Published OnlineFirst January 26, 2010; DOI: 10.1158/1078-0432.CCR-09-1837
Published Online First on January 26, 2010 as 10.1158/1078-0432.CCR-09-1837

Cancer Therapy: Clinical

Clinical Cancer Research

Clinical and Translational Studies of a Phase II Trial of the Novel Oral Akt Inhibitor Perifosine in Relapsed or Relapsed/Refractory Waldenström's Macroglobulinemia

Irene M. Ghobrial[1], Aldo Roccaro[1], Fangxin Hong[1], Edie Weller[1], Nancy Rubin[3], Renee Leduc[1], Meghan Rourke[1], Stacey Chuma[1], Antonio Sacco[1], Xiaoying Jia[1], Feda Azab[1], Abdel Kareem Azab[1], Scott Rodig[2], Diane Warren[1], Brianna Harris[1], Lyuba Varticovski[4], Peter Sportelli[5], Xavier Leleu[1], Kenneth C. Anderson[1], and Paul G. Richardson[1]

Abstract

Background: Waldenström's macroglobulinemia (WM) is a rare, low-grade lymphoproliferative disorder. Based on preclinical studies, we conducted a phase II clinical trial testing the efficacy and safety of the Akt inhibitor perifosine in patients with relapsed/refractory WM.

Patients and Methods: Thirty-seven patients were treated with oral perifosine (150 mg daily) for six cycles. Stable or responding patients were allowed to continue therapy until progression.

Results: The median age was 65 years (range, 44-82). The median number of prior therapy lines was two (range, one to five). Of the 37 patients, 4 achieved partial response (11%), 9 minimal response (24%), and 20 showed stable disease (54%). The median progression-free survival was 12.6 months. Additionally, β2 microglobulin of >3.5 mg/dL was associated with poor event-free survival ($P = 0.002$). Perifosine was generally well tolerated; adverse events related to therapy were cytopenias (grade 3-4, 13%), gastrointestinal symptoms (grade 1-2, 81%), and arthritis flare (all grades, 11%). Translational studies using gene expression profiling and immunohistochemistry showed that perifosine inhibited pGSK activity downstream of Akt, and inhibited nuclear factor κB activity.

Conclusion: Perifosine resulted in at least a minimal response in 35% of patients and a median progression-free survival of 12.6 months in patients with relapsed or relapsed/refractory WM, as well as *in vivo* inhibition of pGSK activity. The results of this study warrant further evaluation of perifosine in combination with rituximab or other active agents in patients with WM. *Clin Cancer Res; 16(3); 1033–41.* ©*2010 AACR.*

Waldenström's macroglobulinemia (WM) is a distinct lymphoproliferative disorder characterized by bone marrow infiltration with lymphoplasmacytic cells, along with an IgM monoclonal gammopathy (1–4). Despite advances in the therapy of WM, the disease remains incurable, thereby necessitating the development of novel therapeutics (3, 5, 6). Current therapies used in the up-front or relapsed settings include alkylator agents (chlorambucil), nucleoside analogues, and rituximab (7–10). In the salvage setting, overall response rate is in the range of 30% to 40%, with median response duration of 6 months to 1 year (8, 11). Therefore, the development of therapies specifically targeting the malignant clone in WM in these patients is a priority.

Increased expression of Akt plays an important role in the initiation and progression of malignancies, specifically in lymphomas. The phosphatidylinositol-3-kinase pathway enhances cell survival by stimulating cell proliferation and inhibiting apoptosis (12–16). Akt, downstream of phosphatidylinositol-3-kinase, regulates multiple signaling pathways controlling cell cycle, proliferation, and resistance to apoptosis (13, 15).

Perifosine (1,1-dimethyl-4 [(octadecyloxy)hydroxyphosphinyl]oxy-piperidinium inner salt; Keryx Biopharmaceuticals) is a novel Akt inhibitor belonging to a class of lipid-related compounds called alkylphospholipids (17, 18). Phase I and phase II studies have been conducted with perifosine (19). The most frequently observed toxicities were gastrointestinal events (nausea, vomiting, and diarrhea) and fatigue. An oral dose of 150 mg/d was determined in phase II studies. We previously did preclinical studies demonstrating that perifosine specifically inhibits Akt in WM primary cells and cell lines (17, 18). Perifosine led to significant inhibition of proliferation and induction of apoptosis in WM cells *in vitro*, but not

Authors' Affiliations: [1]Dana-Farber Cancer Institute, [2]Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts; [3]Community Hospital of Monterey Peninsula, Monterey, California; [4]Center for Cancer Research, National Cancer Institute, Bethesda, Maryland; and [5]Keryx Biopharmaceuticals, Inc., New York, New York

Corresponding Author: Irene M. Ghobrial, Medical Oncology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115. Phone: 617-632-4198; Fax: 617-582-8608; E-mail: irene_ghobrial@dfci.harvard.edu.

doi: 10.1158/1078-0432.CCR-09-1837

Published OnlineFirst January 26, 2010; DOI: 10.1158/1078-0432.CCR-09-1837

Translational Relevance

Waldenström's macroglobulinemia (WM) is a rare, low-grade lymphoproliferative disorder. We conducted a phase II clinical trial testing the efficacy and safety of the Akt inhibitor perifosine in patients with relapsed/refractory WM. We also did correlative studies to examine *in vivo* inhibition of Akt. Perifosine resulted in at least a minimal response in 35% and stable disease in 54% of patients. The median progression-free survival was 12.6 months. Using immunohistochemistry, we were able to show that there was a significant reduction of pGSK3/β at the protein level in the majority of samples tested. Similarly, we found that perifosine significantly inhibited the expression of multiple members of the nuclear factor-κB family of genes, confirming our *in vitro* studies showing activity of perifosine on this pathway. The results of this study warrant further evaluation of perifosine in combination with rituximab or other active agents in patients with WM.

in normal donor peripheral blood and hematopoietic progenitors (20). Perifosine induced significant reduction in WM tumor growth *in vivo* in a subcutaneous xenograft model through inhibition of Akt phosphorylation and downstream targets (20). We also showed that Akt pathway downregulation inhibited migration and adhesion *in vitro*, and homing of WM tumor cells to the bone marrow microenvironment *in vivo* (20). Based on these studies, we tested the clinical and *in vivo* activity of perifosine in patients with relapsed or relapsed/refractory WM.

