

Clinical Cancer Research



A Multicenter Phase II Study of Single-Agent Enzastaurin in Previously Treated Waldenström Macroglobulinemia

Irene M. Ghobrial, Philippe Moreau, Brianna Harris, et al.

Clin Cancer Res 2012;18:5043-5050. Published OnlineFirst August 9, 2012.

Updated Version Access the most recent version of this article at:
doi:[10.1158/1078-0432.CCR-12-0181](https://doi.org/10.1158/1078-0432.CCR-12-0181)

Cited Articles This article cites 30 articles, 19 of which you can access for free at:
<http://clincancerres.aacrjournals.org/content/18/18/5043.full.html#ref-list-1>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.

A Multicenter Phase II Study of Single-Agent Enzastaurin in Previously Treated Waldenström Macroglobulinemia

Irene M. Ghobrial¹, Philippe Moreau², Brianna Harris¹, Tiffany Poon¹, Eric Jourdan³, Hervé Maisonneuve⁴, Karim A. Benhadji⁵, Anwar M. Hossain⁶, Tuan S. Nguyen⁶, James E. Wooldridge⁶, and Veronique Leblond⁷

Abstract

Purpose: Enzastaurin is a serine/threonine kinase inhibitor that showed antiangiogenic, antiproliferative, and proapoptotic properties *in vitro* and antitumor activity *in vivo* in a xenograft Waldenström macroglobulinemia (WM) model. These findings provided the rationale for a multicenter phase II trial of oral enzastaurin in previously treated patients with WM.

Experimental design: Patients who were treated with 1 to 5 prior regimens and who had a baseline immunoglobulin M level 2 times or more the upper limit of normal received oral enzastaurin 250 mg twice daily (500 mg total) after a single loading dose (day 1, cycle 1) of 375 mg 3 times daily (1,125 mg total) for 8 cycles of 28 days each or until progressive disease. Six patients who progressed during treatment with enzastaurin had dexamethasone added per protocol.

Results: From July 2008 to December 2010, 42 patients were enrolled. The objective response rate (RR) was 38.1% (2 partial and 14 minor responses). One patient had grade 3 leukopenia and one patient died during the study from septic shock; both events were considered drug related. A statistically significant association between RR and interleukin 15 (IL-15) was observed, suggesting that higher concentration levels of IL-15 may be associated with better response.

Conclusion: Enzastaurin was active and well tolerated in previously treated patients with WM. Because of the small sample size of this uncontrolled study, further assessment of the relationship between IL-15 and response to enzastaurin in patients with WM is required. These results warrant further investigation of enzastaurin for the treatment of WM. *Clin Cancer Res*; 18(18); 5043–50. ©2012 AACR.

Introduction

Waldenström macroglobulinemia (WM) is an incurable low-grade lymphoplasmacytic lymphoma characterized by the presence of immunoglobulin M (IgM) monoclonal protein in the serum and lymphoplasmacytic cells in the bone marrow (1). It is rare, accounting for approximately 2% of all hematologic malignancies, and is associated with a relatively long survival from the time of diagnosis. The median age of patients at diagnosis is 65 years, and patients over the age of 65 years are considered either intermediate or high risk, which is associated with a shorter median

survival, according to the International Prognostic Scoring System for WM (2). With approximately half of patients dying of causes unrelated to the disease (3), most patients do not require immediate treatment and observation is typically recommended. Following the initiation of first-line treatment of symptomatic disease, the median survival is 5 years (4).

Currently, no specific therapy is approved for the treatment of WM. The treatment options for patients with relapsed or refractory WM depend on the patient's age, prior therapies and response, ability to tolerate therapy, and candidacy for stem cell transplantation. Patients who have a prolonged response of 1 year or more to a therapy can be effectively retreated with the same agents (5). Several agents have been evaluated, including the monoclonal antibody (mAb) rituximab, alkylating agents (such as chlorambucil), nucleoside analogues (cladribine or fludarabine), and proteasome inhibitors (bortezomib; refs. 5, 6). Given that many patients with WM are elderly and commonly used therapies are either intravenous, associated with significant adverse events, or both, safe and effective therapies are needed to manage the disease in symptomatic patients.

An antibody-based protein array analysis identified several proteins, including multiple proteins in the phosphatidylinositol-3-kinase (PI3K)/AKT pathway, that were dysregulated in patients with WM compared with patients with

Authors' Affiliations: ¹Dana-Farber Cancer Institute, Boston, Massachusetts; ²University of Nantes, Nantes Cedex, France; ³Centre Hospitalier Régional Universitaire de Nîmes, Nîmes, France; ⁴Centre Hospitalier Départemental Vendée, La Roche-sur-Yon, France; ⁵Lilly France, Suresnes, France; ⁶Eli Lilly and Company, Indianapolis, Indiana; and ⁷Hôpital Pitié-Salpêtrière, APHP, UPMC, Paris, France

Prior presentations: Study presented in part at the American Society of Hematology 51st Annual Meeting and Exposition, New Orleans, LA, December 5 to 8, 2009.

Corresponding Author: Irene M. Ghobrial, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215. Phone: 617-632-4198; Fax: 617-582-8608; E-mail: irene_ghobrial@dfci.harvard.edu

doi: 10.1158/1078-0432.CCR-12-0181

©2012 American Association for Cancer Research.

