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Phase II trial of nintedanib in patients with bevacizumab-resistant recurrent epithelial ovarian, tubal, and peritoneal cancer

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HIGHLIGHTS

- Nintedanib has minimal activity in unselected bevacizumab-resistant ovarian cancer patients.
- Nintedanib was tolerable and toxicities were manageable.
- Baseline plasma IL6 levels were associated with worse progression-free survival.

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ABSTRACT

Background. Bevacizumab provides benefit in epithelial ovarian cancer (EOC), yet resistance to bevacizumab often occurs. We determined if nintedanib, a tyrosine kinase inhibitor of VEGF, FGF, and PDGF receptors has antitumor activity in bevacizumab-resistant recurrent EOC, tubal, and peritoneal cancer.

Methods. This phase II study evaluated nintedanib 200 mg/day until disease progression or unacceptable toxicity. The primary objective was 6-month progression free survival (PFS6m). Secondary objectives were response rate and toxicity. Simon two-stage optimal design was used. Baseline angiogenic plasma biomarkers were measured.

Results. 27 patients were enrolled evaluable for PFS; 26 were evaluable for PFS6m. The median age was 65 years (range 44–73); 89.9% had high-grade serous EOC; 70% received at least >2 prior chemotherapies; and 81% (22/27) had chemoresistant disease. With median follow up of 15.6 months (range 2–38) the PFS6m rate was 11.5% (3/26). Three participants had long duration of disease control (8–16 months). Median PFS and overall survival were 1.8 and 16 months, respectively. Response rate was 7.4% (2/27 PR). Thirty-seven percent (10/27) had stable disease, while 56% (15/27) had progressive disease. Adverse events included Grade 3 liver enzyme elevation (15%), Grade 3 diarrhea (7%), Grade 2 fatigue (7%), and Grade 2 nausea/vomiting (15%). PD patients exhibited higher levels of CD73, IL6, and VEGFD ($p < 0.05$) compared to PR/SD patients. IL6 was associated with worse PFS ($p = 0.03$).

Conclusions. Single-agent nintedanib has minimal activity in an unselected bevacizumab-resistant EOC population. Nintedanib was tolerable and toxicities were manageable. Plasma CD73, IL6, and VEGFD were identified as prognostic markers for progressive disease, and IL6 was associated with worse PFS confirming similar observations made in patients treated with other anti-angiogenic agents.

1. Introduction

Despite high initial complete response rates to chemotherapy, the majority of patients with advanced epithelial ovarian cancer (EOC) will relapse and die from recurrent disease. Anti-angiogenic agents, such as bevacizumab, a monoclonal antibody that targets vascular

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endothelial growth factor (VEGF), have shown promising activity in EOC. The initial study of bevacizumab in recurrent EOC demonstrated a response rate of 21% with a median response duration of 10 months. Approximately 40% of patients with recurrent EOC remained progression-free at 6 months [1]. The promising activity of single-agent bevacizumab led to pivotal trials of bevacizumab in combination with chemotherapy across ovarian cancer disease settings. Several randomized phase III trials have demonstrated that anti-angiogenic therapy with bevacizumab in combination with chemotherapy in women with advanced as well as recurrent disease yielded higher response rates and progression-free survival (PFS) with an acceptable toxicity profile [2–6]. Bevacizumab has Food and Drug Administration (FDA) and European Medical Agency (EMA) approval in combination with chemotherapy in women with platinum-resistant disease, and in combination with chemotherapy followed by maintenance in those with newly diagnosed advanced EOC as well as those with recurrent platinum-sensitive disease [7]. Most eligible patients with EOC, at some point in their treatment course, will most likely receive bevacizumab. Moreover, bevacizumab is the most commonly used anti-angiogenic agent in EOC.

Despite an initial response to bevacizumab, the development of resistance against bevacizumab-induced VEGF-blockade may occur. The development of resistance is not via the classic mechanisms noted for conventional cytotoxic agents. Tumor vascular endothelial cells are part of the tumor microenvironment but not the malignant clone and therefore are not prone to mutation. Rather the tumor endothelial cells adapt to VEGF inhibition by recruitment of vasculature using multiple secondary signaling pathways, such as those mediated by platelet-derived growth factor (PDGF), basic fibroblast growth factor (FGF), or other cytokines [8]. However, since VEGF is the primary angiogenic pathway, there may be an advantage to continue VEGF-blockade.

