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ORIGINAL REPORT

Phase II Clinical Trial of Bevacizumab and Low-Dose Metronomic Oral Cyclophosphamide in Recurrent Ovarian Cancer: A Trial of the California, Chicago, and Princess Margaret Hospital Phase II Consortia

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ABSTRACT

Purpose
Vascular endothelial growth factor (VEGF) plays an important role in the biology of ovarian cancer (OC). Inhibitors of VEGF suppress tumor growth in OC models. Metronomic chemotherapy, defined as frequent administration of low doses of cytotoxic chemotherapy, suppresses tumor growth, possibly by inhibiting angiogenesis. A phase II trial was conducted to evaluate the antitumor activity and adverse effects of bevacizumab and metronomic oral cyclophosphamide in women with recurrent OC.

Patients and Methods
Patients with measurable disease and prior treatment with a platinum-containing regimen were eligible. Up to two different regimens for recurrent disease were allowed. Treatment consisted of bevacizumab 10 mg/kg intravenously every 2 weeks and oral cyclophosphamide 50 mg/d. The primary end point was progression-free survival at 6 months. Plasma levels of VEGF, E-selectin, and thrombospondin-1 were obtained serially.

Results
Seventy patients were enrolled. The probability of being alive and progression free at 6 months was 56% (± 6% SE). A partial response was achieved in 17 patients (24%). Median time to progression and survival were 7.2 and 16.9 months, respectively. The most common serious toxicities were hypertension, fatigue, and pain. Bevacizumab-related toxicities included four episodes of gastrointestinal perforation or fistula, two episodes each of CNS ischemia and pulmonary hypertension, and one episode each of gastrointestinal bleeding and wound healing complication. There were three treatment-related deaths. Levels of VEGF, E-selectin, and thrombospondin-1 were not associated with clinical outcome.

Conclusion
The combination of bevacizumab and metronomic cyclophosphamide is active in recurrent OC. Further study of this combination is warranted.

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INTRODUCTION

Most patients with ovarian cancer (OC) present with advanced disease.[1] While the current standard first-line chemotherapy is associated with an improvement in median survival,[2] most patients will relapse and long-term survival rates remain poor. Several chemotherapy drugs, such as liposomal doxorubicin, gemcitabine, and topotecan, have modest activity in recurrent OC.[3] However, their use is associated with toxicity and has a limited effect in survival, and their use in first-line therapy has failed to improve overall survival.[4-6] Therefore the development of novel agents with limited toxicity is of high priority. One approach is to identify agents that target mechanisms of tumor progression, such as angiogenesis, which is a critical pathway in the development and progression of cancer.[7]

Angiogenesis is regulated by a balance between various angiogenic and antiangiogenic factors.[8] Vascular endothelial growth factor (VEGF) is the best characterized angiogenic factor and is recognized as a major element in regulating angiogenesis.[9-12]

In OC models, VEGF plays a major role in initiating and mediating tumor growth.[13] Similar findings have been reported in humans where

markers of increased angiogenesis are correlated with poor prognosis.[14-16] Treatment of OC xenograft models with the murine anti-human VEGF monoclonal antibody A.4.6.1. (parent antibody of bevacizumab) completely inhibits ascites formation.[17] Bevacizumab, the recombinant humanized version of this antibody, significantly improves outcome in various tumors when combined with cytotoxic chemotherapy.[18-20]

As the process of angiogenesis is regulated by redundant pathways, one potential approach to maximize antiangiogenic therapies is the use of combination therapy.[21] Metronomic chemotherapy (MC), defined as the frequent administration of low doses of cytotoxic chemotherapy at frequent intervals, suppresses tumor growth in experimental models, possibly by inhibiting angiogenesis by stimulating the release of thrombospondin (TSP).[22-27] These experimental findings are supported by a clinical trial where encouraging activity with minimal toxicity was observed in patients with breast cancer (BC).[28] Furthermore, in experimental models, the combined use of MC with antiangiogenic therapies demonstrates marked inhibition of tumor growth.[29-32] Although this data suggests that metronomic cyclophosphamide acts primarily through blockade of angiogenesis it can not be completely excluded that at least part of the activity may be due to its cytotoxic properties.

Based on these data, we conducted a phase II clinical trial to evaluate the antitumor activity and adverse effects of bevacizumab and MC in patients with recurrent OC or primary peritoneal carcinoma. In addition, we also sought to explore molecular correlates for response and outcome through the analysis of serial markers of angiogenesis: VEGF, E-selectin (E-Sel), and TSP-1.