Translational Relevance

Enzastaurin has antiangiogenic, antiproliferative, and proapoptotic properties *in vitro* and antitumor activity *in vivo* in Waldenström macroglobulinemia (WM). We report the results of a phase II trial of enzastaurin administered orally twice daily to 42 patients with WM who were previously treated with 1 to 5 regimens. Six patients who progressed during treatment with enzastaurin had dexamethasone added per protocol. The objective response rate was 38.1% (2 partial and 14 minor responses). Events that were considered possibly drug related included one grade 3 leukopenia and one death due to septic shock. Correlative studies suggested that a high-baseline concentration level of interleukin 15 may be associated with better response. Enzastaurin was active and well tolerated as a twice daily oral agent in previously treated patients with WM; further studies are warranted.

normal bone marrow lymphocytes and plasma cells (7). Another study using protein array techniques and immunohistochemistry identified that protein kinase C β (PKC β) expression is upregulated in WM (8). There is also evidence that vascular endothelial growth factor is produced by plasma cells in some WM patients (9), and angiogenesis, as measured by microvessel density, is significantly greater in the bone marrow of patients with WM compared with that in controls ($P = 0.002$; 10).

Enzastaurin hydrochloride is an oral serine/threonine kinase inhibitor that targets the PKC and PI3K/AKT pathways to inhibit tumor cell proliferation, induce tumor cell apoptosis, and suppress tumor-induced angiogenesis (11). *In vitro* experiments showed that enzastaurin inhibits 90% (IC₉₀) of PKC β enzymatic activity at an enzastaurin concentration of 70 nmol/L and that enzastaurin and its major metabolite (LY326020) are approximately 97% and 95% protein bound, respectively. Therefore, a mean steady-state concentration of 1,400 nmol/L was targeted for clinical efficacy (12). In preclinical studies, enzastaurin induced apoptosis and inhibited the proliferation of the diffuse large B-cell lymphoma (DLBCL), glioblastoma, and colon carcinoma cell lines and xenografts at comparable doses (11). Based on plasma exposure and safety data from the first phase I study, enzastaurin 525 mg once daily was the recommended dose (12). However, when administered in divided doses of 250 mg twice daily, enzastaurin achieves approximately 40% higher exposure than once daily dosing without additional toxicities (13).

Treatment of WM cell lines with enzastaurin resulted in decreased proliferation, increased apoptosis, and decreased PKC β phosphorylation. In addition, treatment with enzastaurin was cytotoxic in a xenograft human mouse model of WM (8). In previous clinical studies, enzastaurin was well tolerated and showed promising activity in solid tumors and non-Hodgkin lymphoma (12, 14, 15).

We conducted an open-label, multicenter, 2-cohort (parallel), phase II trial in patients with relapsed or refractory WM or multiple myeloma based on the activity of enzastaurin in WM cells *in vitro* and nonclinical xenograft models of WM *in vivo*, as well as the safety profile established in earlier clinical studies. The primary objective was to determine whether enzastaurin was worthy of further evaluation in the treatment of these diseases based on the objective response rate (RR), and secondary objectives included TTP, safety, and the association of biomarkers with clinical outcomes. Herein, we report the results for the WM cohort; the results for patients in the multiple myeloma cohort will be reported elsewhere.

Patients and Methods

Eligibility criteria

Eligible patients had WM previously treated with at least 1 and no more than 5 regimens, an IgM paraprotein level of 2 times or more the upper limit of normal, and detectable lymphoplasmacytic cells in the bone marrow. Patients were symptomatic for WM as defined per the consensus recommendations for WM (1, 2, 16) and at least 18 years of age, with an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0, 1, or 2. Adequate organ function was required, including total bilirubin 1.5 or less \times upper limit of normal and aspartate transaminase 3 or less \times upper limit of normal, and calculated creatinine clearance 30 mL/min or more based on the Cockcroft and Gault formula.

Exclusion criteria included prior allogeneic hematopoietic stem cell transplant; inability to discontinue use of enzyme-inducing antiepileptic drugs (carbamazepine, phenobarbital, and phenytoin); prior treatment with CD20 mAb therapy 8 weeks or less, carmustine 6 weeks or less, other systemic cancer therapy 3 weeks or less, or corticosteroids 2 weeks or less before enrollment in this trial without recovery from nonhematologic toxicities from prior therapy at grade 2 or less unless otherwise stated; and significant cardiac abnormalities or baseline 12-lead electrocardiogram with QTc interval of more than 450 milliseconds in men or more than 470 milliseconds in women, personal or family history of congenital long QT syndrome, or QRS duration of more than 100 milliseconds.

This study was approved by ethical/institutional review board(s) and was conducted according to the Declaration of Helsinki, good clinical practices, and applicable laws and regulations. All patients provided written informed consent.

Treatment plan

The dose of enzastaurin used in this study (clinicaltrials.gov identifier: NCT00718419) was selected based on both safety and pharmacokinetic data from previous studies (12, 13). Steady-state concentrations of enzastaurin and its metabolite are usually achieved after 14 days of daily oral administration of enzastaurin. On the basis of the pharmacokinetic parameters of enzastaurin (12), a loading dose was used to attain near steady-state concentrations in a shorter time (<7 days).