Patients and Methods

Patients. Study participants were at least 18 y of age with relapsed/refractory WM. Patients must have had prior therapy with at least one treatment regimen and any number of prior therapies was allowed. Patients must have had symptomatic disease requiring therapy for WM according to the consensus recommendations for WM (7). Patients had measurable monoclonal IgM immunoglobulin concentration on serum electrophoresis and IgM immunoglobulin protein twice the upper limit of normal by nephelometry, as well as the presence of lymphoplasmacytic cells in the bone marrow. Eligibility criteria included an Eastern Cooperative Oncology Group performance status of 2 or less, a serum concentration of aspartate aminotransferase or alanine aminotransferase <3 times the upper limit of the reference range, a serum total bilirubin level <2 times the upper limit of the reference range, a measured creatinine level <2 times the upper limit of the reference range, a platelet count of ≥75,000/mm^2, and an absolute neutrophil count of at least 1,000/mm^2. Exclusion criteria included cytotoxic chemotherapy ≤3 wk, biological therapy ≤2 wk, or corticosteroids ≤2 wk prior to registration. All patients gave written informed consent before entering the study, which was done in accordance with the Declaration of Helsinki; approval was obtained from the institutional review board at each of the participating centers.

Study design and treatment. Patients received perifosine orally at 150 mg daily after food for 28-d cycles. Patients with progressive disease after two cycles were taken off therapy. Patients with stable or responsive disease continued on therapy. Participants received six cycles of therapy, and were allowed to stay on therapy until disease progression if they had continued clinical benefit or stable disease (see Consort diagram). The primary objective was the proportion of patients with at least a minimal response and secondary end points included safety, event-free survival, and progression-free survival.

Assessment of efficacy. Tumor assessment was done using the consensus panel recommendations (21, 22). Response included complete remission, partial remission (PR), and minimal response (MR) using serum protein electrophoresis. Response was also assessed by IgM using nephelometry. Patients were assessed every 28 d for the first 12 mo on therapy and every 3 mo thereafter. Patients who came off therapy were monitored every 3 mo until they progressed, were treated with another therapy, or died.

Assessment of safety. 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 d after the last dose of perifosine.

Immunohistochemistry. Bone marrow biopsies from 11 patients at pretreatment, during therapy (at cycle 3), and at the end of therapy were fixed in Zenker's formalin, embedded in paraffin blocks, and sectioned. Sections were stained for pGSK3α/β (pGSK; Cell Signaling Technology, Inc.).

Gene expression profiling. Total RNA was isolated from primary CD19+ cells, which were isolated from bone marrow aspirates of patients before (n = 6) and during therapy (cycle 3, n = 5) using RNeasy kit (Qiagen), as described by the manufacturer, and analyzed with Affymetrix U133 plus 2.0 geneChips (Affymetrix). The normalization of arrays and calculation of expression values was done using the DNA-chip analyzer (dChip) program. Functional classification and biochemical pathway maps were evaluated using Database for Annotation, Visualization, and Integrated Discovery software.

Statistical analysis. A two-stage design was used, with 17 eligible patients entered on the first stage and an additional 20 eligible patients added to the second stage if at least 4 of the 17 patients achieved a MR. Patient characteristics were summarized and compared between responders and nonresponders using Fisher's exact test for binary end points and Wilcoxon rank sum test for continuous end points. Estimated response proportions were reported along with exact two-stage binomial 90% confidence intervals (CI). Median time to response and duration of response were reported among responding patients. Estimates of time to progression, event-free survival, progression-free survival, and overall survival were calculated using Kaplan-Meier methodology. Cox proportional hazard model was used

Published OnlineFirst January 26, 2010; DOI: 10.1158/1078-0432.CCR-09-1837

Table 1. Baseline characteristics for all patients and their prior therapies

(A) Baseline characteristics

	n	(%)
Gender, male	27	73
Median age, y	65	Range (44–82)
Median hemoglobin (g/dL)	11.2	Range (7.0–14.9)
Anemia		
Hemoglobin <10 (g/dL)	12	32
Hemoglobin <12 (g/dL)	25	68
Median platelet	215	Range (36–390)
Median IgM (mg/dL)	3,120	Range (870–8,480)
IgM >1,000 mg/dL	34	92
IgM <1,000 mg/dL	3	8
Median M spike by SPEP	2.0	Range (0.5–4.9)
Median percentage of bone marrow involvement	70	Range (10–95)
Evidence of disease by CT scan	24	65
β2 Microglobulin		
>3.5	12	32
>5.5	2	5
Median β2 microglobulin (mg/dL)	2.9	(1.4–6.8)
ISS-WM		
Intermediate/high risk	19	51
Low risk	18	49
ECOG performance status		
0	34	92
1 and 2	3	8
Disease status		
Relapsed	18	49
Refractory	8	22
Relapsed and refractory	11	30
No. of prior treatment(s)		
1	12	32
2	10	37
3	6	16
>3	9	25
Prior therapy		
Chlorambucil, chlorambucil/prednisone	8	21.62
2CdA, fludarabine, pentostatin	20	54.05
CHOP, CVP, cytoxan, mitoxantrone	11	29.73
Rituximab alone or with others	31	83.78
Prednisone, dexamethasone, solumedrol	6	16.22
Others such as bortezomib, sildenafil, melphalan, prednisone, and thalidomide	9	24.32