Nintedanib is a potent small molecule multi receptor tyrosine kinase inhibitor (PDGF receptor (PDGFR) α/β, FGF receptor (FGFR) 1, FGFR 3, and VEGF receptor (VEGFR) 1–3 [9]. At the molecular level, nintedanib is thought to inhibit the signaling cascade mediating angiogenesis by binding to the adenosine triphosphate (ATP) binding pocket of the receptor kinase domain, thus interfering with cross-activation via autophosphorylation of the receptor homodimers. Besides inhibition of neo-angiogenesis, tumor regression may also be achieved by inducing apoptosis of tumor blood vessel endothelial cells. Inhibition of receptor kinases may also interfere with autocrine and paracrine stimulation of tumor angiogenesis via activation loops involving VEGF, PDGF, and bFGF utilized by perivascular cells such as pericytes and vascular smooth muscle cells. Nintedanib has shown anti-tumor activity in recurrent ovarian cancer and combined with chemotherapy followed by maintenance nintedanib improved PFS in women with newly diagnosed EOC [10].

Given the known anti-tumor activity and rationale for continued VEGF inhibition in combination with abrogation of secondary pathways, this clinical trial evaluated single-agent nintedanib in participants with bevacizumab-resistant recurrent or persistent ovarian, peritoneal, and tubal cancer.

2. Patients and methods

2.1. Eligibility criteria

Patients with recurrent or persistent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma and confirmed histologic documentation of the original tumor. Patients had bevacizumab-resistant disease defined as having a treatment-free interval of less than 6 months after last bevacizumab treatment, or had progressed during treatment with a bevacizumab-containing regimen. Patients had either measurable disease based on RECIST 1.1 criteria [11], detectable disease based on ascites and/or pleural effusion attributed to tumor, or solid and/or cystic abnormalities on radiographic imaging not meeting RECIST criteria within the setting of a CA125 >2× upper limit normal (ULN). Additional eligibility requirements included an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of less than or equal to 1 and adequate hepatic, renal and hematologic function. All patients were at least 18 years of age. Participants had to have received at least one platinum-based chemotherapy and may have received up to two additional cytotoxic regimens. Patients receiving only one prior cytotoxic regimen must have had either a platinum-free interval less than 12 months, or persistent disease or progression during platinum-based therapy. Patients must not have received any biologic (non-cytotoxic) therapy for treatment of recurrent or persistent disease other than bevacizumab-containing regimens. Patients were allowed to receive biologic therapy as part of their front-line therapy.

2.2. Exclusion criteria

Exclusion criteria included evidence of active infection requiring antibiotics; coagulopathy; ≥grade 2 neurotoxicity; history of gastrointestinal fistula/perforation; serious uncontrolled diabetes, seizures, hypertension, or other cardiovascular diseases (severe/unstable angina, grade II or greater congestive heart failure, serious cardiac arrhythmia requiring medication); ≥grade 2 peripheral vascular disease; serious non-healing wound, ulcer, or bone factor; brain metastases or primary brain tumors within the last three years; history of cerebrovascular accident, transient ischemic attack, or subarachnoid hemorrhage within 6 months of the first date of study treatment; tumor involving major vessels or transmural bowel involvement; central pulmonary metastases or recent hemoptysis (≥1/2 tsp. of red blood) within 28 days of study therapy; >grade 1 proteinuria or urine protein creatinine ratio >1.0; clinical symptoms or signs of gastrointestinal obstruction; therapeutic anticoagulation. Prior treatment with nintedanib was not permitted. Prior bevacizumab therapy required at least a 4-week “wash-out” period. None of the patients enrolled had reproductive potential. All patients were required to provide written informed consent prior to entry into the study.

2.3. Study design

The study was an open-label, single-arm, phase II multicenter clinical trial (Duke Cancer Institute, Virginia Oncology Associates, University of Virginia) (NCT01669798). The primary end point was the proportion of patients surviving progression-free for at least 6 months (PFS_{6m}) after initiating study therapy. The secondary end points included objective tumor response (complete response (CR) or partial response (PR)) based on RECIST 1.1 [11]; objective tumor response based Gynecologic Cancer InterGroup (GCIG) CA-125 criteria [12]; safety and tolerability, PFS, and overall survival (OS). Translational research objectives included correlation of baseline and on treatment levels of VEGF, additional angiogenic growth factors, and treatment outcomes; and correlation of baseline and on treatment levels of coagulation and endothelial cell activation markers that may predict thrombotic or bleeding risks related to treatment. The protocol was approved by independent ethics committee/institutional review boards at the respective institutions and was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice.

2.4. Study treatment

Participants received nintedanib 200 mg orally twice daily in treatment courses of 4 weeks. In the case of treatment-related adverse events, nintedanib dose reductions were performed accordingly (1-level reduction, 150 mg BID, and 2-level reduction, 100 mg BID). There were no dose escalations or re-escalations on this study. Treatment was continued until disease progression, intolerable toxicity, or withdrawal of consent.

