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A Gynecologic Oncology Group phase II trial of the protein kinase C-beta inhibitor, enzastaurin and evaluation of markers with potential predictive and prognostic value in persistent or recurrent epithelial ovarian and primary peritoneal malignancies

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Abstract

Purpose—Protein kinase C (PKC) activation contributes to proliferation and angiogenesis in epithelial ovarian or primary peritoneal carcinoma (EOC/PPC). A multi-institutional phase II trial was conducted to evaluate the efficacy and safety of PKC β inhibitor enzastaurin in persistent or recurrent EOC/PPC and to explore potential prognostic and predictive biomarkers.

Methods—Eligible women with measurable platinum-sensitive and resistant EOC/PPC were treated with continuous administration of oral enzastaurin until disease progression or

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

unacceptable toxicity. A two-stage sequential design was used to evaluate progression-free survival (PFS) $\geq$ 6-months, tumor response, and toxicity. Translational studies included sequencing of the *TP53*, *PTEN*, *PIK3CA* and *PKC β* genes for somatic mutations, quantitative PCR assays for *AKT2* and *PTEN* copy number alterations, and measurement of circulating VEGF-A plasma levels.

Results—Among 27 eligible and evaluable patients, 3 women with PFS $\geq$ 6-months (11%) and 2 women with partial responses (7%) were observed. One of them achieved a durable response and remains on the study. No grade 4 adverse events were observed. Most common grade 3 adverse events were constitutional (4) and gastrointestinal (3). Mutations in the *TP53* gene and abnormal copy number in the *PTEN* gene were common (56% and 48% of cases, respectively).

Conclusions—Enzastaurin was tolerable but had insufficient activity to proceed with the second stage of accrual. However, 1 patient has been progression-free for 44 months. No association between a biomarker and response to enzastaurin has been found.

Exploratory analysis suggested an association between survival and *PTEN* copy number losses.

Keywords

Enzastaurin; ovarian cancer; VEGF PKC β ; TP53; PTEN; PIK3CA; AKT2

INTRODUCTION

Ovarian cancer remains the most lethal cancer of the female genital tract. The estimated incidence of ovarian cancer in the United States for 2009 is 21,550 with about 14,600 deaths [1]. Current first-line treatment for epithelial ovarian or primary peritoneal carcinoma (EOC/PPC) consists of platinum-based chemotherapy following surgical staging with cytoreduction; however, the majority of patients will develop a recurrence and their median overall survival (OS) is approximately two years [2].

Thus, new agents are needed to improve OS of women with EOC/PPC. Biologic agents with novel mechanisms of action are especially appealing in this disease because of their usually more favorable side effect profile and fewer overlapping toxicities with chemotherapy.

Enzastaurin hydrochloride (LY317615 – Eli Lilly) is an acyclic bisindolylmaleimide developed for the treatment of cancer based on its direct anti-proliferative and anti-angiogenic properties [3,4]. It is a potent selective oral inhibitor of protein kinase C (PKC), especially PKC beta (PKC β), and an inhibitor of PI3 kinase (phosphatidylinositol 3-kinase)/AKT signaling pathway [5]. PKC is a family of serine/threonine protein kinases which are high-affinity intracellular receptors to phorbol esters, natural products known to induce skin carcinogenesis in mice [6,7]. PKC beta (PKC β) is one of the most active PKCs involved in regulating cell proliferation. Activated PKC β has been found in multiple human tumors including EOC/PPC [8]. Enzastaurin is relatively inactive against other kinases, especially the tyrosine kinase family.

PKC β as well as other PKCs activate AKT (also known as protein kinase B) which is frequently activated in a wide array of human cancers [9,10,11]. The activation of PI3 kinase (phosphatidylinositol 3-kinase)/AKT cell signaling pathway promotes tumor cell survival and proliferation [12]. Enzastaurin blocks the key enzymes of this pathway and has demonstrated the ability to suppress the proliferation of cancer cell lines *in vitro* including EOC/PPC cell lines: OVCAR-3, OVCAR-4, and OVCAR-8 when tested against the NCI 60 cell line panel [13].

In addition, enzastaurin has anti-angiogenic activity and has been shown to suppress vascular endothelial growth factor (VEGF) production by human tumor xenografts [13,14]. EOC/PPC secrete large amounts of VEGF which stimulates angiogenesis and plays a crucial role in progression of EOC/PPC [15]. A monoclonal antibody to VEGF, bevacizumab, has demonstrated significant activity in ovarian cancer [16]. Thus, anti-angiogenic agents hold promise in the treatment of EOC/PPC.