PATIENTS AND METHODS

Patient Population

To participate in this study patients were required to have histologically documented recurrent epithelial OC or primary peritoneal carcinoma with measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) criteria.[33] Patients were required to have received a platinum-containing regimen for primary disease and up to two distinct prior regimens for recurrent disease. Patients with an initial treatment-free interval of more than 12 months were required to be retreated with a platinum-based regimen. Patients rechallenged with the same platinum-based regimen (or single-agent platinum), were considered to have had that regimen only once. Prior therapy with a taxane was not required.

Participating patients were required to have adequate bone marrow (absolute granulocyte count $\geq$ 1,500/μL and platelets $\geq$ 100,000/μL), renal (serum creatinine $<$ 1.5 times the upper limit of normal [ULN]), and hepatic (bilirubin $\leq$ 1.5 times ULN and ALT or AST $\leq$ 3 times ULN) function. A performance status of 0 to 2 was required. Patients were required to have provided written informed consent consistent with current institutional, state, and federal regulations. Major exclusion criteria included serious, nonhealing wound ulcers, or bone fractures, major surgical procedure, recent open biopsy or significant traumatic injury, any history of deep venous thrombosis, recent arterial thrombosis, full dose anticoagulants, presence of bleeding diathesis or coagulopathy, history or clinical evidence of CNS disease, and significant cardiovascular or peripheral vascular disease. Prior treatment with an antiangiogenic agent was not allowed. Patients were considered to be platinum resistant if they progressed within 6 months of completing their first platinum-based therapy. All eligible patients were included in the analyses of response, toxicity, progression-free survival (PFS) and overall survival (OS).

Study Design and Treatment Schedule

Patients were treated with intravenous bevacizumab and oral cyclophosphamide. During the first 3 weeks of treatment, bevacizumab was administered intravenously at a dose of 10 mg/kg every week followed by administration every 2 weeks. Oral cyclophosphamide was taken at a daily dose of 50 mg. A cycle of treatment lasted 28 days. Treatment was continued until the patient experienced tumor progression, toxicity (thrombosis and/or the following grade 3 or 4 toxicities: renal; CNS cerebrovascular ischemia; hemorrhage; cardiovascular or allergy/immunology or other grade 4 nonhematologic toxicities) or patient request/physician discretion.

Treatment with bevacizumab was held in the event of proteinuria longer than 2 g/24 hours or other grade higher than 2 toxicities until toxicity resolved to grade 0 to 1. Patients were removed from study if toxicity had not resolved by 4 weeks. Cyclophosphamide was held in the event of grade 3 to 4 toxicities until resolution to grade 0 to 2 and subsequent doses reduced to 25 mg. Patients were removed from study if toxicity had not resolved within 4 weeks. Continuous treatment with bevacizumab was allowed in patients achieving clinical benefit. Toxicities were graded according to the National Institutes of Health common toxicity criteria, version 3.0.

Efficacy Determinations

RECIST criteria were used to evaluate response.[33] Response was evaluated after every two cycles of treatment. Complete response (CR) was defined as the disappearance of all target and nontarget lesions, no evidence of new lesions and normalization of CA-125, lasting at least 4 weeks. Partial response (PR) was defined as a 30% or greater reduction in the sum of the longest dimensions of all target lesions and no unequivocal progression of nontarget lesions, lasting at least 4 weeks. Progressive disease (PD) was defined as a 20% or greater increase in the sums of the longest dimensions of target lesions, or the appearance of new lesions within 8 weeks of study entry. Stable disease (SD) was defined as any condition not meeting the above criteria. Changes in CA-125 alone were not used to determine tumor response or progression. OS was defined as the time from the first day of treatment to the time of death due to any cause; patients remaining alive at their last follow-up were censored at that time. PFS was defined as the time from the first day of treatment to the first observation of disease progression or death due to any cause or last follow-up. PFS was censored for patients who were alive and free of progression at time of last follow-up.

Angiogenesis Markers

Plasma levels of VEGF, E-Sel, and TSP-1 were obtained at baseline and repeated every cycle for the first two cycles and then every two cycles. Plasma concentrations were determined using a quantitative sandwich enzyme immunoassay technique according to the instructions from the manufacturers (R & D Systems, Minneapolis, MN [VEGF and E-Sel] and Chemicon, Temecula, CA [TSP-1]).