2.5. Assessment

The baseline evaluations included history, physical examination, clinical and/or radiographic tumor measurement; imaging with computed tomography or magnetic resonance imaging scan including chest imaging; laboratory tests (hematology, blood chemistry, liver function tests, coagulation, urinalysis, CA125, and research samples), 12-lead electrocardiogram (ECGs). Hematology, blood chemistry, liver function, and CA125 evaluations were performed before each treatment cycle. The RECIST response assessments were performed every two cycles. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria version 4.0.

2.6. Biomarker analyses

Double-spun, platelet poor EDTA plasma was collected at baseline, every other cycle (i.e. 3, 5, etc.), at progression, and one month after study treatment was discontinued and stored at −80 °C until analysis. Multiplex protein arrays were used for the analysis of twenty-two factors related to tumor growth and angiogenesis. The markers analyzed included soluble angiogenic factors (ANG2, BMP9, HGF, PDGFAA, PDGFBB, PlGF, VEGF, VEGFD, VEGFR1, VEGFR2, and VEGFR3); matrix-derived factors (OPN, TGFβ1, TGFβ2, TGFβR3, TIMP1, and TSP2); and vascular activation and inflammation markers (CD73, ICAM1, IL6, SDF1, and VCAM1). All biomarkers were measured using the CiraScan multiplex platform (Aushon Biosystems, Inc., Billerica, MA), except for BMP9, TGFβR3 and CD73. BMP9 [13], TGFβR3 [14], and CD73 [15] were analyzed as previously described.

2.7. Statistical analyses

he primary objective of this phase II trial was to assess the proportion of patients surviving progression-free for at least 6 months (PFS_{6m}) after initiating nintedanib therapy.

If 20% of participants remained progression-free at 6 months, nintedanib would be deemed effective and worthy of further research. The trial utilized a 2-stage Simon optimal design to test the null hypothesis that the 6-month PFS rate is ≤13% against the alternative hypothesis that this rate is ≥27% [16]. For a total of 56 evaluable subjects, 27 were accrued during stage 1, and an interim analysis performed. If fewer than 4 patients during stage 1 were found progression-free at 6 months then the therapy would be deemed ineffective and accrual stopped. If 4 or more of the patients were progression-free at 6 months, another 29 patients would be accrued. If at least 11 of 56 patients (20%) were progression-free at 6 months, the therapy would be deemed effective and worthy of further research. This design had a one-sided Type I error of 0.10 and a power of 0.90. In the event that the PFS_{6m} could not be determined due to early discontinuation or loss to follow up before progression, the probability of surviving to 6 months would be calculated by the Kaplan-Meier method. If the lower 10% confidence bound of this probability is greater than 0.13 the trial would be deemed a success. Efficacy outcomes were based on intent-to-treat analyses. PFS was defined as the length of time from on-study to disease progression or death. OS was measured from the date of study entry until the date of death.

Confidence intervals for proportions were estimated using the exact binomial method.

To test the hypothesis that higher angiogenic-related biomarker levels would be prognostic for improved survival outcomes and response in women treated with nintedanib the following statistical analyses were performed. The association of PFS with baseline values of VEGF (continuous) was tested with the Cox proportional hazards models; VEGF was dichotomized at a clinically meaningful cut point and PFS was plotted using the Kaplan-Meier method. Analogous procedures were used to examine the association of PFS with baseline values of the growth factors listed below. Baseline biomarker levels were compared using Wilcoxon rank sum tests based upon response to treatment.

All statistical analyses were conducted using SAS software version 9.4 (SAS Institute, Inc. Cary, NC) and graphs were created using R software (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Patient characteristics

27 participants were enrolled from 02/2013 to 09/2017 and 26 received at least one cycle of nintedanib and were evaluable for PFS_{6m}. There were 5 screen failures and 1 patient withdrew from the study. The baseline demographics of the enrolled patients are listed in Table 1. The median age was 65 years (range 44–73); 89% had high-grade serous EOC; 70% received at least 2 prior chemotherapies; and 81% (22/27) had chemoresistant disease. All patients had received one platinum-based chemotherapy and exhibited bevacizumab-resistant disease. Patients received a median of 2 cycles (range 1–17) of treatment while on-study.