On day 1 of cycle 1, patients took a loading dose of enzastaurin 375 mg orally 3 times (total dose of 1,125 mg). Starting on day 2 of cycle 1, enzastaurin 250 mg was taken twice daily (500 mg total daily dose) within 30 minutes after a meal. Treatment continued for 8 cycles (cycle length was 28 days), or until the patient experienced progressive disease (PD), as defined by the International Workshop on Waldenström's Macroglobulinemia (IWWM) response criteria (17), or unacceptable toxicity. Patients were allowed to continue on study treatment after cycle 8 at the discretion of the investigator. For patients who progressed during single-agent enzastaurin therapy, dexamethasone was allowed at the discretion of the investigator. Dexamethasone was administered (in combination with enzastaurin) at 20 to 40 mg orally once daily on days 1 to 4, 9 to 12, and 17 to 20 of a 28-day cycle for 4 cycles; after 4 cycles, dexamethasone was administered only on days 1 to 4. Leukocyte colony-stimulating factors and erythropoiesis-stimulating agents were allowed according to the American Society of Clinical Oncology guidelines (18, 19).

Enzastaurin was omitted for any of the following events considered possibly drug related until the event resolved: absolute neutrophil count less than $0.5 \times 10^9/L$ for more than 5 days; absolute neutrophil count less than $1.0 \times 10^9/L$ with fever (temperature of $101^\circ F/38.5^\circ C$ or more); platelet count less than $25 \times 10^9/L$; Common Terminology Criteria for Adverse Events (CTCAE), version 3.0, grade 3 or 4 transaminase elevations; or other CTCAE grade 3 or 4 nonhematologic toxicity considered clinically relevant. If the event resolved to baseline or grade 1 or less for nonhematologic or grade 2 or less for hematologic toxicity, enzastaurin was resumed at a dose of 250 mg (one 125 mg tablet twice daily) per day and may have been reescalated to the full dose if the event did not recur after 21 days. If the event did not resolve within 4 weeks, or another aforementioned event occurred during therapy at the 250 mg reduced dose, the patient was discontinued. At the investigator's discretion, a patient who had a QTc interval of more than 500 milliseconds and a change of QTc interval to baseline of more than 60 milliseconds may also have been discontinued from the study.

Dexamethasone was omitted for any of the following events considered possibly drug related until the event resolved: grade 4 hematologic toxicity or other grade 3 or 4 nonhematologic toxicity considered clinically relevant. If the event resolved to baseline or grade 1 or less for nonhematologic or grade 2 or less for hematologic toxicity, weekly dexamethasone was resumed at 40 mg (days 1, 8, 15, and 22) and may have been reescalated to the full dose (first 4 cycles: 40 mg orally once daily on days 1–4, 9–12, and 17–20; after 4 cycles: 40 mg orally once daily on days 1–4) if the event did not recur after 21 days. If the event did not resolve within 4 weeks, or another event occurred during therapy at the reduced dose, dexamethasone was discontinued (enzastaurin treatment could continue).

Baseline and treatment assessments

Patients were assessed at baseline and at each cycle with a physical examination, ECOG PS, and standard laboratory

tests including chemistry and hematology. Electrocardiograms were obtained at baseline and cycle 2.

Treatment response was assessed using the IWWM response criteria (17) during each cycle by serum protein electrophoresis and nephelometry. As per these criteria (17), complete response (CR) was defined as the disappearance of serum M protein, absence of malignant cells, resolution of lymphadenopathy/organomegaly, and no signs or symptoms attributable to WM. Partial response (PR) was defined as a 50% or more IgM reduction, a 50% or more decrease in adenopathy/organomegaly, and no new signs or symptoms of WM. Minor response (MR) was defined as a 25% or more but less than 50% IgM reduction, no progression of adenopathy/organomegaly, and no cytopenias or clinically significant symptoms. Stable disease (SD) was a less than 25% IgM reduction and less than 25% IgM increase, no progression of adenopathy/organomegaly, and no cytopenias or clinically significant symptoms. PD was defined as a 25% or more IgM increase or progression of clinically significant findings (for example, anemia, thrombocytopenia, leukopenia, or bulky adenopathy/organomegaly) or symptoms (for example, unexplained recurrent fever, drenching night sweats, weight loss, hyperviscosity, neuropathy, or symptomatic cryoglobulinemia). Responses required confirmation 6 or more weeks from the first observation of response. PD required confirmation with a repeat test.

Because a spike in the M-protein is common in patients with WM after treatment initiation, an increase in the M-protein in patients with WM before cycle 3 did not constitute PD unless specifically determined by the investigator. Quantitative immunoglobulins and serum-free light chains were measured at baseline and at each cycle starting with cycle 2. In addition, a bone marrow aspirate was obtained at baseline. Lymphadenopathy and splenomegaly were to be measured via computed tomography imaging at baseline and during treatment as clinically indicated to determine response and document relapse or progression. After 8 cycles of therapy, a formal response assessment was required. Responses were also measured after combination therapy (enzastaurin plus dexamethasone) was initiated.

Due to a high-censoring rate that likely resulted in overestimation of time-to-event endpoints, we conducted *post hoc* analyses of TTP and duration of response, in which both confirmed and unconfirmed PDs were considered as events. TTP was defined as the time from the date of study enrollment to the date of objectively determined PD. For patients without objective PD (including those who died), TTP was censored at the date of the last objective progression-free disease assessment. Patients who received subsequent systemic anticancer therapy (after discontinuation from study treatment) before objectively determined PD were censored at the date of the last objective progression-free disease assessment before postdiscontinuation therapy.

For the safety analyses, all patients who received at least 1 dose of the study drug were evaluated. Adverse events were graded at each cycle according to the CTCAE, version 3.0.

