral blood mononuclear cells is shown in Figure 4B. The numbers were too small to perform correlative studies with response to therapy. Interestingly, we observed an increase in the level of acetylated H3 post-therapy in post-treatment samples, Figure 4C.

DISCUSSION

Current recommendations for relapsed or refractory WM include the use of nucleoside analogues, alkylating agents including the use of bendamustine, as well as novel agents such as bortezomib, everolimus, and immunomodulators(14-17). Despite these advances, most patients show relapse or have significant side effects from current therapeutic agents. Therefore, there is a critical need for the development of novel therapeutic agents that have less long-term toxicities.

Preclinical studies have shown that epigenetic regulators including histone acetylation regulate tumor progression(10, 11). Our prior studies have shown significant deregulation in HDAC activity in primary tumor cells, as well as in vitro and in vivo activity of panobinostat in WM(2, 4). Based on this data, we initiated the phase II trial of single agent panobinostat as a new class of novel therapeutic agents in WM.

This study shows a response rate of MR or better in 47% of patients with 22% partial remissions. Response by paraprotein M spike level included response of MR or better of 55% and PR or better of 30% including 1 VGPR, 10 PR, 9 MR, and 1 unevaluable. Other studies using novel agents in a similar population of relapsed or refractory WM have shown responses of MR or better of 30 to 50% using rituximab, 35% using perifosine, 70% using everolimus, and 26-80% using bortezomib. Therefore, the response rate of 47% of single agent panobinostat indicates that indeed this class of agents is highly active in patients with this low-grade lymphoma.

The response rate of panobinostat in other lymphomas and plasma cell dyscrasias varies(18-21). It is significantly high in Hodgkin lymphoma with 30-60% responses and minimal to no responses of single agent panobinostat in Multiple Myeloma(22). However, significant responses were observed in combinations of panobinostat and bortezomib in Multiple Myeloma(23, 24). Therefore, future studies using panobinostat and bortezomib or other proteasome inhibitors can lead to high responses in patients with WM. The progression free survival (PFS) in this trial was 6.6 months. The median PFS survival for bortezomib was 6.6 months in the Waldenstrom's Macroglobulinemia Clinical Trials Group study in a similar patient population (25).

This study is relatively small and was performed in only 2 centers, one academic and one community-based. The younger median age of 62 (compared to median age of 63-65 in other reported studies) and the relatively lower high-risk ISS of 19% (compared to 35% in newly diagnosed patients) may be due to referral bias of these

patients to clinical trials. Larger studies in community practices are needed to further confirm the responses observed in this study.

The most common grade 3 and 4 therapy related adverse events included thrombocytopenia, neutropenia, and anemia. Fatigue and diarrhea were also common side effects that are commonly seen in other clinical trials using HDAC inhibitors(20, 21). Interestingly, we observed pneumonitis in 5 cases on this trial indicating that this toxicity may also occur with panobinostat. Pneumonitis has also been observed with everolimus and bortezomib in prior studies(26). Dose reductions were required in 72% of patients. In addition, the protocol was modified to allow a starting dose of 25 mg in 12/36 (33%) of patients. Despite the dose reduction to 25 mg, most hematological toxicities remained high, with 67% of patients having grade 3-4 thrombocytopenia. However, responses appeared to be slightly lower in patients who were initiated at the lower dose of 25 mg, although the numbers are too small to make recommendations. Lower initial doses may possibly have lower toxicities but may also have lower responses. An initial high dose followed by dose reduction may also be beneficial for some patients who require an urgent response in the paraprotein level. Future studies are required to examine different starting dose levels in patients with WM and other low-grade lymphomas, as well as combinations of therapy using lower doses. In addition, newer more targeted HDAC inhibitors may provide similar responses with less off-target toxicities.

In summary, we recommend the use of panobinostat as an effective and safe regimen

for therapy in relapsed WM. This clinical trial establishes a role for HDAC inhibitors as a new class of therapeutic agents in the treatment of WM.

Acknowledgement

Acknowledgments of research support for the study: Supported in part by a research grant from Novartis Inc, 1R01F0003743, International Waldenstrom Macroglobulinemia Foundation (IWMF), and by Michelle and Steven Kirsch lab for Waldenstrom Macroglobulinemia and the Heje Fellowship for Waldenstrom.

Authorship: Author contribution statement and conflict of interest.

Irene M.Ghobrial: Research support for clinical trial by Novartis. Advisory board of Millennium, Onyx, BMS, and Novartis.
Kenneth C. Anderson: Stockholder of Acetylon. Advisory Board of Novartis, Millennium, Onyx, Celgene, and BMS.
Paul G. Richardson: Advisory Board of Millennium, Celgene, and Novartis.
Other authors did not declare any conflicts of interest.

References.