3.2. Efficacy

With median follow-up of 15.6 months (range 2.1–37.6) the PFS_{6m} rate was 11.5% (3/26) [95% CI: 2.5–30.2]. One patient discontinued therapy prior to progression and the Kaplan-Meier method was used to calculate probability of surviving to 6 months. The PFS_{6m} point estimate was 0.15 with a 10% lower confidence bound of 0.07. Three participants had long duration of disease control (8–16 months). The median PFS

Table 1
Baseline patient characteristics

Characteristic	
	Median (range)
Age years	65 (44–73)
	Number (%)
Race	
Caucasian	24 (88.9)
African American	2 (7.4)
Unknown	1 (3.7)
Ethnicity	
Non-Hispanic	25 (92.6)
Unknown	2 (7.4)
Primary Tumor Site	
Ovary	26 (96.3)
Peritoneum	1 (3.7)
Histologic Subtype	
Serous	24 (88.9)
Clear cell	1 (3.7)
Adenocarcinoma, NOS	1 (3.7)
Adenocarcinoma, poorly differentiated	1 (3.7)
ECOG Performance Status	
0	14 (52%)
1	13 (48%)
Prior number of regimens	
1	8 (29.6)
2	10 (37.0)
3	7 (25.9)
4	2 (7.4)
Platinum status	
Platinum-resistant	22 (81.5)
Platinum-sensitive	5 (18.5)
Antecedent bevacizumab regimen	
1st-line chemotherapy/Bev with Bev maintenance	9 (33.3)
1st-line chemotherapy/Bev without Bev maintenance	2 (7.4)
2nd-line chemotherapy/Bev with Bev maintenance	5 (18.5)
2nd-line chemotherapy/Bev without Bev maintenance	4 (14.8)
2nd-line single agent Bev	6 (22.2)
3rd-line chemotherapy and Bev	1 (3.7)

and OS were 1.8 and (95% CI: 1.6–3.5) and 16 months (95% CI: 11–25), respectively.

The response rate was 7.4% (2/27 PR) [95% CI: 1.0–24.3]. Thirty-seven percent (10/27) [95% CI: 19.4–57.6] had stable disease, while 56% (15/27) [95% CI: 35.3–74.5] had progressive disease. Disease control rate (PR + SD) was 44% (12/27) [95% CI: 25.5–64.7]. There were no CA125 responses. One patient with stable disease discontinued due to toxicity (grade 1 diarrhea, grade 1 weight loss, and grade 2 anorexia) after receiving 4 months of treatment.

A secondary objective was to compare PFS of patients who had platinum-sensitive bevacizumab-resistant disease to those who had both bevacizumab and chemotherapy resistance. Fourteen patients had antecedent bevacizumab maintenance therapy; while 13 had either chemotherapy plus bevacizumab or single-agent bevacizumab antecedent therapy. Of the 14 patients with antecedent bevacizumab maintenance therapy, only four had platinum-sensitive disease. In the four bevacizumab-resistance only patients, the median PFS was 3.6 months compared to median PFS of 1.8 months for the 23 patients with both bevacizumab and chemotherapy resistance. Of the patients with bevacizumab-resistance only there were 2 confirmed PR, one confirmed SD, and one PD.

An exploratory analysis was performed comparing PFS in women who received front-line vs second-line bevacizumab maintenance therapy. Median PFS was similar in both groups (front-line maintenance bevacizumab: 1.84 months; second-line maintenance: 1.79 months).

3.3. Toxicity

All patients who received at least one dose of therapy were evaluable for toxicity (Table 2). Toxicity was reported as the maximum toxicity experienced during study treatment. Grade 3 adverse events included liver enzyme elevation (ALT, n = 1, 4%; AST, n = 3, 11%; total, n = 4, 15%), diarrhea (n = 2, 7%), abdominal pain (n = 2, 7%), non-cardiac chest pain (n = 1, 4%), kidney infection grade 3 (1, 4%), sinusitis (n = 1, 4%), neutropenia (n = 1, 4%), hypoalbuminemia (n = 1, 4%), hyponatremia (n = 1, 4%), proteinuria (n = 1, 4%), other renal/urinary disorder (n = 1, 4%), urinary tract obstruction grade 3 (n = 1, 4%), dyspnea grade 3 (n = 1, 4%), and hypertension (n = 3, 11%). Other adverse events included grade 2 fatigue (n = 2, 7%), and grade 2 nausea/vomiting (n = 4, 15%). While severe diarrhea was uncommon, grade >2 diarrhea occurred more frequently (n = 12, 44%).

Overall 98 treatment cycles were given. There were 8/27 (30%) patients that required dose delay or modifications during 19 cycles. The primary reason for treatment delay and/or dose modifications were gastrointestinal toxicity (nausea or diarrhea, 9/19 (47%)), followed by elevated transaminases (8/19, 42%) The most common reason for treatment discontinuation was disease progression. No treatment-related deaths were reported.