Enzastaurin was well tolerated in a phase I trial with Grade I chromaturia (red discoloration of urine), fatigue, and GI side effects being the most common toxicities [12]. The observed dose limiting toxicity was changes in QT_c defined as prolongation of QT_c by more than 50 msec over baseline [12].

The clinical development of enzastaurin, a potent inhibitor of the PKC and PTEN/PI3K/AKT signaling pathways, is of great interest in EOC/PPC. A translational research objective has been embedded in this phase II protocol to evaluate a panel of biomarkers relevant to enzastaurin and disease state.

As mentioned earlier, the evidence of deregulation of the PI3K/AKT/PTEN pathway in EOC/PPC has been reported previously and includes gain-of-function mutations and amplifications of *PI-3-kinase* [17]; amplification of *AKT2* [18,19]; allelic imbalance and loss of heterozygosity in *PTEN* genes [20,21,22]. *PTEN* (Phosphatase and *TEN*sin homologue) is a key tumor suppressor and normally opposes the activation of the proto-oncogenic PI3K/AKT signaling pathway. Also, there are multiple nodes of crosstalk between PI3K/AKT/PTEN and p53 signaling pathways [23] which may result in cisplatin resistance in EOC/PPC [24]. Given these data, we assessed mutations and copy number changes in *PIK3CA*, *AKT2*, *PKC*, *PTEN*, and *TP53* genes in this study. We sought to determine whether these genetic aberrations in the tumor might have prognostic value as opposed to the levels or activity of proteins encoded by them which may change in the course of the treatment. We also measured VEGF plasma levels before and after the first cycle of enzastaurin to find out whether the change in VEGF level could serve as a potential marker of early response to the drug.

METHODS

Patients

This was a multi-center phase II trial of enzastaurin as a single-agent in women with advanced EOC/PPC. To be eligible for the trial, patients must have had histologic documentation of diagnosis with measurable disease according to RECIST (1.0) criteria [25].

Patients were required to have 1 platinum-based chemotherapy regimen and allowed to have 1 additional cytotoxic regimen. Patients needed a GOG Performance Status of 0, 1, or 2, or just 0 or 1 depending on prior chemotherapy. Patients were required to have a platinum-free interval of less than 12 months or persistent disease. Patients with platinum-resistant and platinum-sensitive disease (i.e. had Platinum Free Interval between 6 and 12 months) were included. Only patients with adequate bone marrow, renal, and liver functions were eligible.

All women provided written informed consent and participating institutions obtained annual Institutional Review Board (IRB) approval for this trial including the correlative studies.

Drug Administration

Enzastaurin was administered orally in a fed state at a loading dose of 375 mg TID on day 1 followed by continuous treatment with 500 mg daily until disease progression or adverse effects prohibited further therapy. The length of one cycle was 28 days.

Clinical Management, Assessments and Testing

Pretreatment evaluation consisted of history and physical exam, CXR, ECG, CBC, serum chemistries, urinalysis, CA125, PT/PTT, and radiographic documentation of measurable disease by CT or MR scan.

During the study, interval visits, CBC, serum chemistries and CA125 were obtained at the start of each cycle (every 28 days). CT or MR scan to evaluate for response was performed every other cycle for the first 6 months. Evaluation of response was performed by RECIST (1.0) criteria [25]. Dose reduction to 250 mg of Enzastaurin daily was required for severe hematologic and non-hematologic toxicities.

Clinical End Points

The primary efficacy clinical end-points of the study were the frequency of patients who were alive and progression-free at 6 months (PFS at 6 months) or who had a tumor response. Another primary endpoint was the toxicity of enzastaurin. Secondary clinical endpoints included the duration of PFS and OS as well as the assessment of the impact of platinum sensitivity, initial performance status, and age on PFS and OS.

Translational Research Objectives

The translational research objectives were to assess markers that could be relevant to the effectiveness of enzastaurin (Supplemental Figure 1) including somatic mutations in genes such as *TP53*, *PTEN*, *PIK3CA* and *PKCβII* in DNA extracted from tumor before initiation of treatment, number of copies of *PTEN* and *AKT2*, and VEGF-A concentration in plasma pre-cycle 1 and pre-cycle 2.

Specimens

Archival formalin-fixed and paraffin-embedded (FFPE) primary, metastatic and/or recurrent tumor collected prior to starting enzastaurin treatment was a requirement for all patients who consented to the study participation. Twenty-three patients had their biomarkers tested from their primary tumors, and four had it tested from metastatic disease at the primary diagnosis. Only one patient had testing done on recurrent tumor. Therefore the results of this study were obtained primarily from the analysis of tissue procured before initial chemotherapy.