(B) Efficacy

	n = 37	% (CI)
Response by M spike		
MR	9	24 (11.8–41.2)
PR	4	11 (3.0–25.4)
SD	20	54 (36.9–70.5)
PD	4	11 (3.0–25.4)

(*Continued on the following page*)

Published OnlineFirst January 26, 2010; DOI: 10.1158/1078-0432.CCR-09-1837

Table 1. Baseline characteristics for all patients and their prior therapies (Cont'd)

(B) Efficacy

	n = 37	% (CI)
Response by IgM		
MR	11	30 (15.9–47.0)
PR	3	8 (0.7–18.2)
SD	19	51 (36.9–70.5)
PD	4	11 (3.0–25.4)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; PD, primary progressive disease; CT, computed tomography; SPEP, serum protein electrophoresis; CHOP, cyclophosphamide-Adriamycin-vincristine-prednisone; CVP, cyclophosphamide-vincristine-prednisone.

to evaluate the effect of multiple factors on time to event end points. All *P* values were two-sided. Statistical analyses were done using SAS statistical software (version 8.2, SAS Institute).

Results

Patients and treatment

From October 2006 to November 2007, 37 patients were enrolled in two centers. Table 1A shows selected characteristics and prior types of therapy for the 37 patients. The median age at enrollment was 65 years (range, 44-82). The median IgM level was 3,120 mg/dL (range, 870-8,480), and the median M spike by serum protein electrophoresis was 2.0 gm/dL (range, 0.5-4.9). Only three (8%) patients had an IgM level below 1,000 mg/dL and they had symptomatic disease requiring therapy such as progressive anemia with significant involvement in the bone marrow or bulky lymphadenopathy. The median hemoglobin level at enrollment was 11.2 gm/dL (range, 7.0-14.9). Twelve (32%) patients had a hemoglobin level of <10.0 gm/dL, and 25 (68%) had a hemoglobin level of <12.0 gm/dL). The median β2 microglobulin at enrollment was 2.9 mg/dL (range, 1.4-6.8). The median percentage of bone marrow involvement was 70 (range, 10-95). There was evidence of disease in soft tissue assessment including organomegaly or lymphadenopathy in 24 patients (65%). Almost 50% of the patients were intermediate or high-risk by the International Staging System of WM (ISS-WM) staging system at the time of enrollment.

Thirty-one patients (84%) received prior rituximab alone or in combination with other agents. The median duration of treatment with perifosine was 5.6 months (range, 1.8-21.5+). A total of 21 patients (57%) completed the treatment duration with six or more cycles of therapy on perifosine.

Efficacy and time to event end point analysis

Of the 37 patients, 4 achieved PR (11%), 9 achieved MR (24%), and 20 showed stable disease (54%), with only 4 patients who showed progressive disease while on therapy (11%; Table 1B). Among the 13 patients with MR/PR, 54% (n = 7) were low-risk, 31% (n = 4) were intermediate-risk, and 15% were high-risk (n = 2) according to ISS-WM. The median time to first response was 2.0 months (range, 1.1-4.9) and the median time to best response was 2.8 months (1.1-21.4).

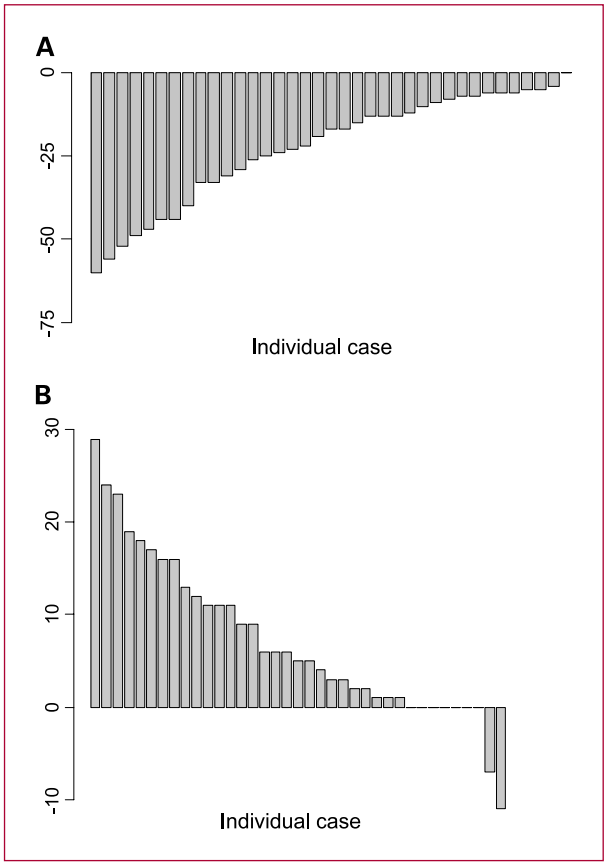

Fig. 1. A, the median decrease in IgM from baseline among all 37 patients was 650 mg/dL (range, 0–3,370) and the median percentage of decrease in IgM in all 37 patients was 22% (range, 0–60%). B, the median improvement in hemoglobin from baseline was 0.6 gm/dL (range, –1 to 2.4 gm/dL) and the median percentage of change in hemoglobin among all 37 patients was 5% (range, –11% to 29%).