1. Dimopoulos MA, Panayiotidis P, Moulopoulos LA, Sfikakis P, Dalakas M. Waldenstrom's macroglobulinemia: clinical features, complications, and management. J Clin Oncol. 2000;18(1):214-26. Epub 2000/01/07.
2. Ghobrial IM, Witzig TE. Waldenstrom macroglobulinemia. Curr Treat Options Oncol. 2004;5(3):239-47. Epub 2004/04/30.
3. Dimopoulos MA, Kyle RA, Anagnostopoulos A, Treon SP. Diagnosis and management of Waldenstrom's macroglobulinemia. J Clin Oncol. 2005;23(7):1564-77. Epub 2005/03/01.
4. Owen RG, Treon SP, Al-Katib A, Fonseca R, Greipp PR, McMaster ML, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol. 2003;30(2):110-5. Epub 2003/04/30.
5. Ghobrial IM, Gertz MA, Fonseca R. Waldenstrom macroglobulinaemia. Lancet Oncol. 2003;4(11):679-85.
6. Morel P, Duhamel A, Gobbi P, Dimopoulos MA, Dhodapkar MV, McCoy J, et al. International prognostic scoring system for Waldenstrom's macroglobulinemia. Blood. 2009.
7. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet. 2007;8(4):286-98.
8. Fraga MF, Esteller M. Towards the human cancer epigenome: a first draft of histone modifications. Cell Cycle. 2005;4(10):1377-81.
9. Ropero S, Esteller M. The role of histone deacetylases (HDACs) in human cancer. Mol Oncol. 2007;1(1):19-25.
10. Roccaro AM, Sacco A, Jia X, Azab AK, Maiso P, Ngo HT, et al. microRNA-dependent modulation of histone acetylation in Waldenstrom macroglobulinemia. Blood. 2010;116(9):1506-14. Epub 2010/06/04.
11. Sacco A, Issa GC, Zhang Y, Liu Y, Maiso P, Ghobrial IM, et al. Epigenetic modifications as key regulators of Waldenstrom's Macroglobulinemia biology. J Hematol Oncol. 2010;3:38. Epub 2010/10/12.
12. Gertz MA, Anagnostopoulos A, Anderson K, Branagan AR, Coleman M, Frankel SR, et al. Treatment recommendations in Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol. 2003;30(2):121-6.
13. Kimby E, Treon SP, Anagnostopoulos A, Dimopoulos M, Garcia-Sanz R, Gertz MA, et al. Update on recommendations for assessing response from the Third International Workshop on Waldenstrom's Macroglobulinemia. Clin Lymphoma Myeloma. 2006;6(5):380-3.
14. Issa GC, Ghobrial IM, Roccaro AM. Novel agents in Waldenstrom macroglobulinemia. Clin Investig (Lond). 2011;1(6):815-24. Epub 2011/10/29.
15. Rourke M, Anderson KC, Ghobrial IM. Review of clinical trials conducted in Waldenstrom macroglobulinemia and recommendations for reporting clinical trial responses in these patients. Leuk Lymphoma. 2010;51(10):1779-92. Epub 2010/08/28.

16. Treon SP. How I treat Waldenstrom macroglobulinemia. Blood. 2009;114(12):2375-85. Epub 2009/07/21.
17. Dimopoulos MA, Gertz MA, Kastritis E, Garcia-Sanz R, Kimby EK, Leblond V, et al. Update on treatment recommendations from the Fourth International Workshop on Waldenstrom's Macroglobulinemia. J Clin Oncol. 2009;27(1):120-6. Epub 2008/12/03.
18. Oki Y, Copeland A, Younes A. Clinical development of panobinostat in classical Hodgkin's lymphoma. Expert Rev Hematol. 2011;4(3):245-52. Epub 2011/06/15.
19. Dickinson M, Ritchie D, DeAngelo DJ, Spencer A, Ottmann OG, Fischer T, et al. Preliminary evidence of disease response to the pan deacetylase inhibitor panobinostat (LBH589) in refractory Hodgkin Lymphoma. Br J Haematol. 2009;147(1):97-101. Epub 2009/08/12.
20. Prince HM, Bishton MJ, Johnstone RW. Panobinostat (LBH589): a potent pan-deacetylase inhibitor with promising activity against hematologic and solid tumors. Future Oncol. 2009;5(5):601-12. Epub 2009/06/13.
21. Giles F, Fischer T, Cortes J, Garcia-Manero G, Beck J, Ravandi F, et al. A phase I study of intravenous LBH589, a novel cinnamic hydroxamic acid analogue histone deacetylase inhibitor, in patients with refractory hematologic malignancies. Clin Cancer Res. 2006;12(15):4628-35. Epub 2006/08/11.
22. Wolf JL, Siegel D, Goldschmidt H, Hazell K, Bourquelot PM, Bengoudifa BR, et al. Phase II trial of the pan-deacetylase inhibitor panobinostat as a single agent in advanced relapsed/refractory multiple myeloma. Leuk Lymphoma. 2012. Epub 2012/02/01.
23. Hideshima T, Richardson PG, Anderson KC. Mechanism of action of proteasome inhibitors and deacetylase inhibitors and the biological basis of synergy in multiple myeloma. Molecular cancer therapeutics. 2011;10(11):2034-42. Epub 2011/11/11.
24. Lonial S. Relapsed multiple myeloma. Hematology Am Soc Hematol Educ Program. 2010;2010:303-9. Epub 2011/01/18.
25. Treon SP, Ioakimidis L, Soumerai JD, Patterson CJ, Sheehy P, Nelson M, et al. Primary therapy of Waldenstrom macroglobulinemia with bortezomib, dexamethasone, and rituximab: WMCTG clinical trial 05-180. J Clin Oncol. 2009;27(23):3830-5. Epub 2009/06/10.
26. Ghobrial IM, Gertz M, Laplant B, Camoriano J, Hayman S, Lacy M, et al. Phase II trial of the oral mammalian target of rapamycin inhibitor everolimus in relapsed or refractory Waldenstrom macroglobulinemia. J Clin Oncol. 2010;28(8):1408-14. Epub 2010/02/10.

Table 1: Baseline characteristics

Patient Characteristic	N=36	%
Median age, years (range)	61.6	(47-80)
Gender, Male	22	61
Race: White	35	97
Median IgM at screening (mg/dL)	3240	Range (804-10,300)
Median M spike at screening (gm/dL)	2.06	Range (0.63-5.1)
Median Hemoglobin (gm/dL)	10.7	range (8.2-14.5)
Median platelets count	242	74-388
Median Beta 2 microglobulin	3.3	range (1.4-7.4)
% bone marrow involvement	70	Range (5-100)
Lymph nodes and HSM assessment by CT scan		
Evidence of disease	29	81
ISS-WM (at enrollment)		
High Risk	7	19
Intermediate Risk	14	39
Low Risk	15	42

Table 2: Response measured by response assessment per protocol and by paraprotein (M spike) measurement based on the starting dose of 30 mg or 25 mg in the patients on this trial.

