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ORIGINAL REPORT

Activity of Sorafenib in Recurrent Ovarian Cancer and Primary Peritoneal Carcinomatosis: A Gynecologic Oncology Group Trial

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ABSTRACT

Purpose
Sorafenib is a kinase inhibitor targeting Raf and other kinases (ie, vascular endothelial growth factor receptor [VEGFR], platelet-derived growth factor receptor [PDGFR], Flt3, and c-KIT). This study assessed its activity and tolerability in patients with recurrent ovarian cancer (OC) or primary peritoneal carcinomatosis (PPC).

Methods
This open-label, multi-institutional, phase II study used a two-stage design. Eligible patients had persistent or recurrent OC/PPC after one to two prior cytotoxic regimens, and they experienced progression within 12 months of platinum-based therapy. Treatment consisted of sorafenib 400 mg orally twice per day. Primary end points were progression-free survival (PFS) at 6 months and toxicity by National Cancer Institute criteria. Secondary end points were tumor response and duration of PFS and overall survival. Biomarker analyses included measurement of ERK and b-Raf expression in tumors and phosphorylation of ERK (pERK) in peripheral-blood lymphocytes (PBLs) before and after 1 month of treatment.

Results
Seventy-three patients were enrolled, of which 71 were eligible. Fifty-nine eligible patients (83%) had measurable disease, and 12 (17%) had detectable disease. Significant grade 3 or 4 toxicities included the following: rash (n = 7), hand-foot syndrome (n = 9), metabolic (n = 10), GI (n = 3), cardiovascular (n = 2), and pulmonary (n = 2). Only patients with measurable disease were used to assess efficacy. Fourteen survived progression free for at least 6 months (24%; 90% CI, 15% to 35%). Two patients had partial responses (3.4%; 90% CI, 1% to 10%); 20 had stable disease; 30 had progressive disease; and seven could not have their tumor assessed. ERK and b-Raf were expressed in all tumors. Exploratory analyses indicated that pERK in post-treatment PBL specimens was associated with PFS.

Conclusion
Sorafenib has modest antitumor activity in patients with recurrent OC, but the activity was at the expense of substantial toxicity.

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INTRODUCTION

Ovarian cancer (OC) is the leading cause of mortality among gynecologic malignancies.[1] Treatment relies on surgical debulking and platinum-based therapy. Unfortunately, most patients experience relapse and become resistant to platinum and subsequent chemotherapy.[2,3] There is a pressing need for more effective therapies that target biologic mechanisms that drive OC progression.[4]

Sorafenib is an oral bisaryl urea that inhibits c-Raf and b-Raf, two kinases that function in the mitogen-activated protein kinase (MAPK) pathway. This pathway is activated in OC as a consequence of growth factor stimulation that activates Ras. Constitutive Ras-Raf-MAPK activation is less common, as Ras or Raf mutations are rare in OC.[5-10] Interestingly, Ras and b-Raf mutations occur with higher frequency in low malignant potential ovarian tumors than in invasive tumors, and constitutive activation of the Ras-Raf-MAPK pathway through mutation or overexpression is prominent in low-grade serous, mucinous, and clear cell ovarian carcinomas.[11-15] Overexpression of c-Raf was reported in greater than half of ovarian tumors and was correlated with unfavorable outcome.[16]

Inhibition of the Ras-Raf-MAPK pathway through genetic or chemical methods blocks the growth and invasion of OC cell lines, which supports the testing of a Raf inhibitor in OC.[17,18]

In addition, sorafenib nonspecifically blocks other receptor tyrosine kinases involved in tumor progression and angiogenesis, specifically the vascular endothelial growth factor receptors (VEGFRs) 2 and 3, the platelet-derived growth factor receptor (PDGFR) β, Flt-3, and c-KIT. The VEGFR and PDGFR are overexpressed and activated in ovarian tumors and play an important role in tumor vascularization.[19-21] In preclinical models, dual inhibition of VEGF and PDGF pathways has potent antiangiogenic effects through destabilization of pericytes.[22] In a hepatocellular carcinoma model, sorafenib inhibited tumor angiogenesis by blocking PDGFR and VEGFR signaling.[23] Sorafenib also induced apoptosis of endothelial cells and blocked angiogenesis by targeting Raf-MAPK signaling.[24,25] These preclinical findings provide strong support for testing sorafenib in OC for which active VEGF and PDGF autocrine and paracrine networks stimulate tumor growth and angiogenesis.[19,21]