Translational research analyses

Plasma samples were collected at baseline, on day 1 of cycles 1 and 3, and after discontinuation of study treatment, and were analyzed for the concentrations of 88 plasma proteins using HumanMAP[®] (multianalyte profile), version 1.6, technology (Rules-Based Medicine; RBM).

Due to a high-censoring rate in the time-to-event endpoints, the translational research (TR) analyses associating biomarkers with clinical outcomes focused only on RR. An association between each marker and RR was assessed using logistic regression. Markers were dichotomized into high- and low-expression groups based on 2 methods: (i) the method of maximal c^2 (20), limiting the search to the central 50% of the RBM values, and (ii) using the least detectable dose (LDD; defined as the mean plus 3 standard deviations of 20 blank readings for each marker) as the cutoff point. The RBM analyte expression was also treated as a continuous variable in the appropriate regression model correlating with each clinical outcome. Because the sample size was expected to be small and the results exploratory, no multiplicity adjustments across markers were carried out for dichotomous or continuous marker analyses in association with clinical outcomes. Repeatedly measured assays were assessed for differences between baseline and postbaseline values using a paired t test.

Statistical considerations

A Simon 2-stage design was used for this study, which assumed a type I error of 0.05 and 80% power. The clinically uninteresting true RR (\geq MR) was considered to be 10%, and the true RR worthy of further development was 30%. Under these conditions, if 2 or more of the first 10 patients (stage 1) experienced an MR or better, the study was expanded with an additional 19 patients enrolled (stage 2). A total of 6 responders (\geq MR) of the 29 total were required to conclude that the true RR was greater than 10% and warranted further investigation. If 6 responders of the first 29 were observed at stage 2, up to 21 additional patients were enrolled (expansion phase) to further assess efficacy and safety.

The objective RR (CR, PR, and MR) and 95% confidence intervals (CI) were estimated using the normal approximation to the binomial. For TTP and duration of response, Kaplan–Meier methods were used, and quartiles and point probabilities were calculated. Interval estimates were calculated using 95% CIs. All tests of treatment effects were conducted at a 2-sided alpha level of 0.05 unless otherwise stated.

Results

Patient characteristics

From July 2008 to December 2010, 42 patients were enrolled. Of these, 6 patients progressed during single-agent enzastaurin therapy and subsequently received dexamethasone. This report presents results for all 42 patients, which includes those who received both enzastaurin and dexamethasone. Baseline patient and disease characteristics are

presented in Table 1. Most patients were Caucasian and male, with an ECOG PS of 0. The median number of prior therapies received was 3.0 (range, 1.0–8.0) and the median time since prior therapy was 11.3 months (range, 0.5–122.5 months). The median time since initial diagnosis was 66.1 months (range, 10.6–248.5 months).

Drug administration

All patients completed at least 1 cycle, 14 patients completed at least 10 cycles, and 10 patients completed at least 12 cycles. The median number of cycles per patient was 8.0 (range, 1.0–19.0). There were no enzastaurin dose reductions; however, 3 patients experienced cycle delays and 7 patients required dose omissions due to adverse events. The mean daily enzastaurin dose was 497.9 mg, and the dose intensity was 99.6%.

Efficacy

The median follow-up time was 9.5 months (range, 2.5–25.4 months). There were no CRs, 2 PRs (4.8%), and 14 MRs (33.3%) for an objective RR (CR+PR+MR) of 38.1% (Table 2). In addition, SD or better was observed in 71.4% of patients. Of the patients who experienced an MR, 2 had received the combination of enzastaurin and dexamethasone.

The analyses of TTP and response duration, conducted according to the statistical analysis plan that was designed before study initiation, resulted in a high degree of censoring, which likely led to an overestimation of the time-to-event endpoints. Therefore, *post hoc* analyses were conducted as described in the Methods. On the basis of these analyses, the median TTP was 10.9 months (95% CI: 7.4, 20.5 months), with a censoring rate of 45.2%. At 6 months, 75% (95% CI: 58%, 86%) of the patients had not progressed, and 44% were progression-free at 12 months (95% CI: 26%, 61%). The median duration of response was 17.7 months (95% CI: 9.0, 23.8 months), with a censoring rate of 62.5%. Of note, no patients were censored for this analysis because of death or subsequent treatment.

Figure 1 shows a waterfall plot of the best percentage change of IgM serum from baseline. Two (4.8%) patients had a confirmed reduction 50% or more (2 additional patients had an unconfirmed reduction \geq 50%), and 12 (28.6%) patients had a confirmed reduction 25% or more [7 (16.7%) additional patients had an unconfirmed reduction \geq 25%]. The mean best percentage of IgM reduction from baseline was 27.3 (SD , 22.0).

Safety

Eleven patients experienced laboratory adverse events and 15 patients experienced nonlaboratory adverse events that were considered possibly study-drug related. All of these adverse events were grade 2 or less, except for grade 3 leukopenia in 1 patient (Table 3). There was 1 on-study death due to septic shock, which was considered possibly drug related and which occurred in a patient who received enzastaurin and dexamethasone. Twenty packed red blood

Table 1. Key patient and disease characteristics at baseline (*N* = 42)