Table 2A: Best overall response.

	All 36 patients		**Pts who started at 30mg (N=24)**		**Pts who started at 25mg (N=12)**	
Best overall response	N	%	N	%	N	%
CR	0	0	0	0	0	0
VGPR	0	0	0	0	0	0
PR	8	22	7	29	1	8
MR	9	25	5	21	4	33
SD	18	50	11	46	8	58
PD	0	0	0	0	0	0
Unevaluable	1	3	1	4	0	0
Total	**36**		**24**		**12**	
PR or better	**8**	**22 % (90%CI:12, 37)**	**7**	**29% (90%CI: 15, 48)**	**1**	**8% (90%CI: 0, 34)**
MR or better	**17**	**47% (90%CI:33,62)**	**12**	**50% (90%CI: 32, 68)**	**5**	**41% (90%CI: 18, 68)**

Table 2B: Best paraprotein response.

	All 36 patients		**Pts who started at 30mg (N=24)**		**Pts who started at 25mg (N=12)**	
Best overall response	N	%	N	%	N	%
CR	0	0	0	0	0	0
VGPR	1	3	1	4	0	0
PR	10	27	9	38	1	8
MR	9	25	5	21	4	33
SD	14	39	7	29	7	58
PD	1	3	1	4	0	0
Unevaluable	1	3	1	4	0	0
Total	36		**24**		**12**	
PR or better	**11**	**30 (90%CI:18, 46)**	**10**	**42% (90%CI: 25, 60)**	**1**	**8% (90%CI: 0, 34)**
MR or better	**20**	**55 (90%CI:41,70)**	**15**	**63% (90%CI: 44, 79)**	**5**	**41% (90%CI: 18, 68)**

Table 3A: Summary of all adverse events >10% of patients (with at least possible attribution), N=36.

TOXICITY TYPE	MAX GRADE					All grades%	Grade 1& 2%	Grade 3 & 4%
	1	2	3	4	Total			
Fatigue	4	22	4	0	30	83	72	11
Platelets	0	4	15	9	28	78	11	67
Neutrophils	0	12	9	4	25	69	33	36
Diarrhea	10	11	0	0	21	58	58	0
Leukocytes	0	12	6	2	20	56	33	22
Hemoglobin	0	8	8	2	18	50	22	28
Nausea	11	4	1	0	16	44	42	3
Anorexia	7	8	0	0	15	42	42	0
Taste disturbance	7	3	0	0	10	28	28	0
Dizziness	7	0	0	0	7	19	19	0
Dyspnea	5	2	0	0	7	19	19	0
Edema limb	6	1	0	0	7	19	19	0
Weight loss	6	1	0	0	7	19	19	0
Abdominal pain	4	2	0	0	6	17	17	0
Headache	3	3	0	0	6	17	17	0
Vomiting	5	1	0	0	6	17	17	0
Alopecia	5	0	0	0	5	14	14	0
Creatinine	0	5	0	0	5	14	14	0
Pneumonitis	4	1	0	0	5	14	14	0
Constipation	4	0	0	0	4	11	11	0
Dry mouth	4	0	0	0	4	11	11	0
Dyspepsia	2	2	0	0	4	11	11	0

Table 3B: All Grade 3 or higher adverse events (at least possible treatment-related): worst grade by type of toxicity in patients who started at 30mg (N=24) or in patients who started at 25mg (N=12)

TOXICITY TYPE	30 mg (N=24)			25 mg (N=12)		
Frequency	**Grade 3**	**Grade 4**	**Total**	**Grade 3**	**Grade 4**	**Total**
Platelets	10	6	16(67%)	5	3	8 (67%)
Hemoglobin	5	2	7(29%)	3	0	3 (25%)
Neutrophils	5	2	7 (29%)	4	2	6 (50%)
Leukocytes	3	1	4(17%)	3	1	4 (33%)
Fatigue	2	0	2(8%)	2	0	2 (17%)
Syncope	2	0	2 (8%)	0	0	0
Hypophosphatemia	1	0	1(4%)	0	0	0
Lower GI-hemorrhage NOS	1	0	1 (4%)	0	0	0
Non-neuropathic generalized weakness	1	0	1(4%)	0	0	0
Bilirubin	0	0	0	1	0	1 (8%)
Total	**32**	**12**	**44**	**18**	**0**	**24**

Figure legends:

Figure 1: Consort Diagram of the design of the study.

Figure 2: Waterfall of the maximum difference in IgM from baseline throughout the study (including follow-up), N=36.

Figure 3A: Kaplan Meier estimation of the duration of response (MR or better) endpoint (N=17).

Figure 3B: Kaplan Meier estimation of the PFS endpoint (N=36): PFS was similar to TTP.

Figure 3C: Kaplan Meier estimation of the EFS endpoint (N=36).

Figure 4A: Immunohistochemistry for CD20 and % involvement on pre and post-treatment samples

Figure 4B: ELISA for acetylated Histone 3 (H3) in peripheral blood mononuclear cells of samples before therapy in 10 patients with the clinical responses that were obtained in these patients.

Figure 4C: ELISA for acetylated H3 in peripheral blood mononuclear cells of samples before and therapy in 3 patients. Although the levels before and after therapy were not statistically significant, there was a trend of increase in the level of acetylated H3 after therapy, consistent with activity of HDAC inhibitors.