Published OnlineFirst January 26, 2010; DOI: 10.1158/1078-0432.CCR-09-1837

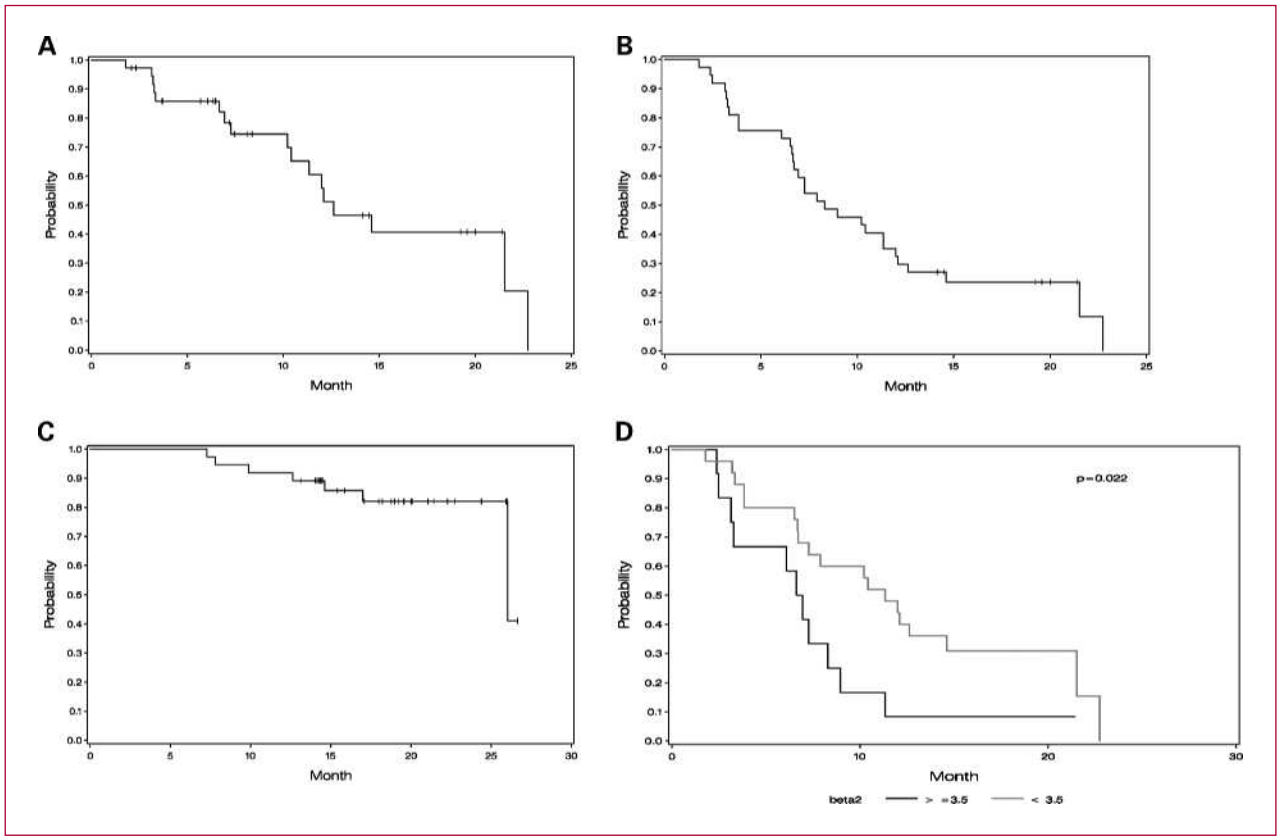

Fig. 2. A, progression-free survival. The median time to progression and progression-free survival were similar among all 37 patients and was at 12.6 mos, 90% CI (10.2–22.7 mos). B, event-free survival. The median time to event-free survival was 8.3 mos (90% CI, 6.7–12.0 mos). C, overall survival. Kaplan-Meier curve for overall survival. The median overall survival was 26 mos, 90% CI (26.0-months, no estimate for upper limit). Death occurred in seven patients. D, the effect of β2 microglobulin (>3.5 mg/dL) on event-free survival. β2 Microglobulin of >3.5 mg/dL was associated with a worse event-free survival in these patients (P = 0.002; hazard ratio, n = 2.42).

Responses based on IgM were similar to that observed by serum protein electrophoresis (Table 1B). The overall response rate (MR + PR) by paraprotein using IgM was 38% (90% CI, 25-53). Patients with low IgM levels (<1,000 mg/dL; n = 3) did not show response to therapy, two patients showed stable disease and one patient showed progressive disease.

The median decrease in IgM among all 37 patients was 650 mg/dL (range, 0-3,370) and the median percentage of decrease in IgM in all 37 patients was 22% (range, 4-60%; Fig. 1A). The median improvement in hemoglobin was 0.6 gm/dL (range, −1 to 2.4 gm/dL) and the median percentage of increase in hemoglobin among all 37 patients was 5% (range,−11% to 29%; Fig. 1B).

Of the 37 patients, 17 progressed (4 with primary progression, 5 progressed after stable disease, and 8 after responding to treatment), 13 started nonprotocol therapy without documented progression, 2 died without documented primary progressive disease but with next therapy, and 18 are still alive without documented primary progressive disease (11 out of 18 started next therapy). Death occurred in seven patients, all of whom were off perifosine at the time of death. Of these, six deaths occurred with causes due to progressive disease and complications related to subsequent therapies, and one death occurred due to a motor vehicle accident.

At a median follow-up of 19.5 months, the median time to progression of disease and progression-free survival were similar among all 37 patients and were 12.6 months with a 90% CI (10.2-22.7; Fig. 2A). The median event-free survival was 8.3 months with a 90% CI (6.7-12.0; Fig. 2B). Primary progressive disease (progression while on therapy) occurred early with a median of 3.2 months. The median treatment duration was 5.6 months (range, 1.8-21.5+). The median overall survival was 26 months, 90% CI (26.0, no estimate for upper limit; Fig. 2C).