Statistical Design and Analysis

A modified two stage Optimum design suggested by Simon[34] was used to plan this study with the following considerations: the primary measures of outcome were PFS at 6 months and overall response rate. We postulated that if 15% or fewer patients were alive and progression free at 6 months, then there would be little interest in studying this combination further. In addition, if there was clear evidence that the true objective response rate was less than 10%, this would also discourage further study. In contrast, if 30% or more patients were to have a clinical benefit with this regimen, and if the objective response rate was at least 10%, then this would encourage further study of this combination. Therefore, 23 patients were entered into the first stage of accrual. The second stage of accrual, with 32 patients, would open if four or more patients were progression-free at 6 months and at least one patient experienced an objective response. This design provided an α error of .10 and a power of 0.81. PFS and OS were estimated using the product-limit method of Kaplan and Meier.

The associations between baseline patient characteristics and clinical outcome were examined using Fisher's exact test for response, and the log-rank test for PFS and OS.

Median baseline levels of plasma VEGF, E-Sel, and TSP-1 were compared between responders and nonresponders by using the Mann-Whitney U test. The maximal χ^2 method of Miller, Siegmund, and Halpern[35,36] was used to dichotomize patients into good and poor prognosis groups in terms of likelihood of remaining alive and progression-free. The

levels of plasma VEGF, E-Sel, and TSP-1 by treatment cycles were compared using the Kruskal-Wallis test.

RESULTS

Seventy patients were entered. Patient characteristics are summarized in Table 1. Most patients were white and had a performance status of 0 to 1. All patients had received prior chemotherapy with platinum and a taxane. Most had received treatment with topotecan, gemcitabine, or liposomal doxorubicin. Forty patients (40%) had platinum-resistant disease based on their initial therapy. Most patients who were initially platinum sensitive became resistant after re-exposure to platinum.

At the time the data were analyzed, the median follow-up period was 23.2 months (range, 3.7 to 32.7). Three patients (4%) remain on study. PD was the most common reason for patients to go off treatment (56%), followed by toxicity (21%) and patient refusal (4%). A total of 608 courses of treatment were administered with a median of five (range, 1 to 31). All patients were eligible and evaluated for PFS, response, toxicity, and OS.

Tumor response. Best response was PR in 17 patients (24%; 95% CI, 15% to 36%), SD in 44 patients (63%; 95% CI, 50% to 74%), and PD in nine patients (13%; 95% CI, 6% to 23%). Response rate according to histology was: 30%, 5%, and 50% for serous tumors, adenocarcinoma not otherwise specified, and clear cell carcinomas, respectively ($P = .049$ based on Fisher's exact test).

Median time to progression was 7.2 months (95% CI, 5.3 to 8.7) while median survival time was 16.9 months (95% CI, 11.4 to 25.2).

Table 1. Patient Characteristics

Characteristic		Value
No. of patients		70
Age at treatment, years		
Median	60	
Range	31-83	
Performance status		
0		31
1		35
2		4
Histology		
Serous		46
Adenocarcinoma NOS		20
Clear cell		4
Prior chemotherapy, %		
Platinum		100
Paclitaxel or docetaxel		100
Liposomal doxorubicin		27
Topotecan		23
Gemcitabine		19
No. of prior chemotherapy regimens		
Median	2	
Range	1-3	
Platinum sensitivity*, %		
Sensitive		60
Resistant		40

Abbreviations: NOS, not otherwise specified.
*Based on initial platinum-based therapy.

PFS and OS curves are shown in Figures 1 and 2. The probability of being alive and progression-free at 6 months was 56% (95% CI, 44 to 67). There was a statistically significant difference in PFS and OS between platinum-sensitive and platinum-resistant patients. There was no statistical significant difference in response rate between platinum-sensitive and platinum-resistant patients (33% *v* 12%, respectively; $P = .074$).

Toxicities. The most common toxicities were lymphopenia, fatigue, nausea, vomiting, increased alkaline phosphatase, pain, hypertension, and proteinuria. Serious toxicities are summarized in Tables 2 and 3. There were 16 episodes of grade 3 hematologic toxicity with grade 3 lymphopenia as the most common and two episodes of grade 4 lymphopenia. There were no episodes of grade 3 anemia and only one episode of grade 3 neutropenia and one episode of grade 3 thrombocytopenia. Forty-four patients experienced grade 3, 4, or 5 nonhematologic toxicities with grade 3 hypertension (11 episodes), pain (13 episodes), and fatigue (6 episodes) being the most common.