Here, we studied the effects of sorafenib in women with OC or PPC recurring within 12 months of a platinum-based regimen. The main objectives were to measure progression-free survival (PFS) at 6 months and tolerability. Biologic activity was assessed by measuring the level of phosphorylated extracellular signal-regulated protein kinase (pERK) in peripheral-blood lymphocytes (PBLs) before and 1 month after of sorafenib treatment by using reverse-phase protein microarrays, a quantitative protein microarray format developed for multiplexed cell signaling analysis.[26,27] Expression of b-Raf and ERK was determined in archival tumors and correlated with clinical outcome.

PATIENTS AND METHODS

Patient Population

Patients with advanced, histologically documented OC or PPC who experienced recurrence within 12 months after platinum-based chemotherapy were eligible. Eligibility included both measurable and nonmeasurable disease. Measurable disease was defined according to Response Evaluation Criteria in Solid Tumor (RECIST).[28] Patients with nonmeasurable disease could enroll if they had ascites or pleural effusions attributable to disease, radiologic abnormalities that did not meet RECIST criteria, and a pretreatment serum CA-125 level higher than twice the upper limit of normal. Only patients with measurable disease were used to formally evaluate the activity of the study agent. Patients with nonmeasurable disease enrolled in parallel with patients who had measurable disease for as long as the trial was open and were assessed descriptively with the intent of gaining insight into the distribution of PFS for this subgroup of patients previously not included in Gynecologic Oncology Group (GOG) trials. All patients were at least 18 years old with a GOG performance status of 0 to 2. Eligibility criteria included the requirement of at least one prior, but no more than two prior, cytotoxic therapy; and adequate hematologic, hepatic, and renal functions. Key exclusion criteria were prior treatment with sorafenib, history of brain metastases, clinical evidence of small bowel obstruction, and use of oral anticoagulation. All patients signed written informed consent, and the protocol was approved by institutional review boards.

Treatment Plan

Treatment consisted of sorafenib orally given as 400 mg orally twice per day continuously. Each cycle was 4 weeks, and treatment was continued until occurrence of disease progression (ie, progressive disease [PD]) or intolerable toxicity.

Efficacy and Toxicity Assessment

When possible, tumor burden was evaluated by clinical examination at baseline and before each cycle. Alternatively, disease was evaluated radiographically at baseline, before each odd cycle, and at the end of treatment. Investigator-determined best overall response was defined by using RECIST 1.0 criteria in patients with measurable tumors.[28] No independent outcome review was performed. CA-125 measurements were scheduled for all patients on day 1 of each cycle. Adverse events (AEs) were assessed on day 1 of each cycle and were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.

Translational Analyses

Levels of pERK were measured by lysate arrays constructed as previously described[26,27] in pre- and post-treatment PBLs that were collected pretreatment (within 14 days of the start of cycle 1) and post-treatment (within 3 days of the start of cycle 2; Appendix, online only). Total b-Raf and ERK expressions were assessed by immunohistochemistry in paraffin-embedded archival tissue, as previously described[29] (Appendix, online only). Immunostaining intensity was scored as 1+, 2+, or 3+, and an H score was calculated as the product between the intensity and the percent of staining cells.