Prognostic factors

We also sought to investigate markers that influenced progression-free survival including age, ISS-WM staging system, β2 microglobulin, number of previous therapies, or percentage of lymphoplasmacytic cells in the bone marrow at enrollment. Of these variables, a significant difference in progression-free survival was detected for β2 microglobulin (P = 0.03; hazard ratio, 1.4). In addition, β2 microglobulin of >3.5 mg/dL was associated

Published OnlineFirst January 26, 2010; DOI: 10.1158/1078-0432.CCR-09-1837

Table 2. Drug-related adverse events

Toxicity type	G_{1-2} (%)	G_{3-4} (%)
Hematologic toxicities		
Anemia	24 (65)	1 (3)
Leukocytes	20 (54)	4 (11)
Neutrophils	18 (49)	4 (11)
Thrombocytopenia	3 (8)	
Gastrointestinal		
Nausea	28 (76)	
Vomiting	24 (65)	
Diarrhea	30 (81)	
Gastritis/dyspepsia	8 (22)	
Abdomen, pain	5 (14)	
Musculoskeletal		
Arthritis	3 (8)	1 (3)
Visual		
Vision, blurred	3 (9)	1 (3)
Eye, pain	2 (5)	1 (3)
Infections		
Fever without neutropenia	1 (3)	
Infection, G_{0-2} neutropenia (lung, bronchi, others)	2 (5)	1 (3)
Constitutional		
Fatigue	23 (62)	1 (3)
Weight loss	5 (14)	
Dizziness	5 (14)	

NOTE: Related includes possibly, probably or definitely.

with poor progression-free survival in these patients (P = 0.002; hazard ratio, 2.4; Fig. 2D).

Safety

The most common adverse events were gastrointestinal symptoms, fatigue, cytopenias, and flare of arthritis/joint effusions (Table 2). Overall, five patients experienced grade 3 or 4 anemia, four were unrelated to therapy and one was possibly related to therapy. Interestingly, arthritis/joint effusions occurred in four patients (three grades 1-2, and one grade 3). All of these patients responded to perifosine. The etiology of this event is not known and it did not seem to be due to hyperuricemia in any of the patients. Dose reductions to 100 mg occurred in 16 patients (43%) due to neutropenia, gastrointestinal symptoms, or arthritis.

Translational studies

Gene expression analysis comparing pretreatment and posttreatment samples. We first did gene expression profiling in bone marrow–derived CD19+ cells of matched samples from six patients before therapy and of five patients during therapy (after two cycles of therapy). Supervised clustering analysis, done by comparing pretreatment and posttreatment samples, showed a significant separation at 1.5-fold difference in gene expression and $P < 0.05$ (Fig. 3A). There were 162 genes significantly changed in expression in response to perifosine. We found reduced expression of several genes involved in the adhesion and migration processes, as well as regulators of the nuclear factor-κB pathway (Table 3). Interestingly, mitogen-activated protein kinase family genes showed increased expression in response to perifosine. Statistical correlation with clinical response was not done given the small number of samples analyzed.

Regulation of GSK signaling by perifosine. Given that our previous studies showed that total Akt did not change in response to perifosine, and that changes only occurred at the posttranslational level at phosphorylation, we therefore investigated whether perifosine inhibits pGSK activity *in vivo* using immunohistochemistry. Phosphorylation of GSK occurs downstream of Akt and indicates Akt kinase activity (20). Immunohistochemistry was done on seven matched samples from pretherapy and on their corresponding samples after two cycles of therapy and at the end of treatment. As shown in Fig. 3B, there was an increased expression of pGSK3 in most of the samples tested (five of seven), and a reduction in the level of pGSK in five of seven posttreatment samples at the end of study, indicating that pGSK was inhibited in response to perifosine therapy. Statistical correlation with response was not done given the small number of samples analyzed.

Discussion

In this phase II study, we showed that 35% of the patients achieved at least a MR to single-agent perifosine, with another 54% of patients showing stabilization of their disease progression while on therapy, and 11% of patients showed progression. Other targeted therapeutic agents that have shown efficacy in WM include thalidomide, bortezomib, and alemtuzumab (8, 23–25). The response rate of MR and better using these agents ranges between 25% and 80% (8, 23–25). Unfortunately, some of these agents have a high toxicity profile such as alemtuzumab therapy in WM (26–28). The use of bortezomib as a single agent in WM has been tested in relapsed WM (26, 27, 29). Chen et al. (26) treated 27 patients with bortezomib in both untreated (44%) and previously treated (56%) patients with WM. The percentage of patients with MR or better to bortezomib was 78%, with major responses (PR or better) observed in 44% of patients; however, sensory neuropathy occurred in 20 of 27 patients, 5 patients with grade >3 disease which occurred following two to four cycles of therapy. In addition, the time to progression was relatively short in studies using single-agent bortezomib, with a median time to progression of 7.9 months in the study by Treon et al. (27). Therefore, there is a need to develop therapeutic agents that do not cause neuropathy and lead to longer progression-free survival in this patient population with relapsed/refractory WM.

The results of this study show promising activity in this agent, especially as it was used in patients with relapsed

Published OnlineFirst January 26, 2010; DOI: 10.1158/1078-0432.CCR-09-1837

or refractory symptomatic disease. In this study, 41% of these patients had three or more lines of prior therapy, which included nucleoside analogues, alkylating agents, and rituximab. The median percentage of involvement of the bone marrow with lymphoplasmacytic cells was 70% and 65% of patients that had organomegaly or lymphadenopathy according to computed tomography scan measurements. These numbers are significantly higher compared with the recent review of 365 patients that presented with WM, in which patients usually had 30% involvement in the bone marrow and only 10% to 15% organomegaly or lymadenopathy (30). All of the patients had to show symptomatic disease requiring therapy at the time of enrollment in this study according to the second consensus recommendations for the therapy of WM (7). Although ISS-WM was not described in patients with relapsed or refractory WM (31), 51% of the patients in our study had intermediate or high-risk ISS-WM at the time of enrollment.