Characteristic	
Age, years	
Median (range)	65 (46–82)
≤65, <i>n</i> (%)	21 (50.0)
>65, <i>n</i> (%)	21 (50.0)
Caucasian/African, <i>n</i> (%)	41 (97.6)/1 (2.4)
Females/males, <i>n</i> (%)	7 (16.7)/35 (83.3)
ECOG PS 0/1, <i>n</i> (%)	39 (92.9)/3 (7.1)
Prior chemotherapy	
Median number of therapies (range)	3 (1–8)
One, <i>n</i> (%)	11 (26.2)
Two, <i>n</i> (%)	17 (40.5)
Three+, <i>n</i> (%)	14 (33.3)
Prior rituximab, <i>n</i> (%)	38 (90.5)
Prior fludarabine, <i>n</i> (%)	12 (28.6)
Prior bortezomib, <i>n</i> (%)	20 (47.6)
Any prior alkylating agent, <i>n</i> (%)	13 (31.0)
Median serum	
M-protein (range), g/dL	2.05 (0.60–4.40)
β2-microglobulin, mg/L (<i>n</i> = 22)	
Median (range)	3.95 (2.00–13.30)
≤3, <i>n</i> (%)	8 (36.4)
>3, <i>n</i> (%)	14 (63.6)
Hemoglobin, g/dL	
Median (range)	11.0 (7.9–15.3)
≤11.5, <i>n</i> (%)	27 (64.3)
>11.5, <i>n</i> (%)	15 (35.7)
IgM, g/dL (<i>n</i> = 39)	
Median (range)	3.66 (0.67–7.44)
≤7, <i>n</i> (%)	37 (94.9)
>7, <i>n</i> (%)	2 (5.1)
Platelets, × 10 ⁹ /L, <i>n</i> (%)	
≤100	1 (2.4)
>100	41 (97.6)
ISSWM risk category (<i>n</i> = 22), <i>n</i> (%) [*]	
Low (≤1 adverse characteristic and age ≤65 yrs)	7 (31.8)
Intermediate (2 adverse characteristics or age >65 yrs)	14 (63.6)
High (≥3 adverse characteristics)	1 (4.5)

NOTE: ECOG PS, Eastern Cooperative Oncology Group performance status; IgM, immunoglobulin M; ISSWM, International prognostic scoring system for Waldenström macroglobulinemia.

^{*}Because the ISSWM (2) was not available when this study was designed, ISSWM risk category scoring was done retrospectively for 22 patients (20 patients were missing β2-microglobulin).

Table 2. Response rates (*N* = 42)

Response	<i>n</i> (%)
CR	0 (0.0)
PR	2 (4.8)
MR	14 (33.3)
Objective RR (CR+PR+MR)	16 (38.1)
95% CI	(23.4, 52.8)
SD	14 (33.3)
PD	3 (7.1)
Other [*]	9 (21.4)

CR, complete response; PR, partial response; MR, minor response; RR, response rate; CI, confidence interval; SD, stable disease; PD, progressive disease.

^{*}Other, patients whose best overall responses could not be determined because of lack of confirmation; 4 patients had SD, 2 had MR, and 3 had PD.

cell transfusions and 3 platelet transfusions were given to 7 patients during the study.

TR

TR analyses focused on all enrolled patients who received at least 1 dose of the study drug and had at least 1 TR sample for the RBM marker of interest (the TR analysis set). Of the 42 total patients, 28 had RBM samples; 17 patients had baseline samples, 20 had postbaseline samples, and 9 had both baseline and postbaseline samples. For the 9 patients with both baseline and postbaseline samples, there was no difference between the 2 distribution means of baseline and postbaseline RBM protein concentration levels ($P > 0.05$).

In the patients included in the TR analysis set, there were 2 PRs and 10 MRs, for an objective RR of 42.9%. For the 17 patients with baseline samples, using the LDD of 0.58 ng/mL as the cutoff point for interleukin 15 (IL-15), RR in the high-concentration subgroup ($n = 8$) was 75.0% compared with 11.1% in the low-concentration subgroup ($n = 9$; high- to low-concentration OR 24.0; 95% CI: 1.74, 330.8; $P = 0.018$), indicating that a high-concentration level of IL-15 was significantly associated with improved RR. Continuous

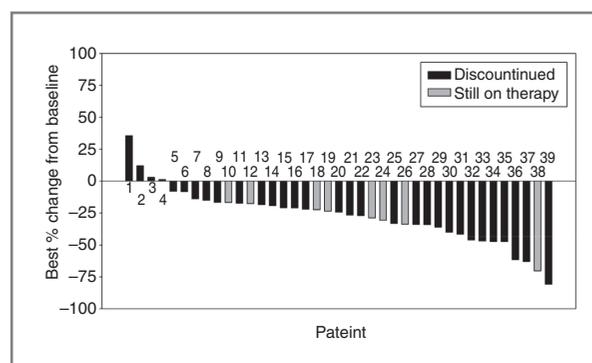
**Figure 1.** Best percentage change of IgM serum from baseline.

Table 3. Maximum grade drug-related CTCAEs (N = 42)

Event	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)
Laboratory				
Neutropenia	3 (7.1)	1 (2.4)	0	0
Anemia	0	5 (11.9)	0	0
Leukopenia	3 (7.1)	1 (2.4)	1 (2.4)	0
Hyperglycemia	1 (2.4)	0	0	0
ALT, SGPT	1 (2.4)	0	0	0
AST, SGOT	0	1 (2.4)	0	0
Nonlaboratory				
Fatigue	5 (11.9)	1 (2.4)	0	0
Any infection*	3 (7.1)	1 (2.4)	0	0
Constipation	2 (4.8)	0	0	0
Cough	2 (4.8)	0	0	0
Dyspnea	2 (4.8)	0	0	0
Anorexia	1 (2.4)	0	0	0
Urine color change	2 (4.8)	0	0	0
Dizziness	1 (2.4)	0	0	0
Flatulence	1 (2.4)	0	0	0
Nausea	1 (2.4)	0	0	0
Vomiting	1 (2.4)	0	0	0

CTCAE, Common Terminology Criteria for Adverse Events; ALT, alanine aminotransferase; SGPT, serum glutamic pyruvic transaminase; AST, aspartate aminotransferase; SGOT, serum glutamic oxaloacetic transaminase.