Statistical Analysis

This was an open-label, multicenter, two-stage, phase II study performed through the GOG (protocol GOG170F). The primary objectives were determining the efficacy of sorafenib as estimated from the probability of surviving progression free for at least 6 months (ie, PFS at 6 months) and characterizing the toxicity of sorafenib with the frequency and severity of AEs. The time to progression or death was assessed from the date of entry onto the study. The first stage targeted a sample size of 25 eligible patients with measurable disease but was allowed to range from 22 to 29 patients. If five or more patients of 25 were progression free at 6 months, the study was allowed to proceed to the second stage. If the study continued to the second stage, the targeted cumulative accrual was 56 but was allowed to range from 53 to 60 patients. If 11 or fewer patients of 56 were progression free at 6 months, then the activity of the agent was deemed uninteresting. The full set of decision criteria for deeming an agent interesting for additional study was previously presented.[30] These decision criteria limit the probability of falsely declaring inactive agents (true probability of PFS at 6 months equal to 15%) as interesting to 10%, with an average probability of early termination of 59%, and the criteria have a probability of correctly declaring active regimens (true probability of PFS at 6 months equal to 30% or more) as interesting with 90% power.[31] Efficacy analysis and calculation of sample size were prospectively defined to include only patients with measurable disease. The a priori exclusion of patients with nonmeasurable detectable disease from the efficacy analysis was based on lack of any historical database on which to judge the agent as being interesting for additional study in this group. The secondary objectives were to measure the proportion of patients with objective responses (ie, partial and complete) to estimate the distribution of PFS and overall survival (OS) and to assess the impact of prognostic variables: platinum-free interval and performance status. An exploratory objective was to assess the effect of measurable disease status on PFS and OS. Translational objectives included evaluation of changes in pERK levels in PBLs before and after treatment and the assessment of b-Raf and ERK expression in archival paraffin-embedded tumors with clinical outcome. The exploratory analyses conducted to evaluate these objectives included paired *t* tests and survival analyses in which transformed levels of b-Raf and ERK expression were included as covariates in Cox modeling.[32] Landmark analyses were occasionally used to help assess the potential prognostic significance of biomarkers obtained after study entry.[33,34] The Spearman coefficient was used to measure the correlation between intensity of staining for ERK and b-Raf in the stained specimens.

RESULTS

Patients

Seventy-three consenting patients were enrolled, of which 71 (97%) were eligible. The two ineligible patients had wrong histology

Table 1. Patient Demographic and Clinical Characteristics

Characteristic	Patients No. (N = 71)	%
Age, years		
Median	60	
Range	33-80	
Performance status		
0	57	80.3
1	14	19.7
Ethnicity		
White	65	91.5
African American	2	2.8
Other/unspecified	4	5.6
Site		
Ovary	58	81.7
PPC	13	18.3
Platinum sensitive		
Yes	21	29.6
No	50	70.4
Measurable disease		
Yes	59	83.1
No	12	16.9
Histologic type		
Serous	64	90.1
Endometrioid	2	2.8
Clear cell	1	1.4
Mixed	3	4.2
Adenocarcinoma	1	1.4
No. of prior chemotherapy regimens		
1	40	56.3
2	31	43.7

Abbreviation: PPC, primary peritoneal carcinomatosis.

Table 2. Toxicity

Adverse Event	No. of Adverse Events by Grade: Grade 1	Grade 2	Grade 3	Grade 4
Leukopenia	10	1	1	1
Thrombocytopenia	13	1	0	1
Neutropenia	7	1	1	1
Anemia	18	3	1	0
Other hematologic	0	0	1	0
Allergy	0	1	0	0
Hearing	1	0	0	0
Cardiovascular	16	6	1	1
Coagulation	2	0	1	0
Constitutional	38	11	2	1
Dermatologic	18	22	14	0
Endocrine	2	0	0	0
GI	35	18	3	0
Genitourinary/renal	1	0	1	0
Hemorrhage	3	1	0	0
Infection	0	3	0	0
Lymphatic	0	1	1	0
Musculoskeletal	3	4	0	0
Metabolic	28	5	8	2
Neuropathy	14	4	0	0
Other neurologic	10	0	1	0
Ocular	4	1	0	0
Pain	22	9	1	0
Pulmonary	7	0	1	1

NOTE. No. evaluated = 71.

(n = 1) or detectable disease with low CA-125 (n = 1). Table 1 indicates that 59 patients (83%) had measurable disease and that 12 patients (17%) had nonmeasurable disease. Data from 71 patients were analyzed for toxicity, and data from 59 patients with measurable disease were utilized for efficacy. The median age was 60 years (range, 33 to 80 years). Fifty-eight patients (82%) had OC, and 13 (18%) had PPC. Serous papillary carcinoma was the most common histology (64 patients [90%]). Fifty patients (70%) had platinum-resistant or refractory OC. Forty patients (56%) received one prior regimen, and 31 (44%) received two prior regimens.