A block or 20 unstained slides for each patient was submitted for analysis. Plasma was prepared from blood drawn before the first (pre-cycle 1) and the second (pre-cycle 2) cycles of enzastaurin treatment respectively, frozen at $\leq -70^{\circ}\text{C}$ and shipped to the GOG Tissue Bank.

DNA Extraction

Genomic DNA was extracted from FFPE tumor tissue using a Trimgen DNA purification kit (Trimgen Corp, Sparks, MD) according to the kit instructions.

Mutation analysis for TP53, PTEN, PIK3CA and PKCβII

Polymerase chain reaction (PCR) primers flanking the desired exons were used to amplify *TP53* (exons 5–9), *PTEN* (exons 2, 3, 5, 6 and 7), *PIK3CA* (exons 1, 9 and 20) and *PKCβII* (exons 2–17). Primer sequences are available in supplemental Table 1. PCR amplicons were

subjected to direct sequencing using same primers for PCR amplification and ABI BigDye Terminator kit v1.1 (Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Sequence variations were determined by using Sequencher software 4.7 (Gene Codes Corporation, Ann Arbor, MI) compared with GenBank genomic sequences for each gene. All of the sequence variations were confirmed by multiple, independent PCR amplifications and repeated sequencing reactions.

Copy number assessments for PTEN and AKT2

Copy number analysis of AKT2, PTEN (using two independent probes, intron 1 and intron 5) was performed using quantitative PCR (qPCR). Custom TaqMan assays were obtained from ABI (Supplemental Table 2): All probes were labeled with FAM as the reporter dye and TAMRA as the quencher. Each sample was run in triplicate on an ABI Prism 7900HT analyzer using *Caveolin-1* as a reference gene. A standard curve was generated using normal human genomic DNA and data was analyzed using the standard curve method.

Enzyme-linked immunosorbent assay for VEGF

VEGF-A concentrations were quantified using the Quantikine Human VEGF ELISA kit, catalog # DVE00 (R&D Systems, Inc; Minneapolis, MN) and the VEGF¹⁶⁵ standard in pre-cycle 1 and pre-cycle 2 plasma specimens according to the kit instructions.

Statistical Design

The study was designed to detect cytostatic activity by examining delays in tumor progression through the frequency of patients PFS at 6 months and cytotoxic activity through the frequency of tumor response. Activity by either measure was considered sufficient to declare the drug worthy of further investigation in a phase III trial. A bivariate 2-stage design was used to limit needless exposure when the drug was inactive [26].

With 27 eligible and evaluable patients, the study required more than 4 patients PFS at 6 months or more than 3 patients with responses before the study could proceed to the second stage accrual. The cumulative targeted sample size for the second stage was 52 patients. The study would require more than 12 patients PFS at 6 months or more than 8 patients with responses before the agent would be declared interesting.

The sample sizes were targeted so that the probability of declaring the regimen active when in fact it is inactive ($p_r \leq 10\%$ and $p_s \leq 15\%$) was limited to 10% with approximately 90% power ($p_r > 25\%$ or $p_s > 35\%$) where p_r and p_s are the probabilities of response and PFS at 6 months respectively. The null probabilities were obtained from an analysis of historical controls where the agents under investigation were deemed to have minimal activity (Figure 1B) [27–37]. The frequency and severity of adverse events were evaluated with National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE v3) and tabulated according to the organ system affected. Cox proportional hazards models were used where feasible to assess any possible association between a biomarker of interest and PFS or OS [38]. Associations within biomarkers and clinical characteristics were examined with Kendall's Tau-b, Spearman's correlation coefficient, and Fisher's Exact Test as feasible [39–41]. Clinical characteristics examined with biomarker data included response, PFS ≥ 6 months, performance status, age group, number of prior regimens, and grade of tumor tissue. Since all except one of the patients had disease progression, exact Wilcoxon or Kruskal-Wallis tests were conducted to assess associations between mutation data and PFS [42]. Any analysis with a p-value less than 5% was designated as being "suggestive" of a possible association. Power to detect true associations was limited. Suggestive associations should be interpreted as hypothesis-generating.