There were three treatment-related deaths. Two patients developed pulmonary hypertension, (during cycles 4 and 14 of therapy); one of them was found to have a thrombus in the right ventricle. The third treatment-related death occurred in a patient who presented with obstruction and gastrointestinal perforation during cycle 18. Two additional patients died during study participation. One patient experienced PD. Another patient developed a bowel obstruction, was treated conservatively, developed sepsis, and eventually died.

The most common bevacizumab-related toxicities were hypertension and proteinuria, occurring in 39% and 44% of patients, respectively, with less than 16% of patients experiencing grade 3. Three patients developed a gastrointestinal perforation, one patient each a gastrointestinal fistula, wound healing complication, and grade 3 gastrointestinal bleeding. A patient with SD developed a grade 2 gastrointestinal perforation during her ninth course of therapy. She recovered uneventfully and resumed treatment with no further perforations. However, she developed pulmonary hypertension and died during her fourteenth course of therapy. A patient with a PR developed abdominal pain during her eighteenth course of therapy. Bevacizumab was interrupted and 1 week later she developed worsening abdominal pain, nausea, vomiting, and an acute abdomen with gastrointestinal perforation. On the same day, she developed

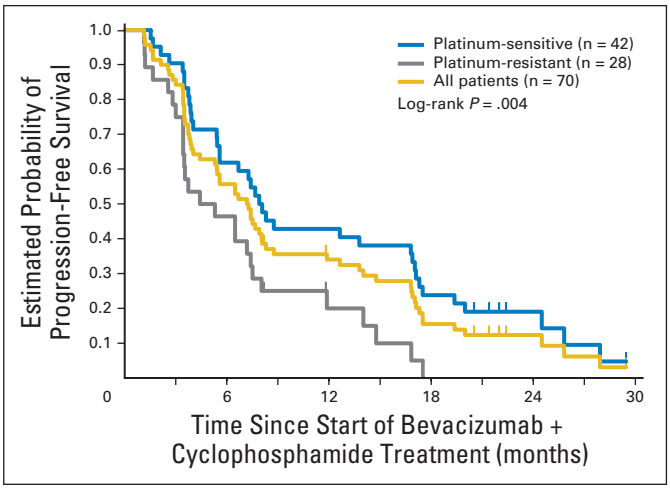

Fig 1. Progression-free survival in all patients and according to platinum sensitivity.

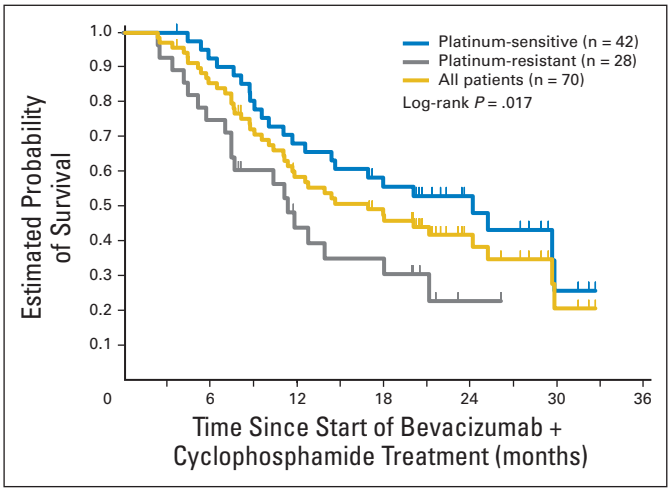

Fig 2. Overall survival in all patients and according to platinum sensitivity.

cardiopulmonary arrest and died. A third patient developed a gastrointestinal perforation during the fourth cycle of treatment. She underwent surgical resection of the jejunum and recovered. One patient with PD developed an enterovaginal fistula during the fourth cycle. One patient with an umbilical hernia experienced rupture of the hernia with leakage of ascites during her seventh course of therapy. She was taken off study and the hernia was repaired surgically 1 month later. One patient with extensive intra-abdominal disease and hemorroids experienced PD after completing four courses of therapy. She began treatment with liposomal doxorubicin and 2 weeks later experienced rectal pain and bloody diarrhea. She was transfused and recovered without sequelae.