Table 2
Adverse events

	Grade 1	Grade 2	Grade 3	Grade 4
	N (%)	N (%)	N (%)	N (%)
Hematologic				
Anemia	2 (7%)	0 (0%)	0 (0%)	0 (0%)
Neutropenia	0 (0%)	1 (4%)	0 (0%)	0 (0%)
Thrombocytopenia	2 (7%)	0 (0%)	0 (0%)	0 (0%)
Non-hematologic[a]				
Abdominal Pain	5 (19%)	2 (7%)	0 (0%)	0 (0%)
ALT elevation	8 (30%)	1(4%)	1(4%)	0 (0%)
AST elevation	7 (26%)	1(4%)	3 (11%)	0 (0%)
ALK phosphatase elevation	7 (26%)	1(4%)	0 (0%)	0 (0%)
Arthralgia	3 (11%)	0 (0%)	0 (0%)	0 (0%)
Anorexia	2 (7%)	2 (7%)	0 (0%)	0 (0%)
Back pain	2 (7%)	0 (0%)	0 (0%)	0 (0%)
Bloating	1(4%)	2 (7%)	0 (0%)	0 (0%)
Constipation	2 (7%)	2 (7%)	0 (0%)	0 (0%)
Diarrhea	6 (22%)	9 (33%)	2 (7%)	0 (0%)
Dyspnea	0 (0%)	2 (7%)	0 (0%)	0 (0%)
Edema	3 (11%)	0 (0%)	0 (0%)	0 (0%)
Fatigue	12 (44%)	2 (7%)	0 (0%)	0 (0%)
Headache	8 (30%)	0 (0%)	0 (0%)	0 (0%)
Hypertension	1(4%)	2 (7%)	1(4%)	0 (0%)
Hypoalbuminemia	2 (7%)	0 (0%)	0 (0%)	0 (0%)
Hypomagnesemia	3 (11%)	1(4%)	0 (0%)	0 (0%)
Hyponatremia	3 (11%)	0 (0%)	1(4%)	0 (0%)
Nausea	13 (48%)	4 (15%)	0 (0%)	0 (0%)
Proteinuria	1(4%)	2 (7%)	1(4%)	0 (0%)
Vomiting	13 (48%)	0 (0%)	0 (0%)	0 (0%)
Weight loss	3 (11%)	0 (0%)	0 (0%)	0 (0%)

[a] Reported if greater than 4% frequency and attributed to study drug.

3.4. Biomarker

Plasma was available from 26 patients for analysis. Twenty-two protein markers were evaluated at baseline. High baseline IL6 levels were associated with increased risk for disease progression based on univariate Cox proportional hazard regression (HR = 2.1; CI: 1.09–3.85, p = 0.03).(Table 3) In contrast, there was no association between baseline levels of either VEGF ligands or soluble VEGFRs with PFS.(Table 3) Three baseline biomarker values were associated with clinical outcome (partial response or stable disease *vs* progressive disease). Specifically, CD73 (median value 3.0 *vs* 3.8, p = 0.02), IL6 (18.2 *vs* 32.6, 0.05), and VEGFD (43.4 *vs* 66.7, p = 0.04) baseline levels were lower in women treated with nintedanib who demonstrated either a PR or had SD compared to those who developed progressive disease.(Table 4, Fig. 1) The patient with the lowest IL6 level had stable disease and received 10 cycles of therapy.

4. Discussion

Bevacizumab in combination with chemotherapy followed by maintenance bevacizumab is currently FDA and EMA approved for the treatment of women with newly diagnosed advanced ovarian, tubal, and peritoneal cancer as well as those with recurrent platinum-sensitive and platinum-resistant disease [7]. Moreover, the randomized phase III MITO16B-MaNGO OV2B-ENGOT OV17 trial reported a 3-month improvement in progression-free survival in women with platinum-sensitive recurrent EOC, after front-line chemotherapy regimen including bevacizumab, who were treated with subsequent bevacizumab

Table 3
Association between baseline biomarker levels and PFS using univariate Cox proportional hazard regression.

Baseline marker	Hazard ratio	Lower HR 95% CI	Upper HR 95% CI	*P*
IL6	2.051	1.093	3.850	0.0253
VEGFR3	1.638	0.950	2.822	0.0756
TGFβ2	1.609	0.897	2.886	0.1106
PDGFbb	1.270	0.944	1.707	0.1140
CD73	2.328	0.804	6.738	0.1191
VEGF	0.413	0.132	1.289	0.1278
VEGFD	1.489	0.891	2.489	0.1291
PDGFAA	1.268	0.929	1.732	0.1351
TGFβ1	0.368	0.093	1.451	0.1532
SDF1	0.730	0.420	1.269	0.2649
ICAM 1	2.463	0.443	13.689	0.3029
TIMP1	1.805	0.553	5.898	0.3280
VEGFR1	0.558	0.166	1.872	0.3446
BMP9	0.418	0.063	2.775	0.3664
Ang2	0.801	0.471	1.363	0.4139
TSP2	1.742	0.460	6.605	0.4142
TGFβR3	1.396	0.527	3.699	0.5018
HGF	1.210	0.641	2.283	0.5568
PlGF	1.542	0.331	7.180	0.5812
VEGFR2	1.168	0.453	3.010	0.7484
OPN	1.065	0.712	1.593	0.7582
VCAM 1	1.152	0.291	4.569	0.8401