Responses were durable and occurred rapidly. The median time to progression and progression-free survival was 12.6 months (90% CI, 10.2-22.7) with a median follow-up of 19.5 months. This was relatively long compared with other targeted agents used in a similar population of relapsed WM such as bortezomib, in which the median time to progression was only 7.9 months in the study by Treon et al. (27). This study represents one of the first phase II clinical trials showing the activity of single-agent perifosine in hematologic malignancies. Based on the safety and activity of single-agent perifosine in this study, and on our preclinical studies of its combination with rituximab and bortezomib (32), we believe that perifosine should be evaluated in combination with other active agents in WM such as rituximab or bortezomib in future clinical trials.

In this study, we found that elevated β2 microglobulin correlated poorly with progression-free survival. Prior studies have evaluated the prognostic relevance of this protein in newly diagnosed patients with WM (31), but have not described its relevance in the relapsed setting. This study, therefore, indicates that elevated β2 microglobulin is an important marker to be assessed in future clinical trials, even in patients with relapsed disease.

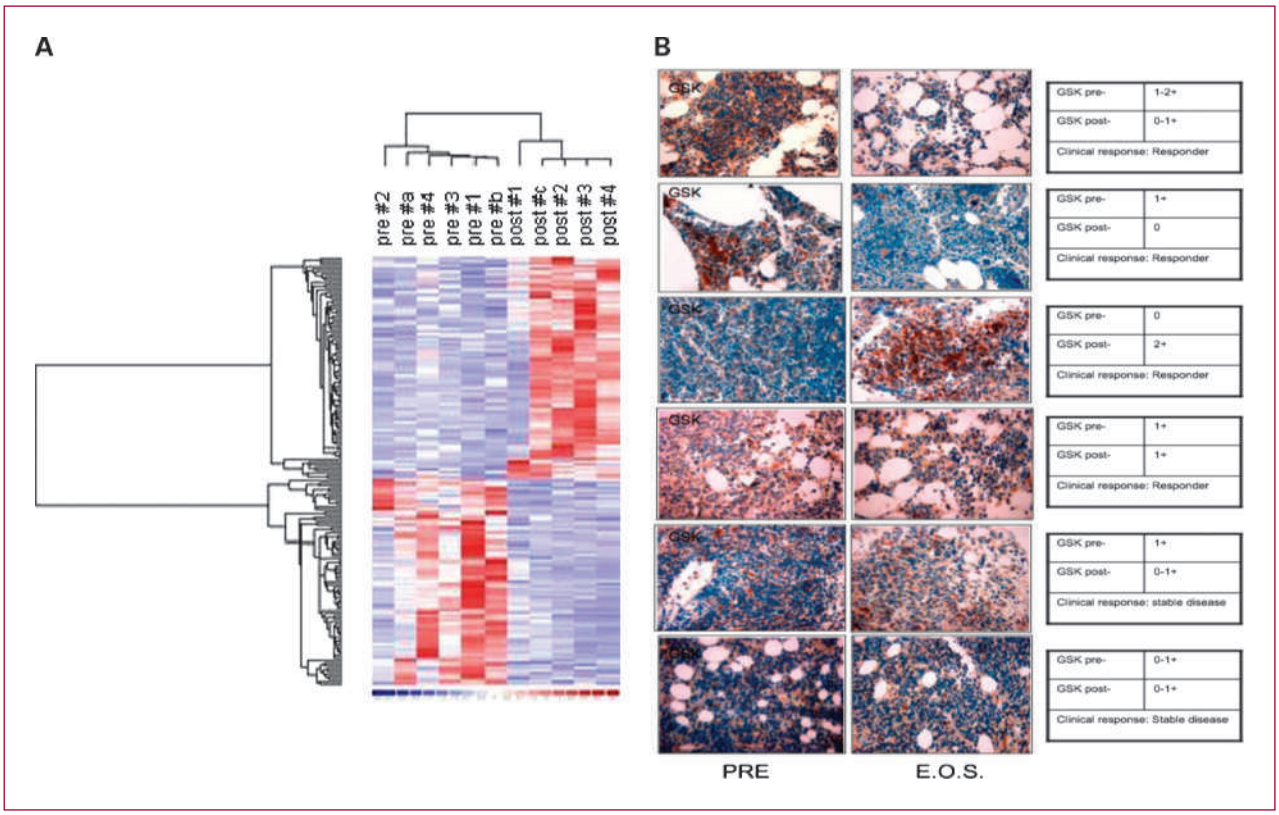

Fig. 3. A, supervised clustering of gene expression profiling of pretreatment versus posttreatment samples. Purified cRNA (15 mg) isolated from primary WM cells was hybridized to HG-U133Plus2.0 GeneChip (Affymetrix). Supervised clustering analysis in six pretreatment and five posttreatment patients. Fold change is shown by the intensity of induction (red) or suppression (blue; $P < 0.05$). Samples 1, 2, 3, and 4 had matching pretreatment and posttreatment samples, whereas samples a and b were available for pretreatment only and sample c was available in posttreatment only. B, immunohistochemistry of phosphorylated GSK in pretreatment and posttreatment samples. Scoring was done by an independent pathologist, who was blinded to the clinical results. Each sample was given a score of 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining of tumor cells) depending on the intensity of pGSK staining in the lymphoplasmacytic cells. For each sample, a table was placed with the score given for the pretreatment and posttreatment samples and the corresponding clinical response observed by monoclonal protein in these patients. Samples at cycle 3 were also obtained and showed similar results to the end of study samples, and therefore, were not included in the figure.