Treatment Administration and Safety

Among all patients, 219 cycles were administered. The median number of cycles completed was two (range, one to 24 cycles). Causes for treatment discontinuation were as follows: disease progression (n = 55), toxicity (n = 9), withdrawal of consent (n = 3), death (n = 1 as a result of sepsis while on treatment, although attribution to treatment was considered highly unlikely), and other reasons (n = 3). Table 2 lists treatment related AEs. The most common AEs were GI (79%), constitutional (73%), dermatologic (76%), metabolic (61%), and pain (45%); the majority were grades 1 to 2. Grade 3 to 4 toxicities affecting more than one patient included the following: dermatologic (n = 14), metabolic (n = 10), constitutional (n = 3), GI (n = 3), cardiovascular (n = 2), leukopenia (n = 2), neutropenia (n = 2), and pulmonary (n = 2). Antiangiogenic class-specific AEs were as follows: hypertension (n = 20 occurrences; one was grade 3) and proteinuria (n = 3; grades 1 to 2). Twenty-nine women developed hand-foot syndrome (nine were grade 3). Unexpected serious AEs included a nonfatal cardiopulmonary arrest possibly related to treatment. No treatment-related deaths or GI perforations were recorded.

Efficacy

Fifty-nine patients (83%) had measurable disease and were therefore included in the analysis of efficacy. At 6 months, 14 patients (23.7%) were without disease progression. There were two partial responses by RECIST (3.4%), and 20 patients (33.9%) had stable disease as best response. Durations of the two responses were 6.77 and 6.14 months, respectively. At a median follow-up of 23.6 months, 18 patients were alive, of which three were without evidence of progression. The median PFS was 2.1 months (95% CI, 1.87 to 3.42 months; Fig 1). The median OS was 16.33 months (95% CI, 11.10 to 22.21 months). Multivariate Cox analysis indicated that neither performance status nor length of the platinum-free interval were predictors for PFS (hazard ratio [HR], 0.94; 95% CI, 0.48 to 1.84) for performance status; and HR, 0.70; 95% CI, 0.36 to 1.38 for platinum sensitivity) or OS (HR, 1.90; 95% CI, 0.93 to 3.89 for performance status; and HR, 0.66; 95% CI, 0.32 to 1.35 for platinum sensitivity).

Because patients with detectable disease had not been included in prior GOG protocols, this subgroup was analyzed separately and only with exploratory intent. Of 12 patients with detectable disease enrolled, 11 had PFS shorter than 6 months. The median PFS was 1.87 months (95% CI, 1.74 to 2.83 months), and the median OS was 22.67

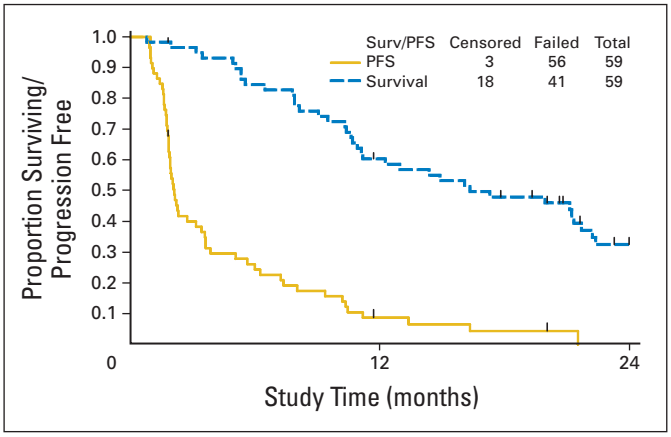

Fig 1. Survival curves: overall survival and progression-free survival (PFS). Surv, survival.

months (Appendix Fig A1, online only). Performance status and platinum sensitivity were not substantially associated with PFS or OS in patients who had unmeasurable disease.

Translational Correlative Analyses

Total ERK and b-Raf expressions were assessed by immunohistochemistry in archived paraffin-embedded tumors from 60 women. Total ERK was expressed in 100% of tumors analyzed; 55% (n = 33) were an intensity of 1+, 25% (n = 15) were 2+, and 20% (n = 12) were 3+. b-Raf was also expressed in 100% of tumors analyzed; 48% (n = 29) were an intensity of 1+, 17% (n = 10) were 2+, and 35% (n = 21) were 3+ (Table 3). Intensity of total ERK and of b-Raf expression in tumors (1+, 2+, or 3+) was positively and notably associated (Appendix Table A1, online only; $\tau = 0.31$). Pre- and post-treatment PBLs were used to explore the pharmacodynamic activity of sorafenib. pERK was measured in pre- and post-treatment PBLs in 37 and 36 women, respectively, by using reverse-phase protein microarray. There was no notable change in pERK levels between pre- and post-treatment PBL specimens (Appendix Table A2, online only; Fig 2).