One patient presented with confusion and aphasia during her third cycle of therapy. She was found to have a brain infarct. Treatment was discontinued, she received anticoagulation and symptoms improved. Another patient experienced grade 3 hypertension during the second course of therapy. She developed headaches and vomiting and was found to have a grade 2 CNS hemorrhage. Treatment was discontinued and she fully recovered. Another patient developed confusion and aphasia during the third course of therapy. She was found to have multiple cerebral infarcts. She was taken off study and placed on anticoagulation therapy. She recovered well but died 5 months later due to PD.

Correlative Studies

Pretreatment plasma levels of VEGF, E-Sel, and TSP-1 were obtained in 28, 29, and 17 patients, respectively. Plasma levels during study are summarized in Table 4. Levels of VEGF and TSP-1 decreased over time, but there were no significant associations between any of these markers and clinical outcome (response, PFS, or OS).

DISCUSSION

It is well established that OC is a chemotherapy-sensitive tumor. However the addition of cytotoxic agents to the standard first-line chemotherapy has not improved survival. Therefore, the identification of biologic or target-specific agents with activity in OC has great importance. Our study shows that the combination of two biologic agents, bevacizumab and metronomic oral cyclophosphamide, has encouraging activity in recurrent OC.

Several biologic agents have been studied in the treatment of OC with rather disappointing results. As summarized in Table 5 these agents (tamoxifen, carboxyamidotriazole, erlotinib, gefitinib, and trastuzumab) have shown, at best, modest activity, usually with response rates of less than 10% and median PFS of only 2 or 3 months.[37-42]

In contrast, it appears that bevacizumab is more active. Burger et al[43] reported the results of a phase II study of single-agent bevacizumab a response rate of 17.7% and median PFS of 4.7 months were observed. In addition, 38.7% of the patients were alive and progression free at 6 months. Cannistra et al[44] evaluated the activity of bevacizumab in a highly resistant patient population and reported a response rate of 15.9%. The 6-month PFS was 27.4%. This study was

Table 2. Grade 3-4 Toxicities

Toxicity	Maximum Toxicity Experienced Over All Courses (No.)	
	Grade 3	Grade 4
Hematologic		
Leukocytes (total WBC)	2	
Lymphopenia	12	2
Neutrophils/granulocytes (ANC/AGC)	1	
Platelets	1	
Nonhemotologic		
Coagulation		
Partial thromboplastin time	1	
Other (specify)	1	
Fatigue (asthenia, lethargy, malaise)	6	
Dermatology/skin		
Rash/desquamation	1	
Wound complication, noninfectious	1	
Hot flashes	1	
Gastrointestinal/hepatic		
Constipation	2	1
Dehydration	1	
Distension/bloating, abdominal	2	
Nausea	2	
Obstruction, gastrointestinal	5	1
Vomiting	4	
ALT	3	
AST	3	
Alkaline phosphatase	2	
Glucose, serum-high (hyperglycemia)	2	
Potassium, serum-low (hypokalemia)	2	
Sodium, serum-low (hyponatremia)	4	1
Pain	13	
Pulmonary/upper respiratory		
Cough	1	
Dyspnea (shortness of breath)	1	1
Hypoxia	1	
Pleural effusion (nonmalignant)	1	
Voice changes/dysarthria (eg, hoarseness, loss or alteration in voice, laryngitis)	1	

NOTE. Other grade 3 toxicities: hyperamylasemia, hypocalcemia, hypomagnesemia, hypermagnesemia, infection, arthritis, cough and urinary incontinence (1 each).Other grade 4 toxicities: anorexia, constipation, hyperkalemia, and elevated creatinine (1 each).
Abbreviations: ANC, absolute neutrophil count; AGC, absolute granulocyte count.

Table 3. Bevacizumab-Related Grade 3 to 5 Toxicities

Toxicity	Grade (No.) 3	4	5
Cardiovascular (arrhythmia)			
Supraventricular and nodal arrhythmia		1	
Cardiovascular (general)			
Cardiac ischemia/infarction	1		
Cardiac troponin T	1		
Hypertension	11		
Hypotension		1	
Pulmonary hypertension		1	1
Edema:limb	1		
Thrombosis/thrombus/embolism	1		1
Thrombosis/embolism (vascular access related)	1		
Gastrointestinal			
Perforation, Gastrointestinal		1	1
Hemorrhage	1		
Neurology			
CNS cerebrovascular ischemia		2	
Dizziness	2		
Neuropathy: sensory	2		
Speech impairment (eg, dysphasia or aphasia)	1		
Renal/genitourinary			
Creatinine		1	
Proteinuria	3		
Incontinence, urinary	1		