RESULTS

Patient Eligibility and Characteristics

Twenty-eight patients were enrolled onto the study from November, 2006 to May, 2007. One was ineligible due to improper treatment prior to study entry, leaving 27 for analysis. Patient characteristics are listed in Table 1. Twenty-three patients discontinued treatment for disease progression. One patient achieved a durable partial response and remains on the study at the time of writing this manuscript. Two refused further treatment, and 1 was discontinued for toxicity (grade 2 vomiting) as permitted by the protocol.

Adverse events

A summary of adverse events is provided in Table 2. Only events deemed at least possibly related to enzastaurin were included. Overall, enzastaurin was well tolerated with no grade 4 toxicities. The most common severe side effects observed in the study were constitutional (4), primarily fatigue, gastrointestinal (3), and anemia (1). One patient had grade 3 dyspnea. The grade 3 neurological and metabolic reactions were dizziness and hyperkalemia, respectively. We had not observed QT_c changes in our study group and only 2 patients developed grade 1 thrombocytopenia. No patient required a dose reduction of enzastaurin.

Clinical Activity

Patients received a median of 2 cycles of therapy (range 1 – 39). Three patients had 5 or more cycles. The median follow-up time among the 11 patients alive at the time of data freeze was 26.5 months (range 17 – 30). The observed number of patients who were progression free at 6 months was 3 (11.1%; 90% CI 3.1% – 26.3%), and the observed number of patients with responses was 2 (7.4%; 90% CI 1.3% – 21.5%). One patient had a response and was progression free at 6 months. Seventeen patients had increasing disease (63%). As a single agent, enzastaurin did not demonstrate sufficient activity to proceed to a second stage of accrual and the study was terminated. The median PFS was observed to be 1.87 months (90% CI 1.54 – 2.73). The median OS was 15.1 months (Figure 1A). Survival analyses involving PFS and OS did not show any suggestive relationships with platinum sensitivity, age, or performance status. Interestingly, as of January 2011, 1 patient remained on the study. She completed 49 cycles of treatment with enzastaurin and achieved a durable partial response lasting for 44 months. This patient was a 52-year-old Caucasian woman with a performance status of 0 at the time of enrollment with adenocarcinoma of unspecified cellular type. She initially completed 8 cycles of Carboplatin and Taxol with a platinum-free interval of about 3.6 months and then, was treated with 3 cycles of Doxil.

Translational Research

Possible associations were detected among some of the biomarkers and the clinical parameters. However, no associations between the biomarkers and tumor response or resistance to enzastaurin were observed. Mutations were common in *TP53* (56%; 15/27), although likely underestimated since only exons 5–9 were analyzed, but were less frequent in *PKCβII* (15%; 4/27), *PIK3CA* (0%; 0/27), and *PTEN* (0%; 0/27) (Table 3). A previously reported mutation in *PIK3CA* (c.1634A>C; p.E545A) [43] was found in 8 of the 27 samples; however, based on its frequency in this sample set, we suspect that this alteration is likely a benign polymorphism. Gene copy number alterations were found for *AKT2* (15%; 4/27) and *PTEN* (48% with a gain or loss; 15% [4/27] with a gain and 33% [9/27] with a loss) (Table 3), which is consistent with our recent studies showing frequent genetic abnormalities of the PI3K/AKT pathway in primary ovarian cancer [unpublished data]. We observed that 1 patient of 9 with *PTEN* loss was alive, while 10 patients were alive among 18 patients with the stable *PTEN* and gain in *PTEN* within 24 months since the enrollment in the study. Of

potential significance, an exploratory analysis suggested an association between copy number loss of the *PTEN* gene and poor overall survival (OS) (Figure 2). There were 4 cases that showed gains in *PTEN* copy number using two independent qPCR probes; however, this may reflect the loss of the reference gene, *Caveolin-1*, in a subset of the tumors. Interestingly, these individuals had the best prognosis (Figure 2). Circulating VEGF levels were also measured. The Q1, M, and Q3 (first, median, and third quartiles) for VEGF at baseline were 33, 53, and 102 pg/ml, respectively. The Q1, M, and Q3 for VEGF pre-cycle 2 were 53, 87, and 127 pg/ml, respectively. These values are in the low range for cancer patients and there were no significant differences noted before and after the first cycle of treatment (pre-cycle 1 versus pre-cycle 2) when the sign test was applied.