*One patient died from septic shock/grade 5 infection.

marker analysis for RR also identified baseline IL-15 concentration levels as significant, with an OR (for 1 unit increase in IL-15 concentration levels) of 13.39 (95% CI: 1.02, 176.24; $P = 0.048$). For the 20 patients with postbaseline samples, and for each of the markers IL-15 and IL-5, using the LDD as the cutoff point (0.58 mg/mL for IL-15 and 4 pg/mL for IL-5), RR in the high-concentration subgroup ($n = 9$) was 66.7% compared with 18.2% in the low-concentration subgroup ($n = 11$; OR 9.0; 95% CI: 1.14, 71.03; $P = 0.037$), indicating that high concentration of each of the markers was significantly associated with improved RR. The distribution means of IL-15 concentration levels were 0.66 (SD, 0.69) at baseline ($n = 17$) and 0.65 (SD, 0.33) after baseline ($n = 20$). This indicates a slight but insignificant numerical decrease of IL-15 concentration levels after baseline.

Discussion

This multicenter phase II study in patients with previously treated WM showed that single-agent enzastaurin was active and well tolerated. Although there were no CRs and only 2 PRs, it is noteworthy that 14 (33.3%) patients had an MR, which is encouraging in a pretreated population who received a median of 3.0 (range, 1.0–8.0) prior regimens.

The objective RR (CR+PR+MR) was 38.1%, and SD or better was observed in 71.4% of patients. In studies of other novel agents that target the PI3K/AKT pathways, the observed RR (\geq MR) in previously treated patients with relapsed WM ranged from 27% to 85% for bortezomib (21–23), 35% for perifosine (24), 70% for everolimus (25), and 51% for rituximab (26); however, such comparisons are risky given the small number of patients and differences in the patient populations and study methodologies.

Despite the small number of patients who achieved a PR in our study, a substantial number achieved an MR, and a reduction in IgM was observed in most patients. MR has been associated with clinically meaningful benefits for patients with WM; patients who achieved an MR had similar overall survival, progression-free survival, and TTP as those who achieved a CR or PR (27). In addition, per a *post hoc* analysis, the median TTP was 10.9 months, which compares favorably with the median TTP reported for other novel single agents, including 6.6 months for bortezomib in the Waldenstrom's Macroglobulinemia Clinical Trials Group study (23) and 12.6 months for perifosine (24).

Enzastaurin was well tolerated. During the study, one grade 3 to 4 adverse event (grade 3 leukopenia) and one death (due to septic shock) occurred that were considered drug related. In addition, no dose reductions were required. A higher incidence of grade 3 and 4 toxicities was reported for other novel single agents in this patient population. Bortezomib caused new or worsening peripheral neuropathy in 20 patients (74%), and 44% discontinued treatment mainly because of neuropathy (22). Perifosine dose reductions occurred in 16 patients (43%) because of neutropenia, gastrointestinal symptoms, or arthritis (24). Everolimus was associated with grade 3 or higher drug-related toxicities in 28 patients (56%), including hematologic toxicities with cytopenias, and 52% of patients required dose reductions because of toxicities (25). For rituximab, grade 3 and 4 toxicities were reported in 39% and 16% of previously treated patients, respectively (26).

Given the tolerability and activity observed in this study, the use of enzastaurin in combination with other agents or as a maintenance treatment should be considered. In a phase I study in 23 patients with relapsed or refractory multiple myeloma who had received 3 or more regimens, the combination of enzastaurin and bortezomib was well tolerated and showed modest activity (28). No dose-limiting toxicities were observed, and thrombocytopenia (26.1%) and anemia (8.7%) were the most common drug-related grade 3 or 4 toxicities. The objective RR observed in this study was 17.4%, with 9 patients (39.1%) achieving SD, and the median TTP was 7.9 months (range, 0.95–19.7 months). In a randomized phase II study of rituximab-cyclophosphamide-Adriamycin-vincristine-prednisone with or without enzastaurin as first-line treatment of intermediate- and high-risk DLBCL, preliminary results suggested that the addition of enzastaurin improved progression-free survival and

response (RRs of 80.4% and 83.3%, respectively; 29). Toxicities were comparable between the arms, with the most frequent grade 3/4 adverse events being neutropenia (56.1% vs. 51.1%, respectively) and thrombocytopenia (17.5% vs. 13.9%, respectively).

The current study suggests that high concentration levels of IL-15 may be associated with objective RR. IL-15 is a cytokine with structural similarity to IL-2. Like IL-2, IL-15 binds to and signals through the IL-2/IL-15 beta chain (CD122) and the common gamma chain (gamma-C, or CD132). IL-15 induces proliferation of natural killer cells and has been shown to enhance the antitumor immunity of CD8⁺ T cells in preclinical models (30, 31). A phase I clinical trial to evaluate safety, dosing, and antitumor efficacy of IL-15 in patients with metastatic melanoma and renal cell carcinoma (kidney cancer) is ongoing at the U.S. NIH (32). This observation warrants further assessment to examine the relationship between IL-15 and enzastaurin in enhancing antitumor immunity in WM.