Table 5. Biologic Agents in Recurrent Ovarian Cancer

Agent	Response Rate (%)	TTP or PFS	Patients Progression Free at 6 Months (%)
Tamoxifen[37,38]	13	—	< 15
Carboxyamidotriazole[39]	3	3.6 months	31
Erlotinib[40]	6	9 weeks	—
Gefitinib[41]	4	—	< 15
Trastuzumab[42]	7.3	2 months	< 20
Bevacizumab[43]	17.7	4.7 months	38.7
Bevacizumab[44]	15.9	4.3 months	27.4
Bevacizumab plus metronomic cyclophosphamide*	24	7.2 months	56

Abbreviations: TTP, time to progression; PFS, progression-free survival.
*Current study.

closed prematurely due to a high frequency (11.4%) of gastrointestinal perforations. However, the true incidence of gastrointestinal perforations in OC is unknown. We identified only four episodes (6%) while there were no episodes in the report by Burger et al. We did not identify any risk factors for gastrointestinal perforations. However, it has been suggested that recent or current bowel obstruction, advanced disease, and chemotherapy-resistant disease represent potential risk factors.

These two studies show that bevacizumab has significant activity in recurrent OC. Despite the inherent limitations of cross-study comparison, our findings suggest that the addition of MC to bevacizumab may enhance the activity of single-agent bevacizumab.

Further data to support the potential clinical value of combining MC with antiangiogenic therapies was provided by Burstein.[45] In a phase II randomized trial women with metastatic BC were allocated to treatment with MC or MC plus bevacizumab. Response rate and PFS were superior for the combination regimen. Buckstein reported a very encouraging response rate of 37% with cyclophosphamide and celecoxib in heavily pretreated lymphoma.[46]

Our data suggest that the combination of bevacizumab and MC has significant activity in recurrent OC. This was a population that was resistant to at least one line platinum therapy having all progressed fewer than 12 months from a prior platinum therapy. The encouraging activity, time to progression, and median survival compare favorably with both, conventional and investigational agents. Despite the small sample size, the response observed in two of four patients with clear cell carcinoma, a histology usually associated with low response to chemotherapy,[47] is particularly encouraging.

Clearly, therapies that target VEGF have been shown to be active in various tumors. However, currently, there is no marker that reliably predicts for benefit to treatment with antiangiogenic therapies. We attempted to evaluate the potential predictive role of various circulating angiogenic factors as it has been suggested that they represent useful surrogate markers.[48] We selected VEGF as this growth factor is the target of bevacizumab, VEGF levels are elevated in patients with OC[49,50] and appear to decrease during therapy.[50] In BC levels of E-Sel may be associated with response to docetaxel and bevacizumab.[51] It has been suggested that elevated TSP-1 levels correlate with response to MC.[52] We also examined the prognostic and predictive potential use of various genetic polymorphisms and the results are the subject of a separate publication.[53] The analysis of the correlative markers

Table 4. Plasma Levels of VEGF, E selectin, and TSP-1

Cycle	VEGF No.	VEGF Median	VEGF Range	E selectin No.	E selectin Median	E selectin Range	TSP-1 No.	TSP-1 Median	TSP-1 Range
0-1	28	83.51	2.33-301.40	29	2.92	0.18-13.04	17	1,368.91	310.66-2,152.87
2-3	26	76.86	9.15-857.36	24	3.46	0.07-10.33	11	1,495.98	0.14-2,100.68
4-5	19	181.08	27.05-1,154.68	16	3.48	1.18-14.33	11	572.86	1.17-1,544.40
6-7	9	79.59	22.61-156.25	9	1.42	1.18-7.33	8	457.25	1.61-1,375.46
8+	7	59.01	31.92-85.28	7	2.13	1.15-5.19	7	731.14	206.59-1,112.11
*P**		.015			.34			.010	

Abbreviation: VEGF, vascular endothelial growth factor; TSP-1, thrombospondin-1.
*Based on the Kruskal-Wallis test.

reported here did not reveal statistically significant patterns; however, these results are limited by the small numbers of patients participating in this portion of the study, and it is possible that stronger patterns would have emerged with larger numbers. The small numbers of specimens provided for analysis reflects the difficulty in procuring repeated specimens.

In conclusion, bevacizumab in combination with MC has significant activity in recurrent OC. Further study of this combination is warranted.

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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