The previously mentioned patient who had achieved durable response had relatively normal levels of biomarkers relative to the cohort under examination (Table 4). Her tumor biomarkers were unremarkable, i.e., wild type for *TP53*, *PKC β II*, *PIK3CA*, and *PTEN*, no evidence of copy number alterations in *AKT* and *PTEN*, and no elevation of plasma VEGF level at baseline. The pre-treatment VEGF fell within the first (Q1) and third quartiles (Q3) of the sample. However, the sample first quartile, median, and third quartile estimates for the second VEGF measurements after 1st cycle of enzastaurin among the 16 available specimens were 52.5, 86.9, and 127.4 pg/ml respectively while the selected patient's level was 26.8 pg/ml (standardized score from a mean of 93.6 pg/ml and standard deviation of 53.2 pg/ml was -1.26). Although not a statistical outlier, this patient's VEGF measurement after 1st cycle of enzastaurin was low.

DISCUSSION

Our result showed that enzastaurin as a single agent was well tolerated by women with ovarian cancer. Although there were 2 cases of partial response and 3 cases of stable disease for longer than 6 months, the study did not meet the criteria for the second stage of accrual and was terminated. These results are consistent with the results of other single-agent phase II trials with enzastaurin in other solid tumors which have been negative to date [44–46]. They are also consistent with phase II trials of other PKC inhibitors, bryostatins and aprinocarsen (PKC α inhibitor), in ovarian cancer [47–49]. Although our results are disappointing as far as targeting PKC in ovarian cancer, it is very likely that insufficient activity of enzastaurin is related to multiplicity of signal transduction pathways in cancer cells. This gives a rationale to using combinations of PKC inhibitors with non-cross resistant inhibitors of other important pathways in ovarian cancer, particularly VEGF inhibitors. A phase I trial using enzastaurin and bevacizumab in heavily-pretreated patients with gynecologic cancers showed very encouraging results with 32% of patients achieving a response and 48% of patients being progression-free at 6 months [50].

Enzastaurin continues to be studied in other cancers. A cytostatic mechanism of action along with favorable side effect profile makes enzastaurin a candidate for clinical trials evaluating it in the maintenance setting for malignant diseases [46, 51]. Enzastaurin in combination with various chemotherapeutic agents is also being evaluated in phase II trials.

Our translational research data have not definitively established associations between the studied biomarkers and clinical outcomes, although interesting observations have been made. The lack of correlation between biomarkers relevant to enzastaurin and clinical outcomes has been noted in another study [52]. It must be stressed that our genomic studies have focused on mutations and changes in gene copy numbers. We have not performed a full assessment of the genes and proteins in the PI3K/AKT/PTEN signaling pathway. If enzastaurin development continues new clinical trials should include downstream evaluation

of these genes and their products including mRNA quantification and direct measurement of protein expression by immunoblotting or immunohistochemistry.

CONCLUSIONS

Single agent Enzastaurin was well tolerated but showed limited activity in unselected persistent or recurrent EOC/PPC. One patient has achieved a PR and remains progression-free on enzastaurin for more than 3 years. None of the evaluated biomarkers showed correlations with tumor response. Potential association was observed between survival and *PTEN* gene copy number alterations in the tumor which requires validation in a larger sample. Trials are needed to evaluate combinations of enzastaurin with cytotoxic chemotherapy and/or targeted agents, especially anti-angiogenic drugs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Kaplan-Meier estimate of progression-free survival (solid line) and overall survival (dashed line) for women treated with enzastaurin, and (B) the PFS¹ for those treated with enzastaurin (solid line) compared with non-randomized GOG historical controls (dashed lines).

Figure 2.

Overall survival of patients in the study cohort based on *PTEN* copy number in tumors. Kaplan-Meier survival distributions for *PTEN* loss (solid line), *PTEN* stable (long dashed line) and *PTEN* gain (short dashed line) and log-rank (permutation test) suggest potential association between copy number alterations (CNAs) in *PTEN* and survival.

Table 1

Characteristics and Outcomes for the 27 Eligible Patients

Characteristics	Cases	%
Age		
40–49	4	14.8
50–59	10	37.0
60–69	6	22.2
70–79	7	25.9
Race		
African American	1	3.7
White	26	96.3
Performance Status		
0	16	59.3
1	11	40.7
Site of Disease		
Ovary	24	88.9
Peritoneum	3	11.1
Cell Type		
Adenocarcinoma, Unspecified.	2	7.4
Endometrioid Adenocarcinoma	1	3.7
Mixed Epithelial Carcinoma	2	7.4
Serous Adenocarcinoma	22	81.5
Grade		
1: Well differentiated	1	3.7
2: Moderately differentiated	6	22.2
3: Poorly differentiated	20	74.1
Prior Regimens		
1	14	51.9
2	13	48.1
Platinum Sensitivity (Platinum-Free Interval)		
Refractory / Resistant (<6 month)	16	59.3
Sensitive (≥6 month)	11	40.7
Tumor Response		
Objective Response	2 ^{†*}	7.4
Stable Disease	6	22.2
Increasing Disease	17	63.0
Non-evaluable	2	7.4
PFS ≥6 months		
No	24	88.9
Yes	3 [‡]	11.1
Cycles		
One	6	22.2