Figure 1

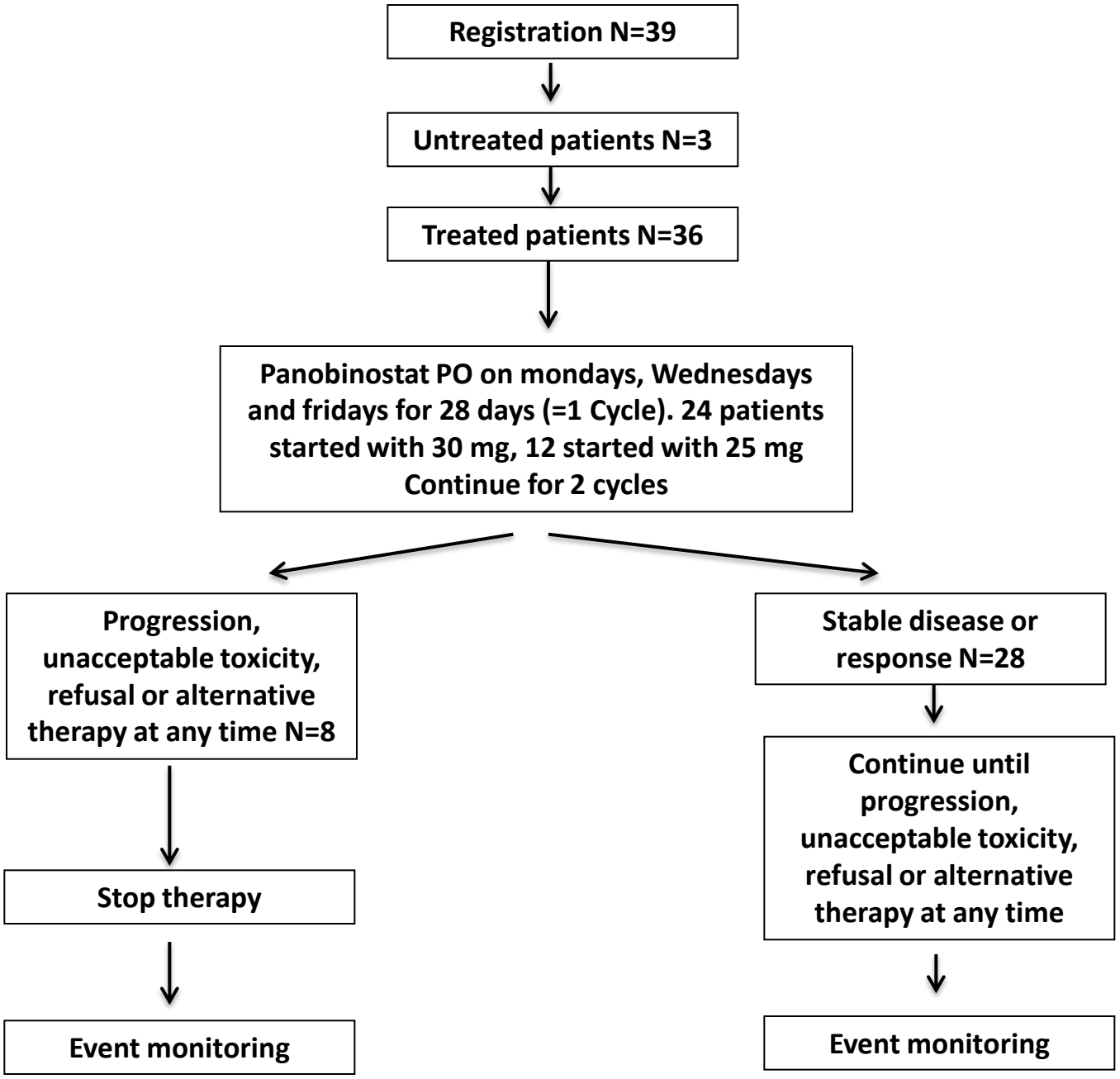

Registration N=39
Untreated patients N=3
Treated patients N=36
Panobinostat PO on mondays, Wednesdays and fridays for 28 days (=1 Cycle). 24 patients started with 30 mg, 12 started with 25 mg
Continue for 2 cycles
Progression, unacceptable toxicity, refusal or alternative therapy at any time N=8
Stop therapy
Event monitoring
Stable disease or response N=28
Continue until progression, unacceptable toxicity, refusal or alternative therapy at any time
Event monitoring

Figure2

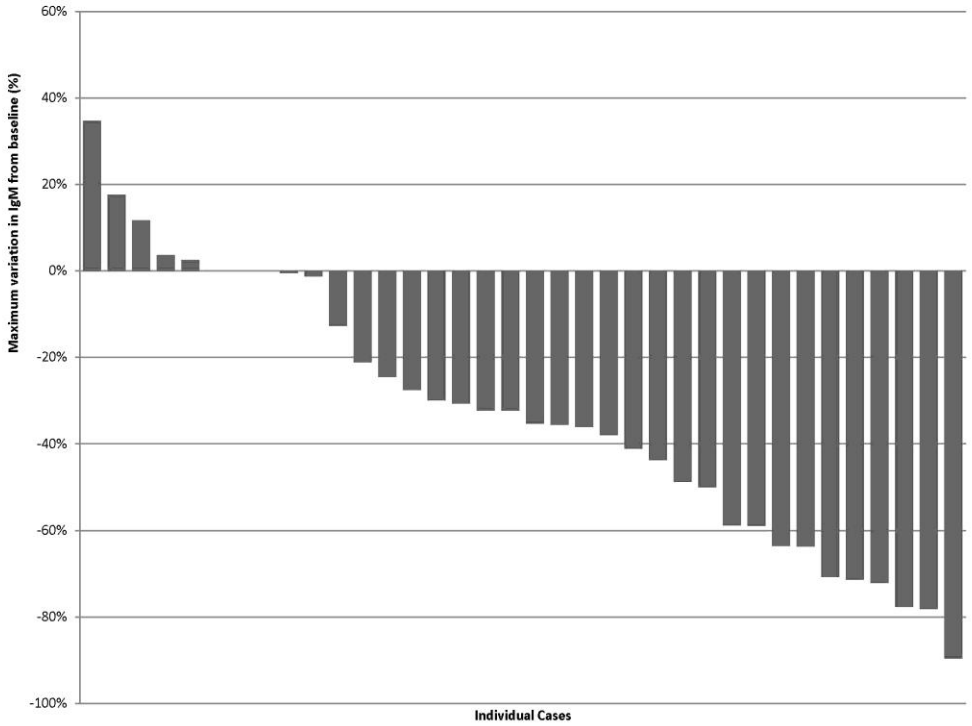

60%
40%
20%
0%
-20%
-40%
-60%
-80%
-100%
Maximum variation in IgM from baseline (%)
Individual Cases