Published OnlineFirst January 26, 2010; DOI: 10.1158/1078-0432.CCR-09-1837

Table 3. Genes that showed changes in expression in response to perifosine

AFFY_ID	DAVID gene name	Category
Perifosine-downregulated genes		
240690_at	Hypothetical protein DKFZP761P0423	Tyrosine protein kinases 238735_at
240613_at	Janus kinase 1	
240850_at	Dual-specificity tyrosine phosphorylation–regulated kinase 1A	
238735_at	Transcription factor 12	Transcription factors
215164_at	Transcription factor 4	
1570299_at, 244414_at, 232333_at	Mastermind-like 2	
232791_at, 240867_at	Regulatory factor X, 3	
1559078_at	B-cell CLL/lymphoma 11A	
242572_at	GAB1 (GRB2-associated binding protein 1)	Adhesion and migration
244061_at	ARHGAP15 (Rho GTPase-activating protein 15)	
237001_at	NIBP (NIK and IKK β-binding protein)	Nuclear factor-κB activators
202987_at	TRAF3IP2 (TRAF3-interacting protein 2)	
243450_at	AKAP13 (a kinase anchor protein 13)	
232210_at, 232614_at	BCL2 (B-cell CLL/lymphoma 2)	Antiapoptotic protein
Perifosine-upregulated genes		
201538_s_at	DUSP3 (dual-specificity phosphatase 3)	Growth factor inhibitors
207704_s_at, 210872_x_at	GAS7 (growth arrest–specific 7)	
244652_at	Immunoglobulin superfamily, member 2	Tyrosine kinase inhibitors
208602_x_at	cd6 antigen	MAPK activators

Abbreviations: DAVID, Database for Annotation, Visualization, and Integrated Discovery; CLL, chronic lymphocytic leukemia; MAPK, mitogen-activated protein kinase.

We were not able to identify prognostic significance for ISS-WM in this relapsed population treated with perifosine. The ISS-WM was described in newly diagnosed previously untreated patients who were subsequently treated with alkylating agents and nucleoside analogues (31). In the current study, patients had relapsed or refractory WM with 41% of these patients having three or more prior lines of therapy. Most of the patients in this study received prior rituximab alone or in combination. In addition, more than 50% of the patients received nucleoside analogues. The use of chlorambucil in this study (20%) was not as high as in the study used to assess ISS-WM in WM (31). This difference may be due to practice differences between the United States and Europe (because alkylating agents such as chlorambucil are not widely used in the United States compared with Europe). We cannot compare the patient population with relapsed and refractory WM in this trial to newly diagnosed untreated patients with WM in the original ISS-WM staging. In our current study of patients with relapsed or refractory WM who were treated with perifosine, we were unable to identify a prognostic significance for ISS-WM. However, β2 microglobulin correlated with poor prognosis. Future studies to further examine the role of ISS-WM as a prognostic indicator in relapsed WM are warranted.

Perifosine was generally well tolerated with minimal grade 3 and 4 toxicities. The main side effects were cytopenias and gastrointestinal toxicities. Dose reduction improved the degree of gastrointestinal toxicities and current studies using perifosine are evaluating doses of 100 or 50 mg. Interestingly, arthritis in the form of large joint effusions, including arthritis of the knee or elbow, occurred in four patients. These patients showed response to perifosine. The etiology of toxicity is not well known and was not observed in previous studies with perifosine. Future studies are warranted to identify the underlying mechanism of arthritic flare in patients receiving perifosine.

To further investigate the *in vivo* activity of perifosine in WM, we did gene expression profiling and immunohistochemistry, and identified a signature that differentiated samples of pretreatment versus posttreatment. The most differentially expressed genes were regulators of adhesion. This highlights the significant effect of perifosine on adhesion, potentially through the activity of this class of agents on lipid rafts. Recent studies have shown that this class of agents could also induce apoptosis through their activity on the lipid rafts (33).

We next examined the activity of perifosine *in vivo* on the Akt signaling pathway. These studies were designed to ask the question, "did we hit the target *in vivo*?" Although the sample size was small, we were able to show, in the majority of samples tested, that there was a significant reduction of pGSK3/β at the protein level using immunohistochemistry. Similarly, we found that perifosine significantly inhibited the expression of multiple members of the nuclear factor-κB

Published OnlineFirst January 26, 2010; DOI: 10.1158/1078-0432.CCR-09-1837

family of genes, confirming our *in vitro* studies showing activity of perifosine on this pathway.

In summary, we conducted a phase II clinical trial of perifosine in WM and showed that single-agent perifosine induces at least a MR in 35% of patients with relapsed or refractory disease, stable disease in 54%, and a median progression-free survival of over 1 year. Future studies using this agent in combination with rituximab or other agents active in WM are warranted.

Disclosure of Potential Conflicts of Interest

I.M. Ghobrial, member of speakers' bureau for Novartis, Millenium, and Celgene and received funding from Keryx Biopharmaceuticals, Millenium, and Novartis; P. Sportelli, employed by Keryx Biopharmaceuticals; K. Anderson, received research funding and member of speakers' bureau for Novartis, Millenium, and Celgene; P. Richardson, member of speakers' bureau for Millenium and Celgene.

Acknowledgments

We thank Jennifer Stedman for editing and reviewing the manuscript.

Grant Support

R21 CA126119-01, International Waldenström Macroglobulinemia Foundation, the Michelle and Steven Kirsch lab for Waldenstrom macroglobulinemia, and Keryx Biopharmaceuticals, Inc.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 7/15/09; revised 11/22/09; accepted 12/3/09; published OnlineFirst 1/26/10.