The expression of total ERK and b-Raf in tumors or pERK in PBLs were examined for associations with PFS or OS. ERK and b-Raf expression and pretreatment pERK level in PBLs were not notably associated with tumor response, PFS for at least 6 months, or OS. However, there was an indication of post-treatment pERK levels being associated with tumor response ($\tau = 0.37$) and PFS for at least 6 months ($\tau = 0.36$). Higher levels of post-treatment pERK correlated with a lower risk of progression (Table 4; Fig 3; Appendix Table A3; HR, 0.45; 95% CI, 0.22 to 0.93) but not with OS (HR, 1.41; 95% CI, 0.64 to 3.07; Fig 3). Landmark analysis of PFS on post-treatment pERK (6 weeks after trial entry) for 29 patients yielded similar results (HR, 0.47; 95% CI, 0.21 to 1.07). One of the two responders for whom pre- and post-treatment PBLs were available had high post-treatment pERK levels (Appendix Table A3, online only). Expression levels of the other phosphoproteins measured on the arrays did not vary between pre- and post-treatment and did not correlate with PFS (data not shown).

Table 3. Biomarker Analyses

Biomarker	No.	Analysis		
		Median	Lower Quartile	Upper Quartile
pERK*				
Pretreatment	37	1,553.09	1,082.47	1,931.40
Post-treatment	36	1,238.93	874.41	1,740.80
ΔpERK†	32	23.78	−756.57	442.46
ERK (pretreatment)‡	60	0.89	0.16	1.89
Raf (pretreatment)‡	60	0.87	0.13	1.90

Abbreviation: pERK, phosphorylated ERK.
*pERK as measured by lysate arrays.
†Post-treatment pERK minus pretreatment pERK.
‡ERK and Raf immunohistochemistry H score.

DISCUSSION

In this phase II trial, sorafenib demonstrated modest antitumor activity in patients with recurrent OC; there were two objective, sustained responses, and 14 patients were free of progression at 6 months. Sorafenib targets the Raf kinases and the receptors, VEGFR and PDGFR, and it exerts antitumor activity through direct effects on cancer cells and indirect effects on endothelial cells. The agent has demonstrated clinical benefit in hepatocellular, renal, and thyroid carcinomas, and its study in OC was supported by the knowledge that the Ras-Raf-MAPK pathway is activated in ovarian tumors, mostly through nonconstitutive mechanisms, and that OC progression is heavily dependent on angiogenesis.[31-33,35-37]

The toxicities observed were substantial and consistent with observations from previous trials.[36,38] Notably, dermatologic toxicity and metabolic abnormalities were frequent. There were nine occurrences of grade 3 hand-foot syndrome, and there were seven patients who experienced grade 3 rash, as significant sorafenib toxicities. One patient developed a superficial squamous skin carcinoma in the context of grade 3 rash within 5 months of treatment with sorafenib. Squamous cutaneous carcinomas, keratoachantomas, and flares of actinic keratoses have recently been reported with sorafenib.[39-41] Decreased cutaneous immune surveillance caused by impairment of dendritic cell function or compensatory hyperactivation of ERK in keratinocytes induced by selective Raf inhibitors are potential factors in the pathogenesis of proliferative skin lesions induced by sorafenib.[39,42-44] In contrast, toxicities specific to antiangiogenic agents (ie, hypertension, proteinuria, or coagulation disturbances) were infrequent. Importantly, grade 3 hypertension occurred only once, and venous or arterial thrombotic events were not recorded. One cardiopulmonary arrest occurred in a patient who developed respiratory failure with wheezing shortly after initiating treatment with sorafenib. GI perforations reported with other anti-VEGF agents in OC were not recorded in this trial.[45-47] Myelosuppression was not frequently observed.