This study has several limitations. The finding that elevated IL-15 is associated with RR requires confirmation because of the small sample size and lack of analytical correction for multiple testing, and thus should be viewed as hypothesis generating. The time-to-event results in the current trial should be interpreted with caution given that these were derived from *post hoc* analyses. Finally, this study is limited by potential selection bias because of the single-arm trial design.

In conclusion, this phase II study showed that enzastaurin is active and well tolerated in previously treated patients with WM. The oral route of delivery, well-tolerated safety profile, and prolonged disease control are encouraging and compare favorably with other novel agents studied in a similar patient population. These results should be confirmed in a larger study, and enzastaurin should be considered in studies as a maintenance therapy or in combination with other agents such as bortezomib or rituximab. Ultimately, randomized trials will be required to

determine whether the encouraging activity will translate into improved outcomes for patients with WM.

Disclosure of Potential Conflicts of Interest

I.M. Ghobrial is a consultant/advisory board member of Millennium, Genzyme, Novartis, Noxxon, BMS, Onyx, and Celgene and has research support from Genzyme, Millennium, Novartis, BMS, and Onyx. P. Moreau has a honoraria from Lilly (advisory board). K.A. Benhadji is employed as a Senior Medical Advisor and has ownership interest (including patents) in Eli Lilly Company. A. Hossain is employed as a Research Scientist in Eli Lilly Company. T. Nguyen is employed as a Research Scientist and has ownership interest (including patents) in Eli Lilly Company. J.E. Wooldridge is employed as a Medical Fellow and has ownership interest (including patents) in Eli Lilly Company. V. Leblond is a consultant/advisory board member of Lilly France and Roche and has a honoraria from speaker's bureau from Mundipharma.

Authors' Contributions

Conception and design: I.M. Ghobrial, K.A. Benhadji, J.E. Wooldridge.
Development of methodology: I.M. Ghobrial, J.E. Wooldridge,
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): I.M. Ghobrial, P. Moreau, B. Harris, E. Jourdan, H. Maisonneuve, V. Leblond.
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): I.M. Ghobrial, P. Moreau, K.A. Benhadji, A. Hossain, T. Nguyen, J.E. Wooldridge, V. Leblond.
Writing, review, and/or revision of the manuscript: I.M. Ghobrial, P. Moreau, E. Jourdan, K.A. Benhadji, A. Hossain, T. Nguyen, J.E. Wooldridge, V. Leblond.
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): I.M. Ghobrial, T. Poon, J.E. Wooldridge.
Study supervision: I.M. Ghobrial, T. Poon, K.A. Benhadji, A. Hossain.

Acknowledgments

The authors thank Noelle Gasco and Svetlana Dominguez, ELS, employees of Eli Lilly and Company, who provided writing and editorial assistance to the authors during preparation of this manuscript.

Grant Support

The research support was provided by Eli Lilly and Company. 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 January 19, 2012; revised May 30, 2012; accepted July 24, 2012; published OnlineFirst August 9, 2012.

References

- Owen RG, Treon SP, Al-Katib A, Fonseca R, Greipp PR, McMaster ML, et al. Clinicopathological definition of Waldenström's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenström's Macroglobulinemia. *Semin Oncol* 2003;30:110-5.
- Morel P, Duhamel A, Gobbi P, Dimopoulos MA, Dhodapkar MV, McCoy J, et al. International prognostic scoring system for Waldenström macroglobulinemia. *Blood* 2009;113:4163-70.
- Dimopoulos MA, Panayiotidis P, Mouloupoulos LA, Sfikakis P, Dalakas M. Waldenström's macroglobulinemia: clinical features, complications, and management. *J Clin Oncol* 2000;18:214-26.
- Dimopoulos MA, Galani E, Matsouka C. Waldenström's macroglobulinemia. *Hematol Oncol Clin North Am* 1999;13:1351-66.
- Gertz MA, Anagnostopoulos A, Anderson K, Branagan AR, Coleman M, Frankel SR, et al. Treatment recommendations in Waldenström's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenström's Macroglobulinemia. *Semin Oncol* 2003;30:121-6.
- 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 Waldenström's Macroglobulinemia. *J Clin Oncol* 2009;27:120-6.
- Hatjiharissi E, Ngo H, Leontovich AA, Leleu X, Timm M, Melhem M, et al. Proteomic analysis of Waldenström macroglobulinemia. *Cancer Res* 2007;67:3777-84.
- Moreau AS, Jia X, Ngo HT, Leleu X, O'Sullivan G, Alsayed Y, et al. Protein kinase C inhibitor enzastaurin induces *in vitro* and *in vivo* antitumor activity in Waldenström macroglobulinemia. *Blood* 2007;109:4964-72.
- Bellamy WT, Richter L, Frutiger Y, Grogan TM. Expression of vascular endothelial growth factor and its receptors in hematopoietic malignancies. *Cancer Res* 1999;59:728-33.
- Rajkumar SV, Hayman S, Greipp PR. Angiogenesis in Waldenström's macroglobulinemia. *Semin Oncol* 2003;30:262-4.
- Graff JR, McNulty AM, Hanna KR, Konicek BW, Lynch RL, Bailey SN, et al. The protein kinase C beta-selective inhibitor, Enzastaurin (LY317615.HCl), suppresses signaling through the AKT pathway, induces apoptosis, and suppresses growth of human colon cancer and glioblastoma xenografts. *Cancer Res* 2005;65:7462-69.
- Carducci MA, Musib L, Kies MS, Pili R, Truong M, Brahmer JR, et al. Phase I dose escalation and pharmacokinetic study of enzastaurin, an