References

1. Dimopoulos M, Panayiotidis A, Moulopoulos P, et al. Waldenstrom's macroglobulinemia: clinical features, complications, and management. J Clin Oncol 2000;18:214–6.
2. Ghobrial I, Witzig M. Waldenstrom macroglobulinemia. Curr Treat Options Oncol 2004;5:239–47.
3. Dimopoulos MA, Kyle R, Anagnostopoulos A, Treon SP. Diagnosis and management of Waldenstrom's macroglobulinemia. J Clin Oncol 2005;23:1564–77.
4. Owen R, Treon G, Al-Katib P, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol 2003;30:110–5.
5. Ghobrial I, Gertz M, Fonseca A. Waldenstrom macroglobulinaemia. Lancet Oncol 2003;4:679–85.
6. Kyle R, Treon A, Alexanian P, et al. Prognostic markers and criteria to initiate therapy in Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol 2003;30:116–20.
7. Gertz M, Anagnostopoulos A, Anderson A, et al. Treatment recommendations in Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol 2003;30:121–6.
8. Treon S, Morel P, Leblond P, Fermand V. Report of the Third International Workshop on Waldenstrom's macroglobulinemia. Clin Lymphoma 2005;5:215–6.
9. Treon S, Emmanouilides P, Kimby C, et al. Extended rituximab therapy in Waldenstrom's macroglobulinemia. Ann Oncol 2005;16:132–8.
10. Dimopoulos M, O'Brien A, Kantarjian S, et al. Fludarabine therapy in Waldenstrom's macroglobulinemia. Am J Med 1993;95:49–52.
11. Dimopoulos M, Weber A, Delasalle D, et al. Treatment of Waldenstrom's macroglobulinemia resistant to standard therapy with 2-chlorodeoxyadenosine: identification of prognostic factors. Ann Oncol 1995;6:49–52.
12. Cantrell DA. Phosphoinositide 3-kinase signalling pathways. J Cell Sci 2001;114:1439–45.
13. Fresno Vara J, Casado A, de Castro E, et al. PI3K/Akt signalling pathway and cancer. Cancer Treat Rev 2004;30:193–204.
14. Hennessy B, Smith T, Ram L, et al. Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat Rev Drug Discov 2005;4:988–1004.
15. Pene F, Claessens Y, Muller E, et al. Role of the phosphatidylinositol 3-kinase/Akt and mTOR/P70S6-kinase pathways in the proliferation and apoptosis in multiple myeloma. Oncogene 2002;21:6587–97.
16. Dancey JE. Molecular targeting: PI3 kinase pathway. Ann Oncol 2004;15 Suppl 4:iv233–9.
17. Hideshima T, Catley L, Yasui H, et al. Perifosine, an oral bioactive novel alkylphospholipid, inhibits Akt and induces *in vitro* and *in vivo* cytotoxicity in human multiple myeloma cells. Blood 2006;107:4053–62.
18. Ruiter G, Zerp A, Bartelink F, et al. Anti-cancer alkyl-lysophospholipids inhibit the phosphatidylinositol 3-kinase-Akt/PKB survival pathway. Anticancer Drugs 2003;14:167–73.
19. Crul M, Rosing H, de Klerk G, et al. Phase I and pharmacological study of daily oral administration of perifosine (D-21266) in patients with advanced solid tumours. Eur J Cancer 2002;38:1615–21.
20. Leleu X, Jia X, Runnels J, et al. The Akt pathway regulates survival and homing in Waldenstrom macroglobulinemia. Blood 2007;110:4417–26.
21. Kimby E, Treon S, Anagnostopoulos P, et al. Update on recommendations for assessing response from the Third International Workshop on Waldenstrom's Macroglobulinemia. Clin Lymphoma Myeloma 2006;6:380–3.
22. Weber D, Treon S, Emmanouilides P, et al. Uniform response criteria in Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol 2003;30:127–31.
23. Dimopoulos M, Zomas A, Viniou A, et al. Treatment of Waldenstrom's macroglobulinemia with thalidomide. J Clin Oncol 2001;19:3596–601.
24. Hunter Z, Branagan A, Treon ST, et al. Campath-1H in Waldenström's macroglobulinemia. Blood 2004;104, Abstract, 2004.
25. Treon S, Hunter Z, Matous J, et al. Phase II study of bortezomib in Waldenstrom's macroglobulinemia: results of WMCTG trial 03-248. Blood 2005;106:Abstract.
26. Chen C, Kouroukis I, White T, et al. Bortezomib is active in patients with untreated or relapsed Waldenstrom's macroglobulinemia: a phase II study of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007;25:1570–75.
27. Treon S, Hunter P, Matous R, et al. Multicenter clinical trial of bortezomib in relapsed/refractory Waldenstrom's macroglobulinemia: results of WMCTG Trial 03-248. Clin Cancer Res 2007;13:3320–5.
28. Hunter Z, Boxer M, Kahl B, et al. Phase II study of alemtuzumab in lymphoplasmacytic lymphoma: results of WMCTG trial 02-079 [abstract]. J Clin Oncol 2006;24:427.
29. Dimopoulos MA, Anagnostopoulos A, Kyrtsonis A, et al. Treatment of relapsed or refractory Waldenstrom's macroglobulinemia with bortezomib. Haematologica 2005;90:1655–8.
30. Treon SP. How I treat Waldenstrom's macroglobulinemia. Blood 2009;114:2375–85.
31. Morel P, Duhamel A, Gobbi P, et al. International prognostic scoring system for Waldenstrom's macroglobulinemia. Blood 2009;113:4163–70.
32. Leleu X, Eeckhoute J, Jia X, et al. Targeting NF-{kappa}B in Waldenstrom macroglobulinemia. Blood 2008;111:5068–77.
33. Van der Luit A, Vink H, Klarenbeek R, et al. A new class of anticancer alkylphospholipids uses lipid rafts as membrane gateways to induce apoptosis in lymphoma cells. Mol Cancer Ther 2007;6:2337–45.

Published OnlineFirst January 26, 2010; DOI: 10.1158/1078-0432.CCR-09-1837

Clinical Cancer Research

Clinical and Translational Studies of a Phase II Trial of the Novel Oral Akt Inhibitor Perifosine in Relapsed or Relapsed/Refractory Waldenström's Macroglobulinemia

Irene M. Ghobrial, Aldo Roccaro, Fangxin Hong, et al.

Clin Cancer Res Published OnlineFirst January 26, 2010.