Greater than two thirds of patients treated on this study had platinum-resistant OC. Of the two responding patients, one had clear cell carcinoma. This is consistent with prior observations that suggest that the clear cell OC subtype may be more responsive to antiangiogenic agents and that supports testing of such an intervention in this subgroup of tumors.[47] There were only two responders to sorafenib, and a total of 14 patients were free of PD for at least 6 months; two patients received treatment for greater than 1 year. This suggests a

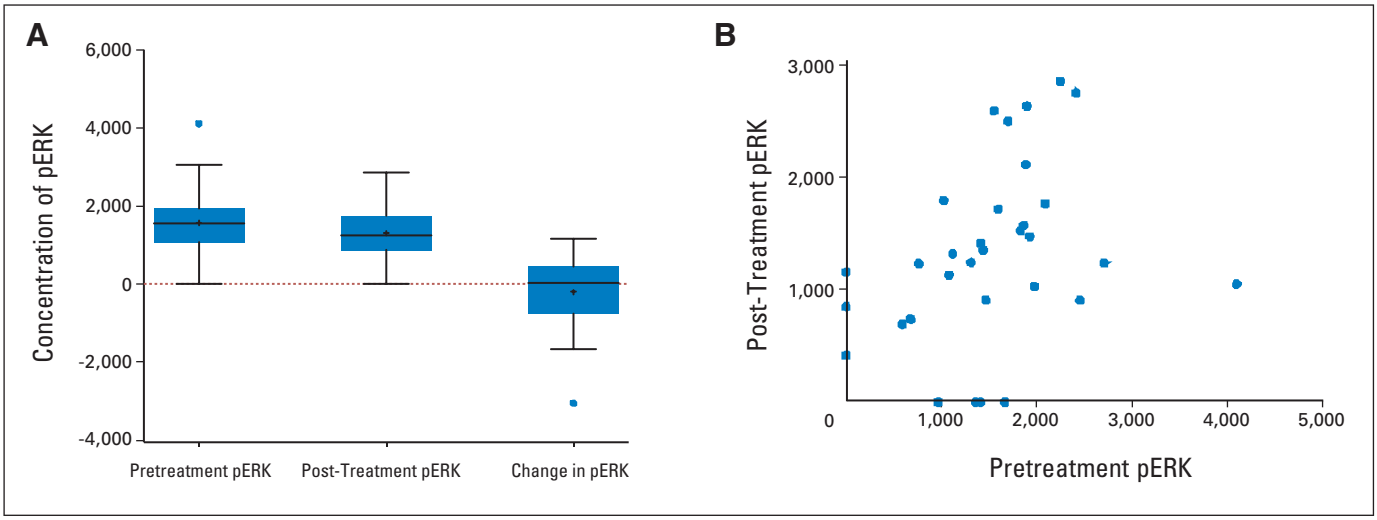

Fig 2. Pre- and post-treatment phosphorylated ERK (pERK) levels in peripheral-blood lymphocytes. (A) Distributions of pre- and post- treatment pERK levels and the change in pERK levels during the course of therapy. Changes in pERK were obtained by subtracting the pretreatment scores from the post-treatment scores for all patients who submitted both samples. (B) Individual values of pre- and post-treatment pERK.

cytostatic effect of sorafenib, consistent with observations from trials in other tumor types.[35,36] Unlike results reported for other anti-VEGF agents (eg, bevacizumab), there was no significant effect of sorafenib in patients with ascites. Interestingly, the outcome of patients who had nonmeasurable disease was not better than that of patients who had measurable disease. Only 12 patients who had detectable disease were enrolled, and only one of these patients remained free of PD for 6 months.

Correlative translational analyses confirmed basal expression of total ERK and b-Raf in all archival ovarian tumors analyzed, of which roughly half demonstrated moderate or intense staining for each protein. Activation of the Ras-Raf-MAPK pathway or b-Raf mutations in ovarian tumors was not analyzed in this study. Pre- and post-treatment PBLs were used to help examine the pharmacodynamic activity of sorafenib. There was no notable change in pERK from pre- to post-treatment in PBLs, consistent with observations from other trials.[48,49] Although pretreatment pERK levels were not notably correlated with post-treatment levels in this analysis (Pearson $r = 0.33$), it is possible that a larger sample size would demonstrate a correlation. Interestingly, there was an indication that higher levels of post-treatment pERK in PBLs were associated with longer PFS. A landmark analysis yielded similar results with a slightly wider CI, perhaps as a result of a smaller sample size.