Table 4
Association between baseline biomarker levels and treatment response

Variable		Baseline value (n = 26)	Baseline value for cohort with PR/SD[a] (n = 11)		Baseline value for cohort with PD[b] (n = 15)		*P*
Name	Unit	Median	Median	FC[c]	Median	FC[c]	
CD73	pg/ml	3.3	3.0	0.8	3.8	1.1	0.02
VEGFD	pg/ml	54.0	43.4	0.5	66.7	0.7	0.04
IL6	pg/ml	23.8	18.2	0.6	32.6	1.1	0.05
TGFβ1	ng/ml	97.9	122.5	1.2	90.3	0.9	0.07
TIMP1	ng/ml	107.0	93.5	0.9	116.4	1.1	0.11
VEGF	pg/ml	1116.3	1405.4	1.2	1033.4	0.9	0.11
PDGFBB	pg/ml	266.5	184.7	0.4	396.4	0.9	0.13
VEGFR2	ng/ml	249.0	183.5	0.7	266.7	1.0	0.15
TSP2	ng/ml	97.7	95.1	0.8	104.9	0.9	0.16
BMP9	pg/ml	46.1	47.0	1.0	45.9	1.0	0.18
ICAM1	ng/ml	663.0	635.1	0.9	686.8	1.0	0.18
HGF	pg/ml	158.8	130.1	0.6	191.0	0.9	0.28
Ang2	pg/ml	191.8	261.1	1.1	189.5	0.8	0.32
TGFβ2	ng/ml	31.8	22.0	0.6	33.4	0.9	0.32
PDGFAA	pg/ml	205.8	89.5	0.4	233.9	0.9	0.35
VEGFR3	pg/ml	131.0	137.3	1.1	124.6	1.0	0.47
VEGFR1	pg/ml	4084.5	4293.0	1.1	3324.0	0.8	0.50
OPN	ng/ml	821.4	643.0	0.3	923.8	0.4	0.57
PlGF	pg/ml	24.1	23.4	0.9	24.3	1.0	0.84
VCAM1	μg/ml	2.1	2.1	1.0	2.3	1.0	0.84
SDF1	pg/ml	2045.3	1968.6	0.9	2121.9	1.0	0.88
TGFβR3	pg/ml	51.6	50.6	0.9	52.5	1.0	1.00

[a] PR = partial response; SD = stable disease
[b] PD = progressive disease
[c] FC = fold change

(11.8 vs 8.8 months; HR 0.51 (CI: 0.41–0.65); p < 0.001)) [17]. Therefore, we anticipate that the use of bevacizumab will continue to increase across all lines of therapy and the development of bevacizumab resistance will become more common. One mechanism of resistance to VEGF-blockade is the induction of secondary pathways such as PDGF and FGF pathways. Despite scientific rationale for the use of nintedanib, that targets secondary PDGF and FGF angiogenic pathways, in patients whose tumors have demonstrated bevacizumab resistance our findings did not demonstrate sufficient anti-tumor activity to warrant further evaluation in an unselected patient population.

Even though our study did not meet the prespecified criteria to proceed to the second stage after interim analysis (Kaplan-Meier PFS_{6m} probability lower 10% confidence bound was 0.07), our findings did reveal that nintedanib had a longer duration of median PFS in select patients with platinum-sensitive disease. However, it is unlikely that this small increase is clinically meaningful. More importantly, those with a partial response and disease stabilization with nintedanib were more likely to have lower baseline levels of CD73, IL6, and VEGFD. Unfortunately, an assessment of the predictive value of biomarkers could not be performed due to the single-arm study design. However, VEGFD and IL6 have been identified as prognostic and predictive markers for survival in other solid tumors, and similar observations have been made in patients treated with bevacizumab, pazopanib, and ramucirumab in various cancers [18–20]. We are currently exploring these and other biomarkers in trials evaluating angiogenic agents such as bevacizumab and cediranib.