Figure 3A

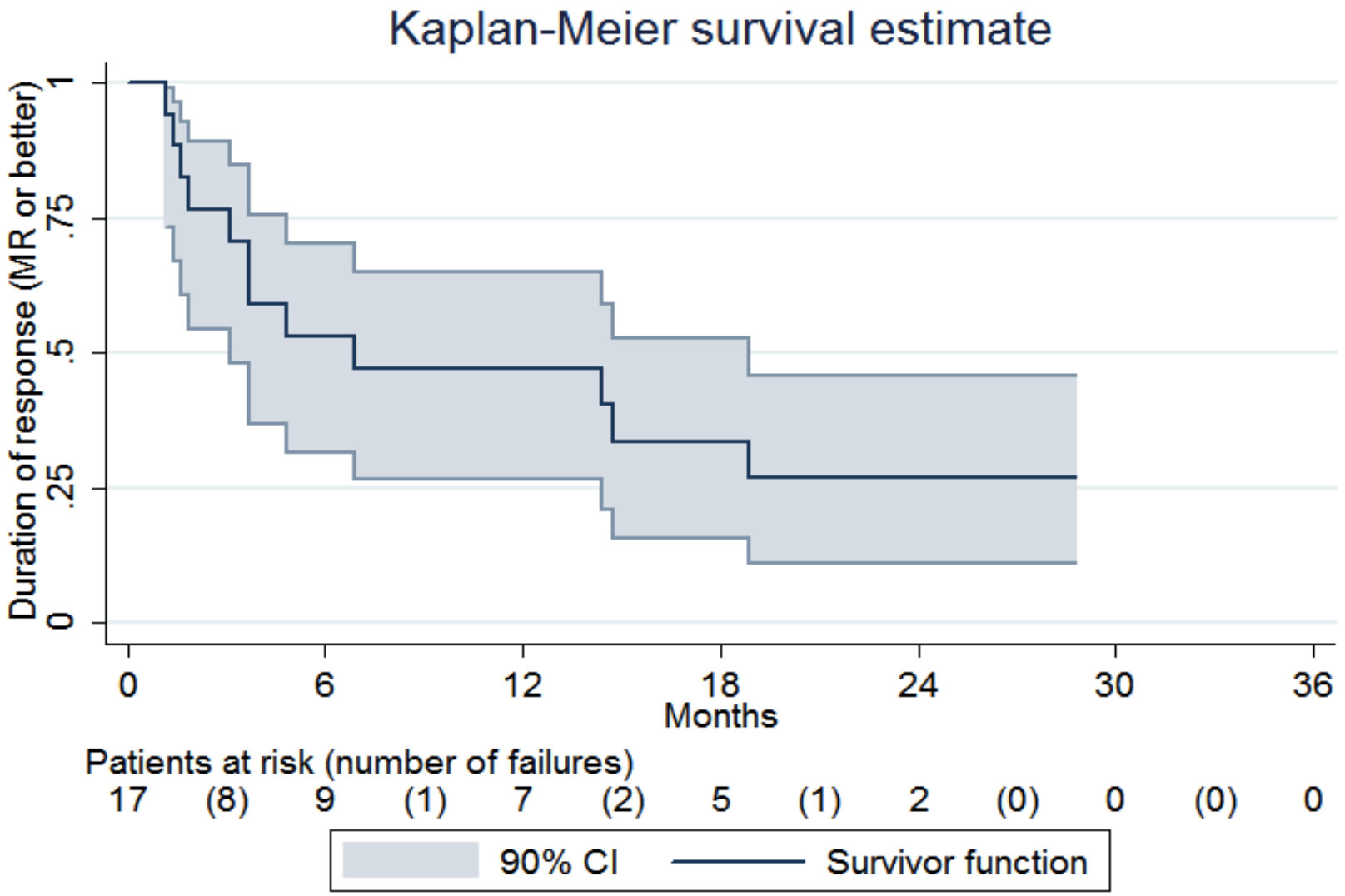

Kaplan-Meier survival estimate
Duration of response (MR or better)
0
.25
.5
.75
1
0
6
12
18
24
30
36
Months
Patients at risk (number of failures)
17 (8) 9 (1) 7 (2) 5 (1) 2 (0) 0 (0) 0
90% CI
Survivor function