Table 4. Association of Biomarkers With Survival

	Progression-Free Survival		Overall Survival	
Biomarker	HR	95% CI	HR	95%CI
pERK				
Pretreatment	0.79	0.40-1.57	0.88	0.41-1.90
Post-treatment	0.45	0.22-0.93	1.41	0.64-3.07
ERK (pretreatment)	1.09	0.64-1.84	0.74	0.40-1.39
Raf (pretreatment)	0.85	0.50-1.45	1.02	0.55-1.90

Abbreviations: HR, hazard ratio; pERK, phosphorylated ERK.

These findings may be explained by the emerging data suggesting complex regulation of the MAPK pathway through feedback loops. Several reports show that selective Raf or MEK inhibitors hyperactivate the Raf kinase through feedback and induce ERK activation.[50,51]

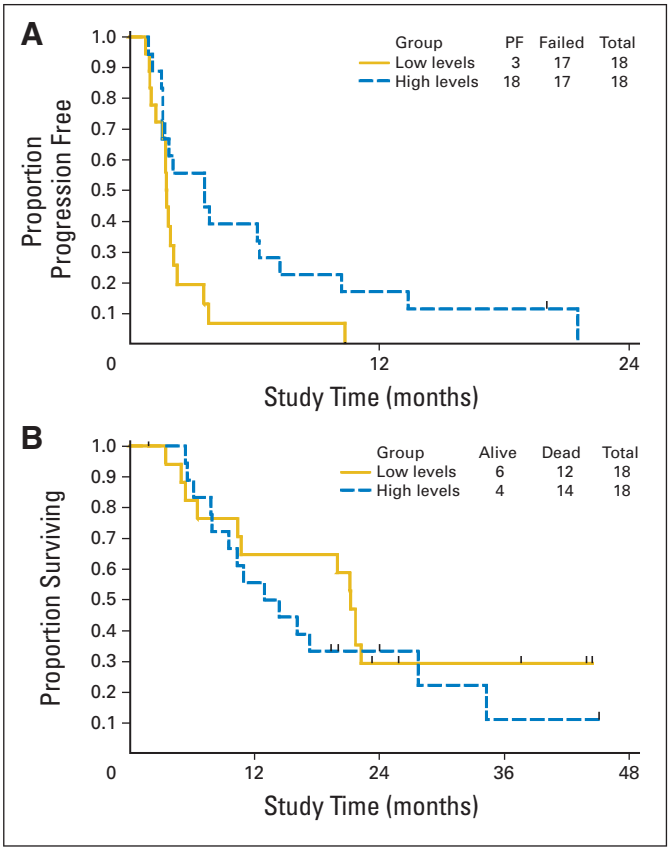

Fig 3. Post-treatment phosphorylated ERK (pERK) levels and survival. Post-treatment pERK levels and survival. Higher levels of post-treatment pERK were notably associated with (A) longer progression-free survival (PF) but not (B) overall survival.

A recent study showed that c-Raf inhibits b-Raf in vivo and that pharmacologic inhibition of one Raf protein can cause compensatory activation of the other.[43] Because sorafenib is a potent c-Raf inhibitor but is less active against wild-type or mutant b-Raf, selective inhibition of c-Raf by sorafenib could alter the b-Raf/c-Raf interaction that allows b-Raf to activate MEK and ERK. Interestingly, this does not appear to occur in cancer cells, because activated b-Raf does not coexist with mutant Ras or with high levels of inhibitory c-Raf.[42] However, in normal cells (ie, PBLs, keratinocytes), b-Raf and c-Raf coexist by controlling, through feedback, the level of ERK activation. Therefore, selective inhibition of c-Raf by sorafenib in PBLs may cause engagement of b-Raf and downstream MEK and ERK activation. Additional evaluation of post-treatment pERK in PBLs as a surrogate marker of sorafenib activity may be warranted.

The results of this trial do not support additional investigation of sorafenib as a single agent in recurrent OC. Evaluation of sorafenib in combination regimens are ongoing.[52,53]

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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