Characteristics	Cases	%
Two	12	44.4
Three	3	11.1
≥ Four	6	22.2
Disease and Vital Status		
Alive without progression	1	3.7
Alive with progression	10	37.0
Dead	16*	59.3

[†] 90% confidence interval (CI): 1.3 to 21.5%.

[‡] 90% CI: 3.1 to 26.3%.

* The cause of death was attributed to disease progression in 15 cases and was unknown in one patient.

Table 2

Adverse Events Sorted by Grade and Number of Patients Experiencing Events

CTCAE/ v3.0 Grade						
	0	1	2	3	4	5 Total
Leukopenia	25	2	0	0	0	27
Thrombocytopenia	25	2	0	0	0	27
Neutropenia	26	1	0	0	0	27
Anemia	9	10	7	1	0	27
Other Hematologic	26	1	0	0	0	27
Cardiac	25	1	1	0	0	27
Constitutional	9	9	5	4	0	27
Dermatologic	22	3	2	0	0	27
Nausea	18	6	2	1	0	27
Vomiting	22	3	2	0	0	27
Gastrointestinal	11	8	6	2	0	27
Genitourinary/Renal	24	3	0	0	0	27
Hemorrhage	26	0	1	0	0	27
Infection	26	0	1	0	0	27
Lymphatics	20	4	3	0	0	27
Metabolic	20	5	1	1	0	27
Neurosensory	26	1	0	0	0	27
Other Neurological	25	1	0	1	0	27
Pain	19	6	2	0	0	27
Pulmonary	23	2	1	1	0	27

J CTCAE v3.0 National Cancer Institute's Common Terminology Criteria for Adverse Events Version 3

Table 3

Results of translational research for the study cohort

High VEGF ¹	Mutations				Copy Number Alterations			
	TP53 ²	PKCβII	PIK3CA ⁴	PTEN ⁵	AKT2 Gain ⁶	PTEN		
						Gain ^{6,7}	Loss ⁸	
10/20	15/27	4/27	0/27	0/27	4/27	4/27	9/27	13/27

¹ Plasma VEGF was categorized at the median (value provided in pg/ml) as low or high

² Exons 5–9 for TP53

³ Exons 2–17 for PKCβII

⁴ Exons 1, 9, 20 for PIK3CA

⁵ Exons 2, 3, 5, 6, 7 for PTEN

⁶ Gain represents ≥2.5 copies relative to Caveolin-1 copies

⁷ Using two independent probes, one for intron 1 and the other for intron 5

⁸ Loss represents <1.6 copies relative to Caveolin-1 copies for both intron 1 and intron 5 probes. One sample showed loss for only the intron 5 probe and was not counted as a PTEN loss.

Table 4
Descriptive marker data for 2 patients who experienced partial responses (PRs) on Enzastaurin

Two PRs	VEGF ¹ Pre cycle 2	Genomic Alterations ²				Copy Number Alterations ³	
		PIK3CA	PTEN	TP53	PKCβII	AKT2	PTEN
PFS ≥ 39 months; Still on study	Low 26.82 Z = -1.25	<i>c.1658G>C p.S553T; c.1634A>C p.E545A; c.1659del T p.H554TfsX5; c.352+40 A>G; c.352+136 ins 3</i>	<i>c.210-34 del T</i>	WT ⁴	<i>c.380 + 31 G>A</i>	None	None
PFS < 6 month PFS = 5.32	Normal 57.24 Z = -0.68	<i>c.1658G>C p.S553T; c.1634A>C p.E545A; c.1659del T p.H554TfsX5</i>	<i>c.210-34 del T</i>	<u><i>c.818G>A p.R273H</i></u>	<i>c.380 + 31 G>A</i>	None	None

¹ Vascular-Endothelial Growth Factor

² Suspected deleterious mutation is in bold and underlined; suspected benign polymorphisms, such as intronic, common missense, or silent mutations are in italics.

³ A gain is considered ≥ 2.5 copies relative to *Caveolin-1* copies; a loss represents < 1.6 copies relative to *Caveolin-1* copies.

⁴ Wild type