Figure 3B

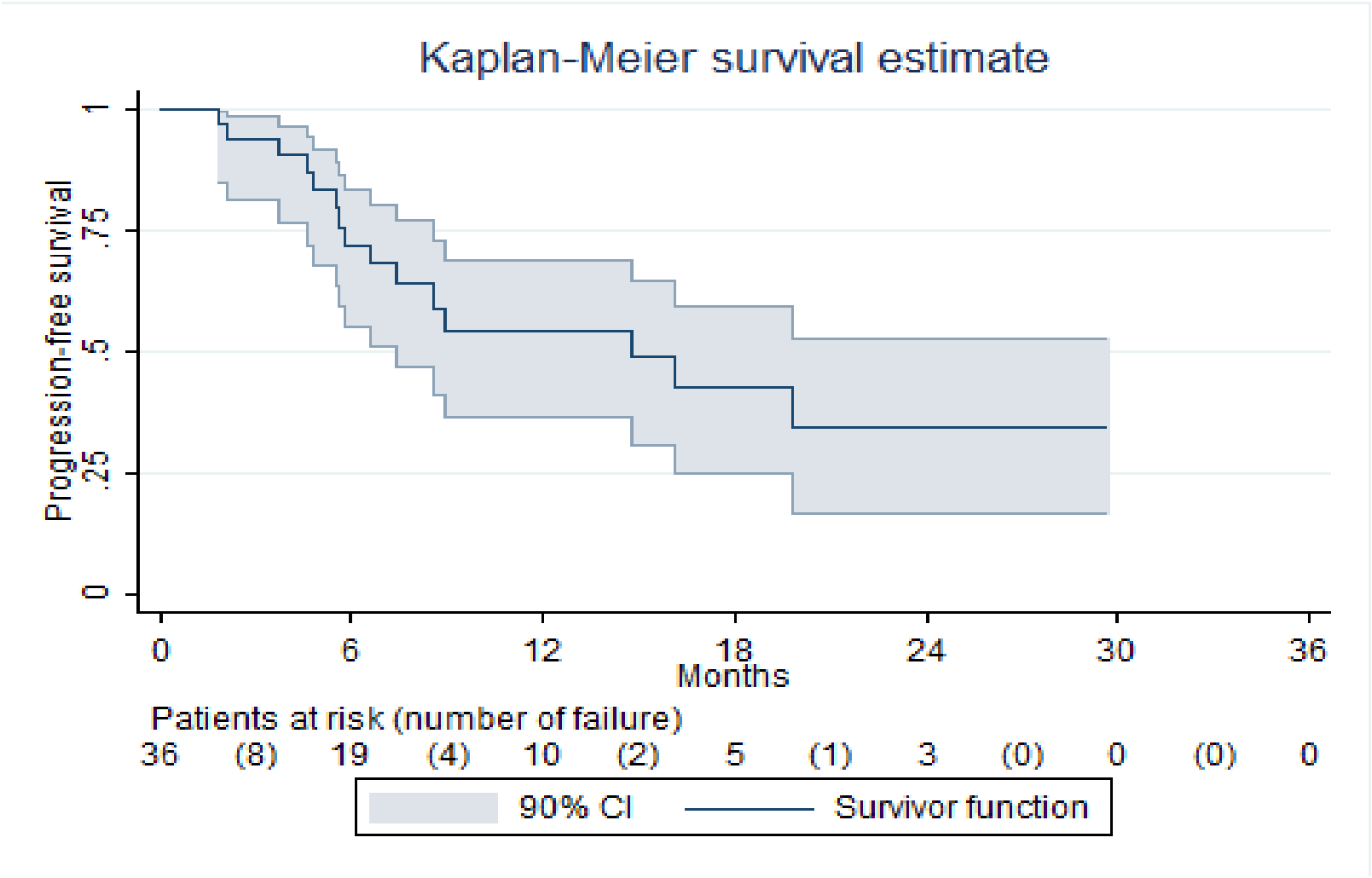

Kaplan-Meier survival estimate
Progression-free survival
0
.25
.5
.75
1
0
6
12
18
24
30
36
Months
Patients at risk (number of failure)
36 (8) 19 (4) 10 (2) 5 (1) 3 (0) 0 (0) 0
90% CI
Survivor function