Our biomarker data revealing a prognostic association between high baseline IL6 levels and worse survival outcomes is consistent with our recent study of IL6 in chemotherapy naïve, newly diagnosed EOC. We previously reported that IL6 was a prognostic and predictive maker in women with advanced EOC treated on GOG-0218 [21]. High IL6 baseline plasma levels were predictive and prognostic of progression-free and overall survival outcomes. IL6 has been reported to play an important role in carcinogenesis in various solid tumors and regulates proliferation, adhesion, angiogenesis, tumor invasion and immunologic functions [22]. Our findings in the current study align with other reports in the literature regarding the prognostic significance of IL6 in EOC [23]. Furthermore, IL6 levels have been predictive of anti-angiogenic therapeutic efficacy in patients with metastatic renal cancer and pancreatic cancer in independent randomized trials. The studies in renal cancer included one evaluating bevacizumab combined with interferon alfa and the other assessing pazopanib [19,24]. In patients with pancreatic cancer, a risk score based on IL6 and hepatocyte growth factor (HGF) identified those who benefitted most from the addition of bevacizumab [18]. However, Richardson et al. reported no association between IL6 and treatment response in a randomized phase II trial of weekly paclitaxel with and without pazopanib in women with recurrent ovarian, tubal, or peritoneal cancer [25]. These findings across different angiogenic therapies (bevacizumab, pazopanib, and nintedanib) and tumor types reinforce exploration of IL6 as a potential predictive biomarker for anti-angiogenic therapy in different cancers.

Baseline VEGFD and CD73 levels were also associated with response and stable disease outcomes. Patients with low levels were more likely to have a partial response or disease stabilization, while those with high levels more frequently had disease progression. High VEGFD expression was associated with poor prognostic features, such as lymph node and extra-pelvic peritoneal metastasis, as well as worse survival [26]. In the GOG-0218 study, VEGFD was not associated with survival outcomes or predictive of bevacizumab efficacy [21]. Interestingly, data suggests that VEGFD predicts benefit from anti-angiogenic agents in GI-related cancer (pancreas, colon) while IL6 predicts benefit from renal and ovarian cancers. Additional analyses are currently underway to explore the predictive potential value of these markers in both prostate cancer (CALGB90401) and bladder cancer (CALGB90601). CD73 is a cell surface enzyme expressed on numerous cell types including tumor cells that can upregulate adenosine (an immunosuppressive factor) in the

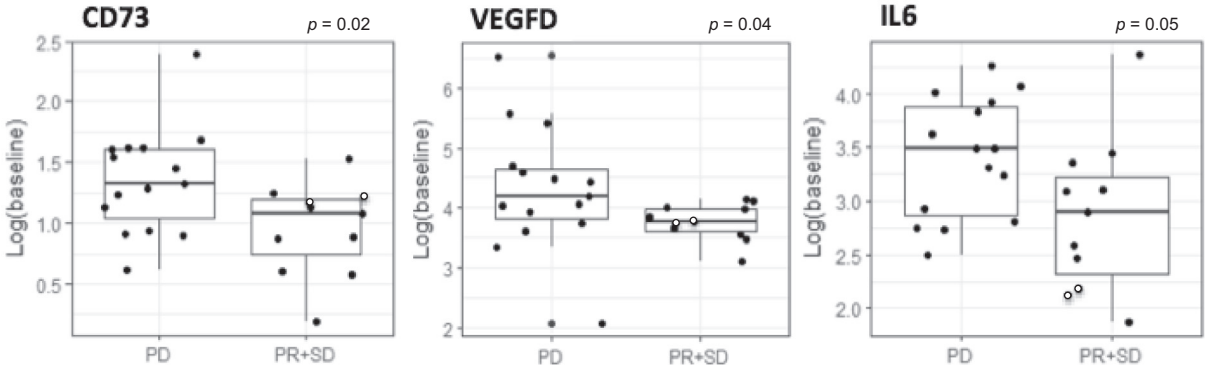

Fig. 1. Box and whisker plots demonstrating association between representative baseline biomarker values and response status. CD73, IL6, and VEGFD baseline biomarker values were associated with clinical outcome. Specifically, CD73 (median value 3.0 vs 3.8, p = 0.02), IL6 (18.2 vs 32.6, p = 0.05), and VEGFD (43.4 vs 66.7, p = 0.04) baseline levels were lower in women who demonstrated either a partial response or had stable disease compared to those with progressive disease on nintedanib therapy. oRepresent those with partial response.

tumor microenvironment. Overexpression of CD73 is known to be immunosuppressive and associated with worse survival outcomes in several solid tumors, including ovarian cancer [27]. CD73 may also be involved in angiogenesis and coordinated with other inflammatory and angiogenic biomarkers. Inhibition of CD73 via siRNA decreased tumor angiogenesis and IL6 levels in an *in vivo* model of breast cancer [28]. The CD73 assay is a new assay developed by our group; early data evaluating circulating levels is now being generated. VEGF is a known immunomodulator; however, the role of CD73 as a potential biomarker in patients treated with anti-angiogenic agents is still not well understood [29,30].