- oral protein kinase C beta inhibitor, in patients with advanced cancer. *J Clin Oncol* 2006;24:4092–9.
13. Hanauske AR, Lahn M, Musib LC, Weigang-Köhler K, Yilmaz E, Graefe T, et al. Phase Ib safety and pharmacokinetic evaluation of daily and twice daily oral enzastaurin in combination with pemetrexed in advanced/metastatic cancer. *Ann Oncol* 2009;20:1565–75.
 14. Robertson MJ, Kahl BS, Vose JM, de Vos S, Laughlin M, Flynn PJ, et al. Phase II study of enzastaurin, a protein kinase C beta inhibitor, in patients with relapsed or refractory diffuse large B-cell lymphoma. *J Clin Oncol* 2007;25:1741–6.
 15. Morschhauser F, Seymour JF, Klün-Nelemans HC, Grigg A, Wolf M, Pfreundschuh M, et al. A phase II study of enzastaurin, a protein kinase C beta inhibitor, in patients with relapsed or refractory mantle cell lymphoma. *Ann Oncol* 2008;19:247–53.
 16. Kyle RA, Treon SP, Alexanian R, Barlogie B, Björkholm M, Dhodapkar M, 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.
 17. 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;6:380–3.
 18. Rizzo JD, Lichtin AE, Woolf SH, Seidenfeld J, Bennett CL, Cella D, et al. American Society of Clinical Oncology, American Society of Hematology. Use of epoetin in patients with cancer: evidence-based clinical practice guidelines of the American Society of Clinical Oncology and the American Society of Hematology. *J Clin Oncol* 2002;20:4083–107.
 19. Ozer H, Armitage JO, Bennett CL, Crawford J, Demetri GD, Pizzo PA, et al. American Society of Clinical Oncology. 2000 update of recommendations for the use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. American Society of Clinical Oncology Growth Factors Expert Panel. *J Clin Oncol* 2000;18:3558–85.
 20. Miller R, Siegmund D. Maximally selected chi-square statistics for small samples. *Biometrics* 1982;38:1011–6.
 21. Dimopoulos MA, Anagnostopoulos A, Kyrtonis MC, Castritis E, Bitsaktsis A, Pangalis GA. Treatment of relapsed or refractory Waldenstrom's macroglobulinemia with bortezomib. *Haematologica* 2005;90:1655–8.
 22. Chen CI, Kouroukis CT, White D, Voralia M, Stadtmayer E, Stewart AK, 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;25:1570–5.
 23. Treon SP, Hunter ZR, Matous J, Joyce RM, Mannion B, Advani 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.
 24. Ghobrial IM, Roccaro A, Hong F, Weller E, Rubin N, Leduc R, et al. Clinical and translational studies of a phase II trial of the novel oral Akt inhibitor perifosine in relapsed or relapsed/refractory Waldenstrom's macroglobulinemia. *Clin Cancer Res* 2010;16:1033–41.
 25. 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;28:1408–14.
 26. Gertz MA, Rue M, Blood E, Kamimer LS, Vesole DH, Greipp PR. Multicenter phase 2 trial of rituximab for Waldenström macroglobulinemia (WM): an Eastern Cooperative Oncology Group Study (E3A98). *Leuk Lymphoma* 2004;45:2047–55.
 27. Gertz MA, Abonour R, Heffner LT, Greipp PR, Uno H, Rajkumar SV. Clinical value of minor responses after 4 doses of rituximab in Waldenström Macroglobulinemia: a follow-up of the Eastern Cooperative Oncology Group E3A98 trial. *Br J Haematol* 2009;147:677–80.
 28. Ghobrial IM, Munshi NC, Harris BN, Shi P, Porter NM, Schlossman RL, et al. A phase I safety study of enzastaurin plus bortezomib in the treatment of relapsed or refractory multiple myeloma. *Am J Hematol* 2011;86:573–8.
 29. Hainsworth JD, Arrowsmith E, McCleod L, Fayad L, Hamid O, Davis L, et al. Randomized phase II study of R-CHOP plus enzastaurin versus R-CHOP in the first-line treatment of patients with intermediate- and high-risk diffuse large B-cell lymphoma (DLBCL): preliminary analysis. *J Clin Oncol* 2011;29:15(suppl):Abstract 8016.
 30. Klebanoff CA, Finkelstein SE, Surman DR, Lichtman MK, Gattinoni L, Theoret MR, et al. IL-15 enhances the *in vivo* antitumor activity of tumor-reactive CD8⁺ T cells. *Proc Natl Acad Sci U S A* 2004;101:1969–74.
 31. Teague RM, Sather BD, Sacks JA, Huang MZ, Dossett ML, Morimoto J, et al. Interleukin-15 rescues tolerant CD8⁺ T cells for use in adoptive immunotherapy of established tumors. *Nat Med* 2006;12:335–41.
 32. ClinicalTrials.gov. A phase I study of intravenous recombinant human IL-15 in adults with refractory metastatic malignant melanoma and metastatic renal cell cancer. Available from: <http://www.clinicaltrials.gov/ct2/show/NCT01021059>. Accessed 2011 Sept 30.