Figure 3C

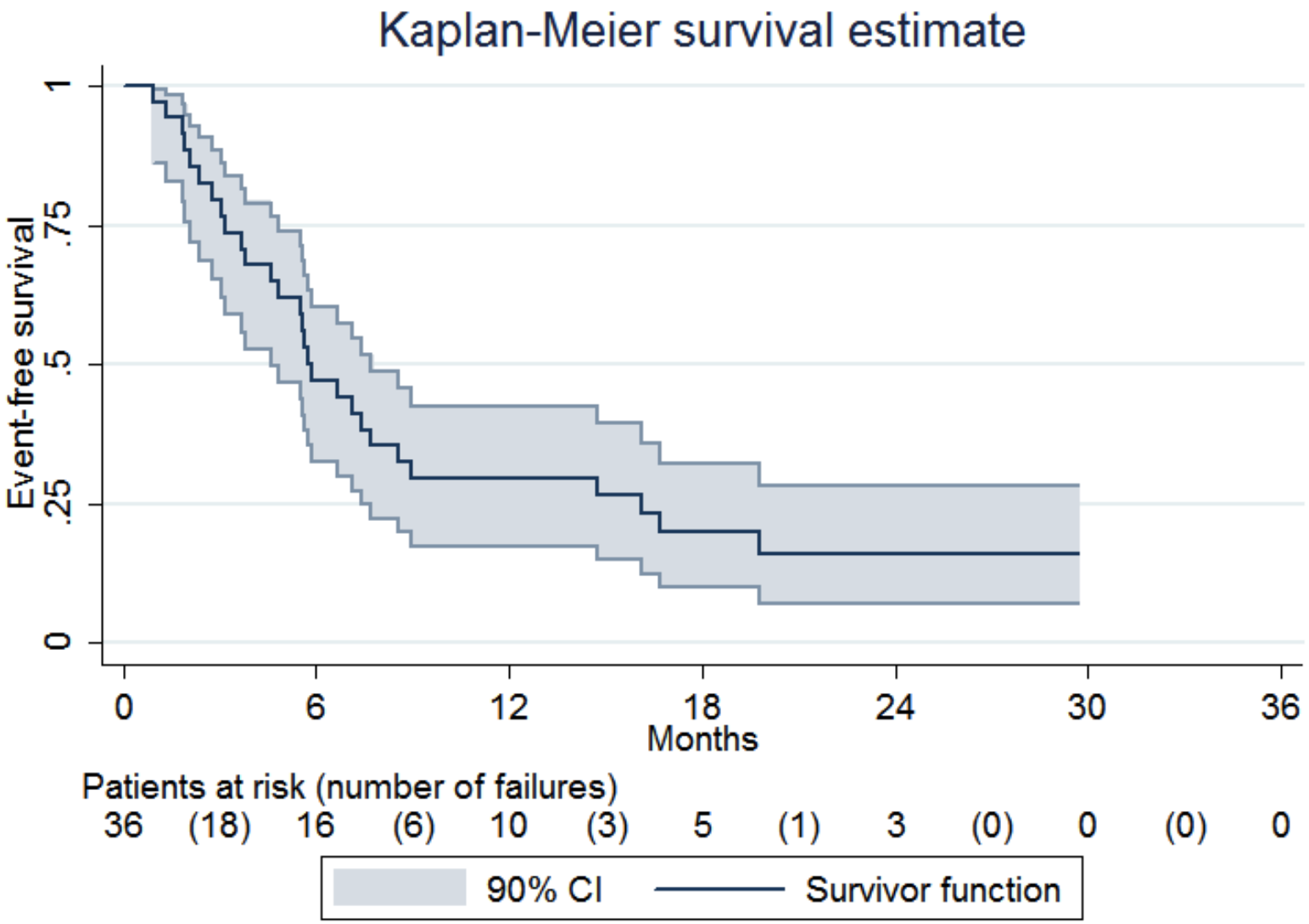

Kaplan-Meier survival estimate
Event-free survival
0
.25
.5
.75
1
0
6
12
18
24
30
36
Months
Patients at risk (number of failures)
36 (18) 16 (6) 10 (3) 5 (1) 3 (0) 0 (0) 0
90% CI
Survivor function