The lack of nintedanib efficacy, in our study, indicates that resistance to VEGF-blockade is multifactorial and complex. We hypothesized that targeting secondary angiogenic pathways in addition to VEGF could overcome bevacizumab resistance. However, resistance to anti-angiogenic therapy occur via mechanisms beyond secondary pathway recruitment such as vessel cooption, intussusception or vessel splitting, endothelial cell differentiation, vascular mimicry, and vasculogenesis [8]. For the purpose of this study bevacizumab-resistance was arbitrarily defined as a treatment-free interval following response to bevacizumab of <6 months, or progression during treatment with a bevacizumab-containing regimen. However, over 80% of recruited patients had both bevacizumab and chemotherapy-resistant disease; a challenging patient population with historically low response rates to cytotoxic chemotherapy and shorter duration of survival. The high frequency of patients with chemotherapy resistant disease possibly contributed to the lack of efficacy in our study. Of the four patients with platinum-sensitive bevacizumab-resistant disease there were 2 confirmed PR, one confirmed SD, and one PD. While a single-agent anti-angiogenic strategy may not have significant anti-tumor activity in this patient population, combination therapeutic options may be more efficacious. Pignata et al. reported improved PFS with the addition of pazopanib to weekly paclitaxel compared to paclitaxel only in women with platinum-resistant ovarian cancer (median PFS 6.4 vs 3.5 months; HR, 0.42; 95% CI 0.25–0.69; p = 0·0002) [31]. In contrast, a similar trial (GOG-0186J) trial of weekly paclitaxel with and without pazopanib in women with either platinum-resistant or -sensitive recurrent disease revealed similar response rates (31.8% vs 22.7%), PFS (7.5 vs 6.2 months), and overall survival (20.7 vs 23.3 months) for pazopanib plus paclitaxel vs paclitaxel alone [25]. The conflicting trial results may be due to differences in eligibility and inclusion of a population more likely to respond to chemotherapy in the GOG-0186J study. The TAPAZ trial, a phase II trial evaluating weekly paclitaxel with or without pazopanib in platinum-resistant EOC patients who relapse during bevacizumab maintenance is currently recruiting (NCT02383251).

Most patients were able to tolerate nintedanib treatment and the safety profile was similar to what has been reported. In our study, the more common reasons for treatment delay and dose modifications were increased AST/ALT levels and gastrointestinal toxicity. Gastrointestinal side effects and elevated liver enzymes represented the leading AEs in other clinical trials of nintedanib. Gastrointestinal AEs rarely were of CTCAE grade >2. Neutropenia has been reported with nintedanib; however, only one of our patients required dose adjustment due to hematologic toxicity. Overall no new concerning safety signals were identified and nintedanib was tolerable with a manageable toxicity profile.

In conclusion, single-agent nintedanib has minimal activity in an unselected bevacizumab-resistant EOC population. While the primary end point of PFS_{6m} rate to proceed with second stage of accrual was not achieved, three participants with platinum-sensitive disease had long duration of disease control (8–16 months). This finding highlights the importance of designing adaptive trials that can be efficiently conducted to continue drug evaluation in an enriched population that may have benefit. Further evaluation of biomarker directed therapy is warranted in ovarian, tubal, and peritoneal cancer patients treated with anti-angiogenic therapy.

Conflict of interest statement

This investigator-initiated study was supported by Boehringer Ingelheim and philanthropic anonymous funding for ovarian cancer research. Dr. Secord reports honoraria for consulting and advisory boards for Alexion, Aravive, Astex, AstraZeneca, Boehringer Ingelheim, Clovis, Janssen/Johnson & Johnson, Merck, Mersano, Myriad, Oncoquest, Precision Therapeutics, Roche/Genentech, and TESARO. Her institution has received clinical trial funding from AbbVie, Amgen, Astellas Pharma Inc., Astex Pharmaceuticals Inc., AstraZeneca, Bristol Myers Squibb, Eisai, Endocyte, Exelixis, Incyte, Merck, PharmaMar, Prima Biomed, Roche/Genentech, TapImmune and TESARO. Dr. Nixon reports honoria for consulting and advisory boards for Eli Lilly, Pfizer, and Kanghong Pharma. He has received grant support from Acceleron Pharma, Amgen, AstraZeneca/MedImmune, Eureka Therapeutics, Genentech, Leadiant Biosciences, MedPacto Inc., Novartis, Seattle Genetics, and Tracon Pharma. Dr. Duska received funding from Duke University to conduct this study. She reports honoraria from advisory boards for Merck, Genentech, and MedImmune as well as from Parexel for independent oncology review. Her institution has received clinical trial funding from Merck, Novartis, AbbieVie, Millenium, Cerulean, Pfizer, Tesaro, Morab, and Ludwig. Dr. Havrilesky reports that her institution has received grant funding from AstraZeneca. The remaining authors have no financial disclosures and conflicts of interest.

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All authors were integral for the study and contributed to the research as noted below.

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