Figure 4A

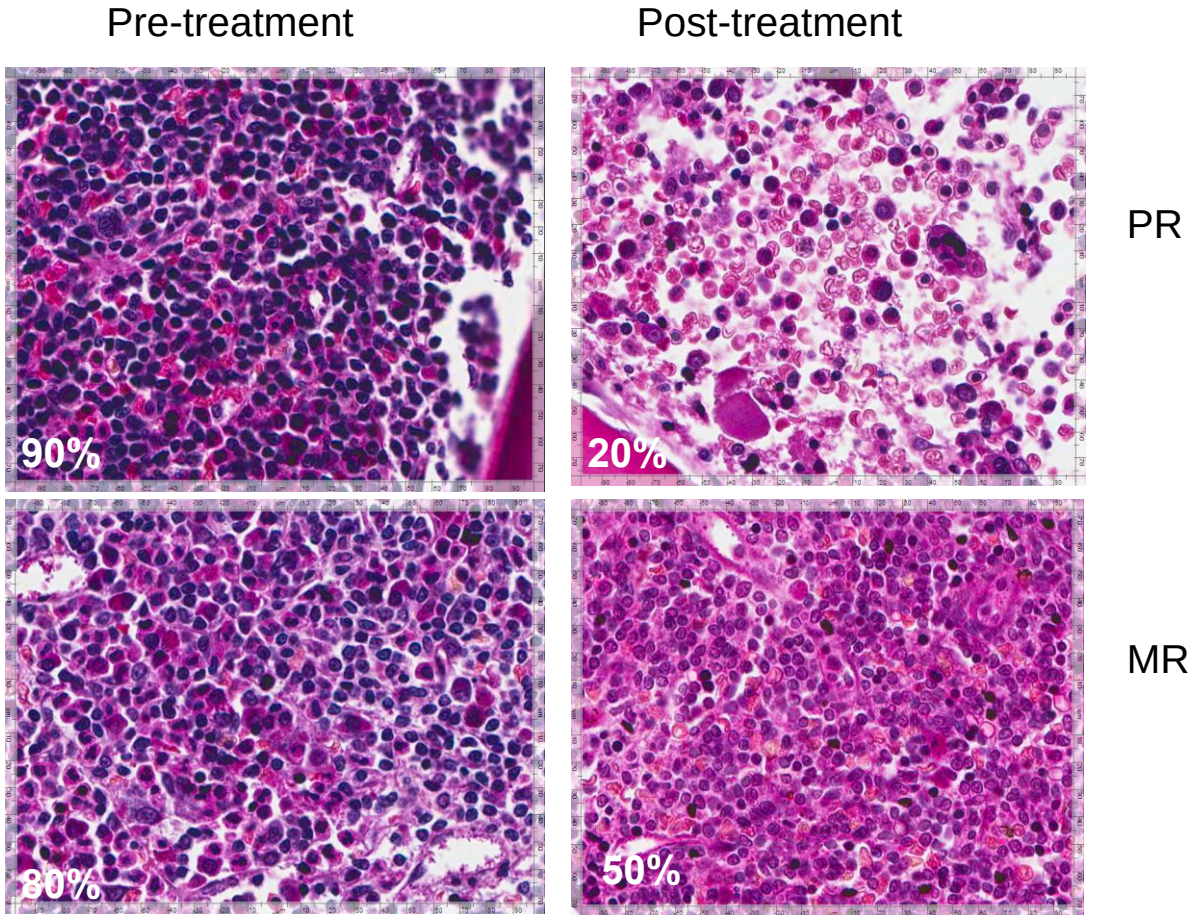


Figure 4B

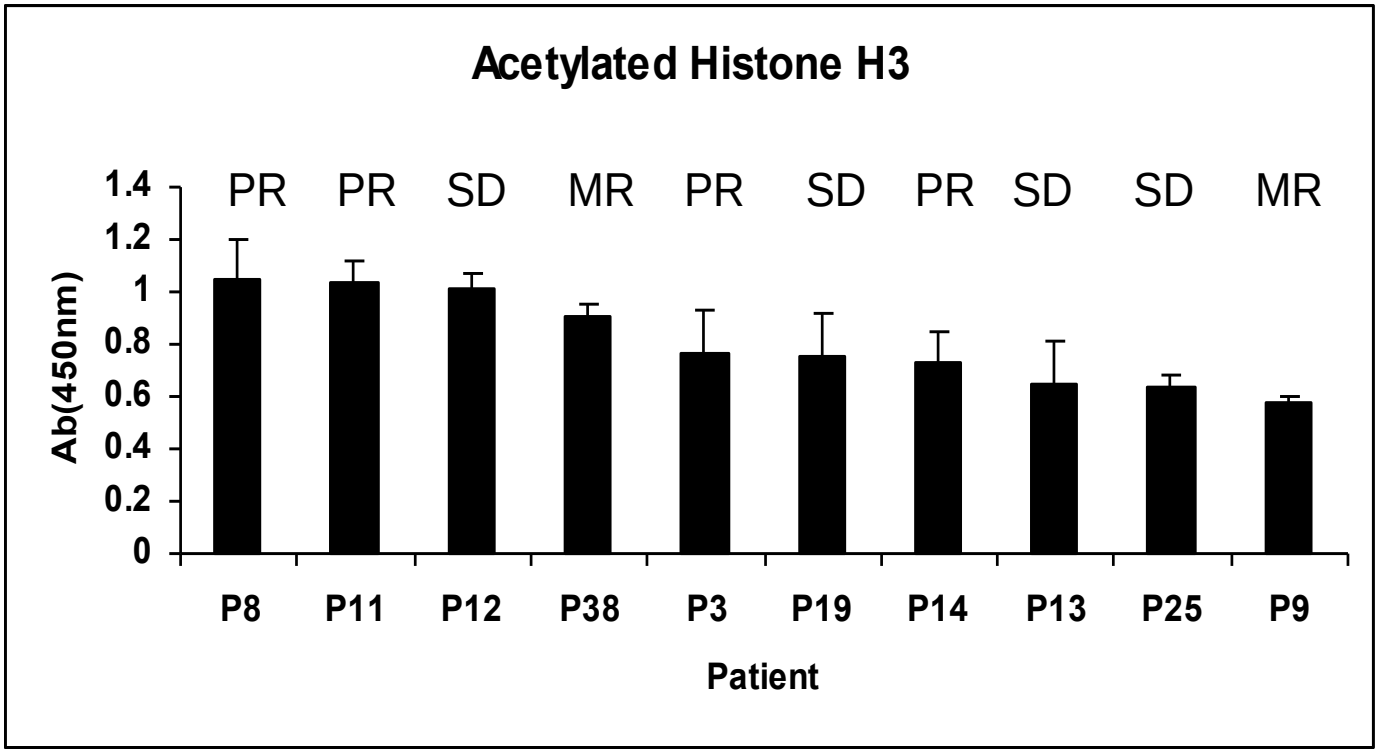
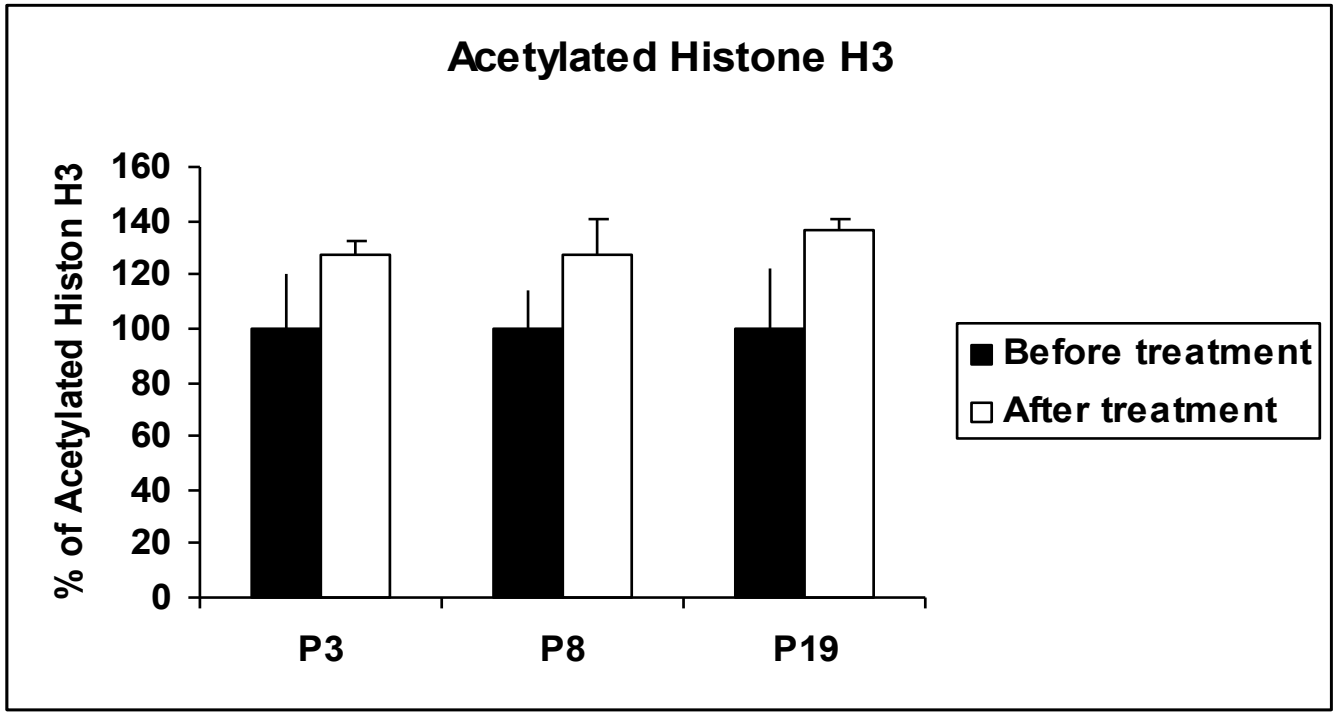

Acetylated Histone H3
PR PR SD MR PR SD PR SD SD MR
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Ab(450nm)
P8 P11 P12 P38 P3 P19 P14 P13 P25 P9
Patient

Figure 4C


Acetylated Histone H3
% of Acetylated Histon H3
160
140
120
100
80
60
40
20
0
P3
P8
P19
Before treatment
After treatment